2026 Genetic Testing Policies

MVP Health Care® Policy and Procedure Guidelines

Policies effective January 1, 2026



Table of Contents

1. Chromosomal Microarray and Low-pass Whole Genome Sequencing
2. Esophageal Pathology Testing
3. Fibromyalgia Testing
4. Gene Expression Profiling and Protein Biomarkers for Prostate Cancer
5. General Genetic Testing, Germline Disorders
6. General Genetic Testing, Somatic Disorders
7. Genetic Markers for Assessing Risk of Cardiovascular Disease
8. Genetic Testing and Genetic Expression Profiling in Patients with Uveal
9. Genetic Testing For Alpha- and Beta- Thalassemia
10. Genetic Testing for Breast, Ovarian, Pancreatic, and Prostate Cancers
11. Genetic Testing for CHARGE Syndrome
12. Genetic Testing for Connective Tissue Disorders
13. Genetic Testing for Cystic Fibrosis
14. Genetic Testing for Diagnosis of Inherited Peripheral Neuropathies
15. Genetic Testing for Duchenne, Becker, Facioscapulohumeral, and Limb-Girdle Muscular Dystrophies
16. Genetic Testing for Epilepsy
17. Genetic Testing for Familial Alzheimer Disease
18. Genetic Testing for Familial Hypercholesterolemia
19. Genetic Testing for Fanconi Anemia
20. Genetic Testing for FMR1 Mutations
21. Genetic Testing for Germline Mutations of the RET Proto-Oncogene
22. Genetic Testing for Hereditary Hearing Loss
23. Genetic Testing for Hereditary Hemochromatosis
24. Genetic Testing for Hereditary Pancreatitis
25. Genetic Testing for Inherited Cardiomyopathies and Channelopathies
26. Genetic Testing for Lactase Insufficiency
27. Genetic Testing for Li-Fraumeni Syndrome
28. Genetic Testing for Lipoprotein(a) Variant(s) as a Decision Aid for Aspirin Treatment and/or CVD Risk Assessment
29. Genetic Testing for Neurodegenerative Disorders
30. Genetic Testing for Neurofibromatosis and Related Disorders
31. Genetic Testing for Ophthalmologic Conditions
32. Genetic Testing for Polyposis Syndromes
33. Genetic Testing for PTEN Hamartoma Tumor Syndrome
34. Genetic Testing for Rett Syndrome
35. Genetic Testing of CADASIL Syndrome
36. Genetic Testing of Mitochondrial Disorders
37. Genomic Testing for Hematopoietic Neoplasms
38. Liquid Biopsy
39. Lynch Syndrome
40. Microsatellite Instability and Tumor Mutational Burden Testing
41. Minimal Residual Disease (MRD)

42. Molecular Analysis for Gliomas
43. Molecular Diagnostics for Breast Cancer Prognosis
44. Molecular Markers in Fine Needle Aspirates of the Thyroid
45. Molecular Profiling for Cancers of Unknown Primary Origin
46. Molecular Testing for Cutaneous Melanoma
47. Molecular Testing of Pulmonary Specimens
48. Mutation Analysis in Myeloproliferative Neoplasms
49. Pancreatic Cancer Risk Testing Using Pancreatic Cyst Fluid
50. Pharmacogenetic Testing
51. Precision Genetic Test Management Reimbursement Policy
52. Pre-Implantation Genetic Testing
53. Prenatal Screening (Genetic)
54. Prenatal Screening for Fetal Aneuploidy
55. Proteogenomic Testing of Individuals with Cancer
56. Red Blood Cell Molecular Testing
57. Testing for Alpha-1 Antitrypsin Deficiency
58. Testing for Colorectal Cancer Management
59. Testing for Targeted Therapy of Non-Small-Cell Lung Cancer
60. Testing of Homocysteine Metabolism-Related Conditions
61. Transplant Rejection Testing
62. Use of Common Genetic Variants (single nucleotide polymorphisms) to Predict Risk of Non-Familial Breast Cancer
63. Venous and Arterial Thrombosis Risk Testing
64. Whole Genome and Whole Exome Sequencing

Chromosomal Microarray and Low-pass Whole Genome Sequencing

Policy Number: AHS – M2033 – Chromosomal Microarray and Low-pass Whole Genome Sequencing	Prior Policy Name and Number, as applicable: AHS – M2033 – Chromosomal Microarray
Initial Policy Effective Date: 01/01/2026	

I. POLICY DESCRIPTION
II. RELATED POLICIES
III. INDICATIONS AND/OR LIMITATIONS OF COVERAGE
IV. TABLE OF TERMINOLOGY
V. SCIENTIFIC BACKGROUND
VI. GUIDELINES AND RECOMMENDATIONS
VII. APPLICABLE STATE AND FEDERAL REGULATIONS
VIII. APPLICABLE CPT/HCPCS PROCEDURE CODES
IX. EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Chromosomal microarray (CMA) testing refers to the use of comparative genomic hybridization (CGH) arrays to detect small (10 to 100kb) duplications or deletions of chromosomal DNA (copy number variants or CNVs), similarity in single nucleotide sequences (homozygosity), and triploidy when chromosomal abnormality is suspected based on clinical presentation (Schrijver & Zehnder, 2024). Genetic counseling is strongly recommended for individuals being considered for chromosomal microarray testing. Low-pass whole genome sequencing (low-pass WGS) is a method of WGS that is less expensive and has lower coverage than standard WGS. Low-pass WGS maintains a high accuracy for detecting single nucleotide sequences by using imputation algorithms (Gencove, 2023).

For guidance on whole exome sequencing, please see AHS-M2032-Whole Genome and Whole Exome Sequencing.

II. Related Policies

Policy Number	Policy Title
AHS-G2055	Prenatal Screening for Fetal Aneuploidy
AHS-M2032	Whole Genome and Whole Exome Sequencing
AHS-M2075	Genetic Testing for Epilepsy
AHS-M2176	Testing for Autism Spectrum Disorder and Developmental Delay
AHS-M2179	Prenatal Screening (Genetic)

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) To evaluate any second-trimester or later pregnancy loss **or** the second consecutive first-trimester pregnancy loss, chromosomal microarray (CMA) testing **or** low-pass whole genome sequencing (low-pass WGS) of the products of conception (e.g., fetal tissue and/or the fetus) **MEETS COVERAGE CRITERIA**.

2) Prenatal CMA testing **or** low-pass WGS **MEETS COVERAGE CRITERIA** when **any** of the following conditions are met:
   a) When diagnostic testing for fetal aneuploidy is needed for pregnant individuals undergoing invasive prenatal testing (i.e., amniocentesis, chorionic villus sampling, or fetal tissue sampling).
   b) When non- invasive prenatal screening (NIPS) results require confirmation.
   c) As a follow-up test for any smaller copy-number changes that were reported as positive by NIPS.
   d) When ultrasound examination reveals one or more structural abnormalities.
   e) When, regardless of gestational age, fetal growth restriction is detected and a fetal malformation or polyhydramnios (or both) are also present.
   f) When unexplained isolated fetal growth restriction is diagnosed at <32 weeks of gestation.
   g) When there is intrauterine fetal demise or stillbirth in the third trimester and CMA is indicated to determine the potential cause.
   h) When the fetus is at high risk for a chromosome abnormality detectable by CMA, based on family history.

3) Evaluation with CMA testing **or** low-pass WGS **MEETS COVERAGE CRITERIA** for **any** of the following situations:

a) For individuals with multiple congenital anomalies that are not specific to a well-delineated genetic condition and cannot be identified based on clinical evaluation alone.

b) For individuals with non-syndromic developmental delay/intellectual delay.

c) For individuals with autism spectrum disorder.

d) For individuals with a suspected inherited seizure disorder.

e) When sex determination by NIPS is discordant with physical examination or clinical findings are suggestive of a disorder of sexual differentiation.

f) For individuals with proportionate short stature with other physical or structure defects.

4) When performed in parallel with fetal diagnostic testing, maternal cell contamination (MCC) analysis **MEETS COVERAGE CRITERIA**.

5) For central nervous system (CNS) tumors and pediatric solid and soft tissue tumors, CMA testing **MEETS COVERAGE CRITERIA.**

6) When a chromosomal trisomy is suspected, postnatal CMA testing **DOES NOT MEET COVERAGE CRITERIA**.

7) Co-testing CMA and low-pass WGS **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

8) For all other situations not described above, CMA testing **DOES NOT MEET COVERAGE CRITERIA**.

IV. Table Of Terminology

Term	Definition
AAN	American Academy of Neurology
AAP	American Academy of Paediatrics
ACMG	American College of Medical Genetics and Genomics
ACOG	American College of Obstetricians and Gynaecologists
AMP	Association For Molecular Pathology
ASD	Autism Spectrum Disorder
ASRM	American Society for Reproductive Medicine

CCMG	Canadian College of Medical Geneticists
CGH	Comparative genomic hybridization
CLIA	Clinical Laboratory Improvement Amendments
CM	Chromosomal mosaicism
CMA	Chromosomal microarray analysis
CMS	Centers for Medicare and Medicaid
cnLOH	Copy-neutral loss of heterozygosity
CNS	Central nervous system
CNVs	Copy number variants
CSCA	Chromosomal abnormalities
CVS	Chorionic villi sampling
DD	Developmental delay
DD/ID	Developmental disability/Intellectual disability
ES	Endocrine Society
FGR	Fetal growth restriction
GDD/ID	Global developmental delay
ID	Intellectual disability
ISCA	International standard cytogenetic array
ISPD	International Society for Prenatal Diagnosis
ISS	Idiopathic short stature
IUGR	Intrauterine growth restriction
LDTs	Laboratory developed Tests
LOH	Loss/Absence of Heterozygosity
LP-GS	Low-pass genome sequencing
MCC	Maternal cell contamination
NIHF	Non-immune hydrops fetalis
NIPS	Non- invasive prenatal screening
POC	Products of conception
PQF	Perinatal Quality Foundation
QF-PCR	Quantitative fluorescent polymerase chain reaction
RAD	Restriction-site associated DNA sequencing
SMFM	Society for Maternal-Fetal Medicine
SNP	Single nucleotide polymorphism
SOGC	Society of Obstetricians and Gynaecologists of Canada
STR	Short tandem repeat
UPD	Uniparental isodisomy
VOUS/VUS	Variant of unknown significance
VPS	Variant of possible significance

WES	Whole exome sequencing
WGS	Whole genome sequencing

V. Scientific Background

Chromosomal abnormalities are associated with a variety of disorders including developmental delay (DD), intellectual disability (ID), and congenital anomalies (Miller et al., 2010), as well as pregnancy loss (Reddy, Page, Saade, et al., 2012).

Chromosomal microarray (CMA) testing to detect copy number variations (CNVs), homozygosity, and triploidy has replaced karyotyping as the first-tier diagnostic tool for many cases where chromosomal abnormality is suspected (Miller et al., 2010; Pivalizza & Lalani, 2024; Schrijver & Zehnder, 2024). CMA is significantly more sensitive (10 to 100 kb) than traditional karyotyping (5 to 10 Mb); additionally, CMA does not require cell culture, which reduces the turnaround time for results (Miller, 2022), and provides an alternative to karyotyping when dividing cells are not available for analysis. This technique may be used for several different purposes, such as identifying a cause of pregnancy loss or identifying other aneuploid conditions, such as Down Syndrome (ACOG, 2016; Reddy, Page, & Saade, 2012). **"**Emerging studies suggest that low-pass genome sequencing (GS) provides additional diagnostic yield of clinically significant copy-number variants (CNVs) compared with chromosomal microarray analysis (CMA). However, a prospective back-to-back comparison evaluating accuracy, efficacy, and incremental yield of low-pass GS compared with CMA is warranted" (Wang et al., 2020).

Chromosomal microarray (CMA) uses comparative genomic hybridization (CGH) to compare the DNA of a patient with a normal control using standard sets of DNA probes immobilized on a glass slide or glass beads (Aradhya & Cherry, 2007). CGH arrays have been designed to cover the entire genome, for targeted analysis of known microdeletion/microduplication syndromes, and for known loci of inherited mutations (Schrijver & Zehnder, 2024). Array sensitivity varies based on the size and type of probes used. Oligonucleotide probes (~60 base pairs) or single nucleotide polymorphism (SNP) probes (32-40 base pairs) are most common. Oligonucleotide probes can be used to cover the entire genome at an average resolution of about 35 kb. Current arrays generally use a combination of copy number probes (oligonucleotide) to detect copy gains and losses and single nucleotide polymorphism (SNP) probes to detect similarity in single nucleotide sequences (homozygosity). The combination of probes detect runs of homozygosity between the maternal and paternal copy of each chromosome, enabling diagnosis of triploidy, uniparental disomy, and consanguinity as well as improving the detection of low levels of mosaicism (Miller, 2022).

Chromosomal microarray (CMA), as all genetic tests, can have variable clinical sensitivity due to the numerous types of genetic abnormalities that can impact gene expression. Some genetic conditions are caused by a change in copy number and/or a sequence change in the gene that is undetectable by CMA. If a genetic condition in which a subset of cases are caused by sequence changes, then other testing should be considered either in place of, or in addition to, CMA (Miller, 2022).

Low-pass WGS is a less expensive but lower coverage method of standard WGS. Low-pass WGS typically has less than 1x coverage, while standard WGS has a depth of 30x to 50x (Illumina, 2024). Alternatively, compared to traditional low-cost microarrays, low-pass WGS results have over ten times the amount of information. Low-pass WGS maintains accuracy with reduced coverage by using imputation algorithms, a statistical analysis method that assigns values to missing data based on known information. With imputation algorithms, the low-pass WGS method uses known genetic variants within a representative population to genotype an individual without measurements from every locus (Gencove, 2023).

CMA and Seizures

A seizure occurs due to erroneous electrical activity in the brain and may strike for many reasons including a brain injury or infection, abnormal sodium or glucose levels in the blood, congenital brain defects, epilepsy, and electric shock.

Epilepsy is a neurological disorder associated with abnormal electrical brain activity. CMA is often the first genetic tool used to obtain more information about a patient's epilepsy (Poduri et al., 2014) and has a diagnostic yield of approximately 8% with several studies reporting higher values (Dubbs, 2022). Testing a specific gene may be appropriate in some epileptic cases as more than 80 genes have been associated with epilepsy and hundreds more associated with disorders that are accompanied by seizures (Dubbs, 2022). However, if results are negative, CMA or gene testing should commence as this is likely more appropriate than testing several more genes individually (Mefford, 2015). If CMA testing is negative, gene panel and exome testing are appropriate.

Olson et al. (2014) have found that in many patients, CNVs identified through CMA were able to explain an epileptic phenotype. The authors concluded that "Because the diagnostic yield of CMA for epilepsy patients is similar to the yield in autism spectrum disorders and in prenatal diagnosis, for which published guidelines recommend testing with CMA, we recommend the implementation of CMA in the evaluation of unexplained epilepsy" (Olson et al., 2014).

CMA and Short Stature

Short stature is a general term used to describe individuals whose height is two standard deviations or more below the mean compared to peers of the same age and racial-ethnic group (Richmond & Rogol, 2024). The most common causes of short stature are genetic and delayed growth; these are considered normal or nonpathologic variants of growth (Richmond & Rogol, 2024).

Intrauterine growth restriction (IUGR) is a condition which describes when an unborn baby is growing abnormally slow in the womb; this could be due to either genetic or environmental factors and may cause significant morbidity and mortality in infants (Mandy, 2024). CMA has been used for diagnostic purposes in fetuses due to IUGR (Daum et al., 2019).

Idiopathic short stature (ISS) describes individuals whose height falls below two standard deviations of the mean for age, but no metabolic, endocrine, or other diagnosis has been identified to cause the height disorder (Richmond & Rogol, 2024). Regarding the genetic evaluation of short stature, some researchers suggest that for patients with ISS, patients born small for gestational age, or patients with growth hormone deficiency, "Targeted evaluation of a single gene or panels of genes is recommended... For those patients who do not fit into a distinct subgroup or for whom initial genetic testing is inconclusive, we recommend consideration of genome-wide evaluation through exome sequencing and chromosomal microarray to detect both sequence variants and CNVs" (Dauber et al., 2014). A significant association has been identified between CNVs and short stature (Yu et al., 2015), and many report that CMA is a promising tool to identify pathogenic CNVs in patients with ISS (Richmond & Rogol, 2024).

Proprietary Testing

The only FDA-approved commercial CMA test is CytoScan® by Affymetrix. This test helps identify the underlying genetic causes of developmental delay, intellectual disability, congenital anomalies, or dysmorphic features in children. CytoScan® detects chromosomal copy number variants (CNV) in genomic DNA from peripheral whole blood (Affymetrix, 2013).

Several other commercial CMA tests are available including the GenomeDx CMA by GeneDx. The GenomeDx is able to confirm clinical diagnoses, differentiate between de novo and familial cases, and assist with prenatal diagnoses in at-risk pregnancies (GeneDx, 2024). This test has a three-week turnaround time and may utilize both blood (preferred) and buccal swabs (cheek swabs) as the tested specimen. The GenomeDx is a whole-genome CMA, containing 118,000 oligonucleotide probes that detect CNVs (GeneDx, 2024).

Quest Diagnostics has developed the ClariSure® Postnatal CMA Test; the ClariSure® consists of over 2.6 million probes that detect 1,900,000 CNVs and 750,000 SNPs (Quest, 2024). With a 10- to 15-day turnaround time, this test can help to determine the genetic cause of developmental delay or mental retardation. Blood is the preferred specimen for this test, but saliva may also be used.

LabCorp has developed Reveal®, an SNP microarray aimed for pediatric purposes. This test uses a blood or salivary sample to detect chromosomal abnormalities that may be associated with congenital anomalies or developmental delay (LabCorp, 2023). Results are provided in 14-17 days.

The FirstStep^{DX} PLUS®, developed by Lineagen (2024), is a CMA test that uses a buccal sample to identify developmental disabilities. Lineagen claims that cheek swabs are a more effective way to ensure accurate CMA results than blood-based samples, and that "mosaicism is better diagnosed through DNA collected by cheek swab than by blood draw" (Lineagen, 2024).

Invitae released a Pregnancy Loss Chromosomal Microarray Analysis test which analyzes products of conception tissue to determine whether a chromosomal abnormality caused a miscarriage, intrauterine fetal demise, or a stillbirth. The average turnaround time is 10-12 days. This test can detect whole and segmental aneuploidies, submicroscopic gains and losses that cannot be detected using karyotype, size and gene content of copy number variations, regions of homozygosity, uniparental isodisomy, and triploidy and complete molar pregnancies (Invitae, 2024).

CentoArrayCyto® is a CMA test released by Centogene which detects known structural aberrations such as CNVs, chromosomal imbalances, regions exhibiting loss/absence of heterozygosity (LOH), uniparental isodisomy (UPD), and mosaicism. The test has a turnaround time of 15 days. CentoArrayCyto® can be used as a first-step analysis for detection of intellectual disability and multiple malformation, in conjunction with whole exome sequencing to complement large CNVs, for deletion/duplication analysis of extremely large genes, and for prenatal testing to help determine a cause of ultrasound-detected abnormalities (CentoGene, 2024).

Analytical Validity

Although chromosomal microarray testing can vary widely in technology, resolution, and the likelihood of producing results of unknown significance, studies have demonstrated that CMA provides chromosomal evaluation at a much higher resolution than karyotyping (Miller et al., 2010; Schrijver & Zehnder, 2024). Miller et al. (2010) noted that most clinical CMA platforms available in 2010 could detect copy number changes at a resolution of 400 kb; this was considered at least a "10-fold"

improvement over G-banded karyotyping.

In a retrospective study, Zhu et al. (2021) compared the utility of non-invasive prenatal screening (NIPS) with chromosomal microarray analysis (CMA) for the detection of chromosomal abnormalities in high-risk pregnancies. Of the 774 high risk pregnancies, 550 (71.1%) had a positive NIPS result, while 308 (39.8%) had a positive CMA result. The rate of full or partial concordance was 82.2% between NIPS and CMA. CNV's were more often detected by CMA, with an incidence of 7.9% by CMA and 3.1% by NIPS. In addition, a genetic aberration was detected by CMA in 1 in 17 high risk pregnancies that had a negative NIPS result. Overall, the authors conclude that "CMA should be offered instead of expanded NIPS for high-risk pregnancies" (Zhu et al., 2021).

Chaubey et al. (2020) compared the diagnostic utility of low-pass genome sequencing (LP-GS) to chromosomal microarrays (CMAs) in its ability to detect copy number variants (CNVs). 409 DNA samples were studied for CNV accuracy, precision, specificity, and sensitivity using both techniques. The LP-GS test accurately detected 40 positive control samples with clinically relevant CNVs, absence of heterozygosity, deletions and duplications, and translocations and accurately reported 38 control samples without any clinically significant CNVs. In addition, 331 clinical specimens were tested for developmental delay, autism, intellectual disability, congenital anomalies, and dysmorphic features. Of the 331 cases, pathogenic CNVs were detected in 57 cases relating to microdeletion/microduplication syndromes, intragenic deletions and intragenic duplications, uniparental isodisomy, triploidy, and whole chromosome aneuploidies. The other cases were classified as variants of unknown significance or did not have a reportable CNV finding. The authors conclude that "LP-GS was able to reliably detect absence of heterozygosity, microdeletion/microduplication syndromes, and intragenic CNVs with higher coverage and resolution over the genome" (Chaubey et al., 2020). The authors suggest that this study could be used by professional societies to recommend replacing CMAs with LP-GS.

Mazzonetto et al. (2024) preformed a study evaluating the performance of low-pass whole genome sequencing (LP-WGS) to detect copy number variants (CNVs) in clinical cytogenetics. The DNA samples selected for this study were obtained from 44 unrelated individuals previously referred to molecular investigation in clinical cytogenetics. The patients were investigated by CMA (either array-CGH or SNP-array), currently considered the gold standard diagnostic test for CNV analysis DNA panel, with 12 prenatal and 32 postnatal samples, comprising a total of 55 genomic imbalances. The CNVs were chosen to represent a wide range of clinically relevant CNVs detected by CMA in diagnostic routine, being the vast majority of them associated with intellectual disability or recognizable syndromes. The selected CNVs contained at least one coding sequence. They were mapped to a variety of

chromosomes ranging in copy number state from zero to 3/4, and ranging in genomic size from 75 kb to 90.3 Mb, including aneuploidies and two mosaic cases. Particularly, for methodology evaluation and quality control metrics, we used DNA extracted from several types of biological samples. The data shows "the potential use of LP-WGS to detect CNVs in clinical diagnosis and confirm the method as an alternative for chromosome imbalances detection" (Mazzonetto et al., 2024).

Clinical Utility and Validity

A review of 33 studies, comparing traditional karyotyping to CMA, has shown that CMA increases the detection rate for chromosomal abnormalities in individuals with DD/ID (developmental disability/intellectual disability) or autism spectrum disorder (ASD). CMA detected pathogenic genomic imbalances with an average diagnostic yield of 12.2% across all studies in this patient population, which is about 10% more than karyotyping alone (Miller et al., 2010).

Hillman et al. (2013) performed both a meta-analysis and cohort study evaluating CMA's detection rate of chromosomal abnormalities. The authors investigated 243 pregnant individuals who had both a CMA and karyotype performed, and the meta-analysis included 25 primary studies. Overall, CMA was found to detect 4.1% more abnormalities compared to karyotyping in the cohort study and 10% more in the meta-analysis (Hillman et al., 2013).

Reddy, Page, Saade, et al. (2012) compared the detection rates of microarray and traditional karyotyping. A total of 532 stillbirths were examined. The authors found that microarrays provided more results than karyotyping (87.4% compared to 70.5%) and identified more genetic abnormalities (8.3% vs 5.8%). Microarray analysis also found more genetic abnormalities among 443 antepartum stillbirths (8.8% vs 6.5%) and among 67 stillbirths with congenital abnormalities (29.9% vs 19.4%). Overall, microarray analysis provided a relative increase in the diagnosis of genetic abnormalities of 41.9% in all stillbirths, 34.5% in antepartum stillbirths, and 53.8% in stillbirths with anomalies compared to karyotyping (Reddy, Page, Saade, et al., 2012).

Coulter et al. (2011) assessed impact of CMA results on clinical decision making. A total of 1792 patients were examined, and 235 of them had either an "abnormal" result (n = 131) or a "variant of possible significance" (VPS) (n = 104). Clinical action was recommended for 54% of the patients in the "abnormal" cohort and 34% of the patients in the VPS cohort (Coulter et al., 2011).

Brady et al. (2013) performed a prospective study of fetuses with abnormalities detected on ultrasound. A total of 383 prenatal samples were examined. Causal imbalances were found in 37 samples, submicroscopic CNVs were found in 10 of the

37 samples, and arrays added "valuable information" over conventional karyotyping in 15 of 37 samples. The authors concluded that there was added value of chromosomal microarrays for prenatal diagnosis in the presence of ultrasound anomalies (Brady et al., 2013).

Borrell et al. (2017) performed a meta-analysis of literature to estimate the incremental yield of CMA over karyotyping in fetal growth restriction (FGR). The authors identified 10 studies and found a 4% incremental yield of CMA over karyotyping in "nonmalformed growth-restricted fetuses" and a 10% incremental yield in FGR when associated with fetal malformations (Borrell et al., 2017).

Robson et al. (2017) compared karyotyping and CMA in fetuses with ultrasound anomalies. Out of 629 cases with structural anomalies, CMA detected copy number variants (CNVs) and more pathogenic CNVs than karyotyping. CMA was also found to have a turnaround time of five days quicker than karyotyping. Finally, CMA was found to be £113 more expensive per patient than karyotyping. The authors conclude, "CMA is a robust, acceptable and probably cost-effective method to detect more clinically significant chromosomal imbalances in the anomalous fetus. The results suggest that CMA should replace karyotyping in these care pathways" (Robson et al., 2017).

Li et al. (2018) performed a study investigating the cost effectiveness of karyotyping, CMA, and NGS in genetic diagnosis of unexplained global developmental delay. The authors found that: "CMA testing results in more genetic diagnoses at an incremental cost of US $2692 per additional diagnosis compared with karyotyping, which has an average cost per diagnosis of US $11,033" (Li et al., 2018). The authors also found that performing both tests sequentially result in the same number of diagnoses but costs less when CMA testing is done first and karyotyping second. The authors also analyzed the cost-effectiveness of a variant of unknown significance. When CMA testing yields a variant of unknown significance, additional genetic diagnoses can be obtained "at an incremental cost of US $4220 by CMA testing of both parents, and when parents are not available or the patient had a normal CMA result, targeted NGS of the patient can add diagnoses at a further incremental cost of US $12,295." The authors concluded that "These results provide a cost effectiveness rationale for the use of CMA as the first-tier test for the genetic diagnosis of unexplained GDD/ID [global developmental delay/intellectual disability] and further indicate that testing of both parents may be cost effective when a variant of unknown significance is detected in the patient" (Li et al., 2018).

Hydrops fetalis occurs when fluid accumulates in fetal serous cavities and soft tissues; nonimmune hydrops fetalis (NIHF) develops when red cell alloimmunization does not cause the hydrops fetalis case in question. A retrospective study of all prenatally

diagnosed NIHF cases identified at the University of California, San Francisco from 2008 to 2018 was performed. A total of 131 cases were identified. The researchers found that "In 43/44 cases with a CMA performed, results were categorized as normal or likely benign. One case was found on CMA to have a large pathogenic duplication" (Mardy et al., 2020). This shows that CMA is not an effective diagnostic tool for NIHF.

Another study aimed to assess the diagnostic capabilities of CMA among pregnancies terminated due to fetal malformations identified with ultrasounds. CMA was performed on 71 pregnancies using fetal DNA or placental DNA. The authors noted that "Findings were abnormal in 17 cases (23.9%), of which 13 were detectable by karyotype. The incremental yield of CMA was 4/71 (5.6%); 1/32 (3.1%) for cases with an isolated anomaly and 3/39 (7.7%) for cases with nonisolated anomalies" (Pasternak et al., 2020). CMA identified more chromosomal abnormalities than karyotype and did not require dividing cells, making it a more practical option after termination.

Rodriguez-Revenga et al. (2020) studied the diagnostic yield of CMA in prenatal diagnosis. 2905 prenatal samples with a normal rapid aneuploidy detection test were referred for CMA testing. The study revealed pathogenic CNVs associated with syndromic disorders in 4.8% of the 2905 cases, adding a 2.8% diagnostic value of CMA over karyotyping. This study shows that "CMA increases 2-fold the diagnostic yield achieved by conventional karyotyping." Therefore, "chromosome microarray analysis should be offered to all invasive prenatal diagnostic testing following a normal rapid aneuploidy test result" (Rodriguez-Revenga et al., 2020).

Bajaj Lall et al. (2021) evaluated the diagnostic yield and clinical utility of CMA by comparing it to karyotyping results. CMA and karyotyping were performed on consecutive referrals of 370 prenatal samples of amniotic fluid (n = 274) and chorionic villi (n = 96) from Indian pregnant individuals with high maternal age (n = 23), biochemical screen positive (n = 61), previous child abnormal (n = 59), abnormal fetal ultrasound (n = 205) and heterozygous parents (n = 22). The overall diagnostic yield of abnormal results was higher via CMA than by karyotyping (9.18% vs. 5.40%, respectively). As such, the authors argue that "CMA must be used as the first tier test in all cases with abnormal fetal ultrasound and if cost is not an issue, it can be offered to all pregnant [individuals] undergoing the invasive test, as the test results are faster and the diagnostic yield is higher by CMA than by karyotyping even in other groups" (Bajaj Lall et al., 2021).

In cases of isolated fetal growth restriction, the clinical utility of chromosomal microarray analysis has yet to be evaluated fully, leading Monier et al. (2021) to evaluate the use of chromosomal microarray analysis as compared to karyotyping in these situations. Both karyotyping and chromosomal microarray analysis were

performed in 146 fetuses, and the researchers found that the detection rate of genetic abnormalities by CMA was 7.5%, with an incremental yield of 3.6% as compared to karyotyping. Moreover, CMA was able to identify a variant of unknown significance in three fetuses boasting normal karyotypes. Consequently, the level of granularity of CMA in "these results support the use of chromosomal microarray analysis in addition to karyotype for isolated fetal growth restriction" (Monier et al., 2021).

Zhang et al. (2021) investigated the incidence and clinical significance of chromosomal mosaicism (CM) in a single-center retrospective study of invasive prenatal diagnosis of CM. From a sample of 5758 G-band karyotyping results and 6066 CMA results, the authors deduced that their findings "demonstrate that increased risk in genetic counselling is due to discordant CM from different specimens or testing methods" and so "It is highly recommended to use more comprehensive assays such as a combination of CMA, FISH [fluorescence in situ hybridization analysis] and karyotyping to detect mosaicism in AF [amniotic fluid] and CB [cord blood] before any irreversible decision is made in regard to the pregnancy (Zhang et al., 2021).

Chau et al. (2020) conducted a retrospective back-to-back comparison study of "low-pass GS versus routine CMA for 532 prenatal, miscarriage, and postnatal cases, the overall diagnostic yield was 22.4% (119/532) for CMA and 23.1% (123/532) for low-pass GS. Thus, the overall relative improvement of the diagnostic yield by low-pass GS versus CMA was ~ 3.4% (4/119). Identification of cryptic and clinically significant CNVs among prenatal, miscarriage, and postnatal cases demonstrated that CNV detection at higher resolutions is warranted for clinical diagnosis regardless of referral indications. Overall, [the] study supports low-pass GS as the first-tier genetic test for molecular cytogenetic testing" (Chau et al., 2020).

VI. Guidelines and Recommendations

American College of Medical Genetics and Genomics (ACMG)

In the Clinical Practice Resource for Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities, the ACMG recommends CMA testing "as a first-line test in the initial postnatal evaluation of individuals with the following (Manning & Hudgins, 2010):

A. "Multiple anomalies not specific to a well-delineated genetic syndrome."
B. "Apparently nonsyndromic DD/ID."
C. "Autism spectrum disorders."

The ACMG also recommends "further determination of the use of CMA testing for the evaluation of the child with growth retardation, speech delay, and other less well-

studied indications…, particularly by prospective studies and after- market analysis." Additionally, ACMG recommends "appropriate follow-up … in cases of chromosome imbalance identified by CMA, to include cytogenetic/FISH studies of the patient, parental evaluation, and clinical genetic evaluation and counseling"(Manning & Hudgins, 2010). These statements were reaffirmed in 2020 by the ACMG Board of Directors.

An update from ACMG states that a stepwise or tiered approach to the clinical genetic diagnostic evaluation of autism spectrum disorder is recommended, with the recommendation being for first tier to include fragile X syndrome and chromosomal microarray analysis (CMA).

In 2016 the ACMG published guidelines on the use of noninvasive prenatal screening for the diagnosis of fetal aneuploidy which recommends (Gregg et al., 2016):

- "Informing all pregnant [individuals] that NIPS is the most sensitive screening option for traditionally screened aneuploidies (i.e., Patau, Edwards, and Down syndromes)."
- "Allowing patients to select diagnostic or screening approaches for the detection of fetal aneuploidy and/or genomic changes that are consistent with their personal goals and preferences."
- "Offering diagnostic testing (CVS or amniocentesis) with CMA when NIPS identifies a CNV"
- "Offering diagnostic testing with CMA when a no-call result is obtained after NIPS due to possible UPD or parental consanguinity" (Gregg et al., 2016).

In 2017 the ACMG published a laboratory practice resource outlining an algorithm for diagnostic cytogenetic testing following positive noninvasive prenatal screening results which recommends (Cherry et al., 2017):

- "CMA testing on either CVS or amniotic fluid may be used as confirmatory diagnostic testing in cases with positive NIPS results, or as reflex testing in cases with initial normal results from chromosome analysis."
- CMA is recommended as follow-up testing for any smaller copy-number changes that are reported as positive by NIPS.
- They also suggest that when "prenatal diagnostic testing may not be performed due to loss of the pregnancy before testing is possible. In such instances, testing of the products of conception and/or the fetus by either chromosome analysis or CMA should be considered on a case-by-case basis."
- In newborns for whom the screen is suggestive of aneuploidy, but further testing is declined a genetics consultation with physical examination is sufficient for

neonates, however, "if the neonate has an abnormal physical examination that is not suggestive of the trisomy in question, CMA is recommended."

- CMA is also recommended when the sex determination by NIPS is discordant with physical examination, or clinical findings suggestive of a disorder of sexual differentiation.

In 2009, the ACMG published guidelines on the genetic evaluation of short stature. These guidelines provide recommendations for genes associated with short stature and intrauterine growth restriction (IUGR) and state that high resolution chromosome analysis and/or array CGH can be used to evaluate IUGR (Seaver & Irons, 2009).

In 2018, the ACMG published a clinical practice report on genetic testing after CMA for the diagnosis of neurodevelopmental disability and congenital anomalies. These guidelines state that "Chromosomal microarray (CMA) is recommended as the first tier test in evaluation of individuals with neurodevelopmental disability and congenital anomalies. CMA may not detect balanced cytogenomic abnormalities or uniparental disomy (UPD), and deletion/duplications and regions of homozygosity may require additional testing to clarify the mechanism and inform accurate counseling" (Waggoner et al., 2018).

In 2020, the ACMG published guidelines on the use of fetal exome sequencing in prenatal diagnosis. These guidelines recommend that "Exome sequencing may be considered for a fetus with ultrasound anomalies when standard CMA and karyotype analysis have failed to yield a definitive diagnosis. If a specific diagnosis is suspected, molecular testing for the suggested disorder (with single-gene test or gene panel) should be the initial test" (Monaghan et al., 2020).

The *ACMG Technical Standards for Clinical Genetics Laboratories (2021 Revision)* state, "the contamination of both direct and cultured cells from AF and CVS with maternal cells is well documented and therefore represents a potential source of error in prenatal diagnosis. Prenatal samples should be examined in parallel with a maternal sample to rule out error due to maternal cell contamination (MCC). Laboratories should understand how their testing methods are affected by the presence and the amount of MCC. For example, prenatal detection of a deletion using PCR, as is the case in testing for DMD and SMA, is expected to be more sensitive to maternal contamination, since a normal maternal allele could mask the deletion. A prenatal test using an allele-specific PCR reaction to detect a paternal RhD gene in the fetus of a RhD-negative mother is much less sensitive to maternal contamination" (ACMG, 2021).

American Academy of Pediatrics (AAP)

The 2014 AAP guidance for the comprehensive evaluation of children with intellectual disability or global developmental delays noted that "chromosome microarray is designated as a first-line test and replaces the standard karyotype and fluorescent in situ hybridization subtelomere tests for the child with intellectual disability of unknown etiology" (Moeschler & Shevell, 2014). It further added that "CMA is now the standard for diagnosis of patients with GDD/ID, as well as other conditions, such as autism spectrum disorders or multiple congenital anomalies."

Another clinical report published by the AAP on the identification of infants and young children with developmental disorders states that "The child with suspected global developmental delay or intellectual disability should have laboratory testing done, including chromosomal microarray and fragile X testing" (Lipkin & Macias, 2020). Further, the authors also note that "The initial genetic workup of the child with suspected ASD is evolving; current recommendations also include chromosomal microarray and fragile X testing" (Lipkin & Macias, 2020).

The AAP has an epilepsy webpage overview and on the genetic testing for epilepsy page states that "The genetic tests most utilized in the evaluation of children with epilepsy include chromosomal microarray (CMA), epilepsy gene panels, and whole-exome sequencing (WES). Each test has its own specific benefits and limitations, and the utility of different tests may vary for a given individual. Decisions regarding testing need to consider the clinical indication including associated symptoms, turn-around time, insurance coverage, and cost" (Dubbs, 2022).AAP published guidelines on the evaluation of children with autism spectrum disorder. According to the guidelines, CMA is recommended if the etiology for developmental disability is not known. Since Fragile X Syndrome increases the risk for autism spectrum disorder, DNA testing for Fragile X should be recommended in all children with ASD, especially for boys and children with a family history of intellectual disability. "The cytosine-guanine-guanine trinucleotide repeat expansion that is responsible for fragile X syndrome is not detected on CMA and must be ordered as a separate test. When the history and physical examination, CMA, and fragile X analysis do not identify an etiology, the next step at this time in the etiologic evaluation for [autism spectrum disorder] is whole-exome sequencing (WES)." AAP does not recommend the use of commercially marketed tests as they do not provide a molecular etiologic diagnosis (Hyman et al., 2020).

American Academy of Neurology (AAN)

The AAN published coverage policies for chromosomal microarray analysis for intellectual disabilities in 2015 (Satya-Murti, 2015). The policy document notes the

criteria do not represent a binding standard of care and that the criteria are proposed as clinical contexts that readily support the use of microarray testing. The authors note that chromosomal microarray analysis is reasonable and medically necessary for diagnosing a genetic abnormality when all of the following conditions are met (Satya-Murti, 2015):

- "In children with developmental delay/intellectual disability (DD/ID) or an autism spectrum disorder (ASD) according to accepted Diagnostic and Statistical Manual of Mental Disorders-IV criteria;

  AND
- If warranted by the clinical situation, biochemical testing for metabolic diseases has been performed and is negative;
- Targeted genetic testing, (for example: *FMR1* gene analysis for Fragile X), if or when indicated by the clinical and family history, is negative;
- The results for the testing have the potential to impact the clinical management of the patient;
- Face-to-face genetic counseling with an appropriately trained and experienced healthcare professional has been provided to the patient (or legal guardian(s) if a minor child). Patient or legal guardians have given their consent for testing. Cognitively competent adolescent patients have given their assent for testing as well."

The document notes the presence of major and minor congenital malformations and dysmorphic features should be considered evidence that microarray testing will be more likely to yield a diagnosis. However, dysmorphic and syndromic features are not required for testing (Satya-Murti, 2015).

American College of Obstetricians and Gynecologists (ACOG) and Society for Maternal-Fetal Medicine (SMFM)

Originally published 2013 reaffirmed in 2020, the ACOG and SMFM issued joint guidelines recommending the following use of CMA for prenatal diagnosis:

- "Most genetic changes identified by chromosomal microarray analysis that typically are not identified on standard karyotype are not associated with increasing maternal age; therefore, the use of this test can be considered for all women, regardless of age, who undergo prenatal diagnostic testing.
- Prenatal chromosomal microarray analysis is recommended for a patient with a fetus with one or more major structural abnormalities identified on ultrasonographic examination and who is undergoing invasive prenatal diagnosis. This test typically can replace the need for fetal karyotype.

- In a patient with a structurally normal fetus who is undergoing invasive prenatal diagnostic testing, either fetal karyotyping or a chromosomal microarray analysis can be performed.
- Chromosomal microarray analysis of fetal tissue (i.e., amniotic fluid, placenta, or products of conception) is recommended in the evaluation of intrauterine fetal death or stillbirth when further cytogenetic analysis is desired because of the test's increased likelihood of obtaining results and improved detection of causative abnormalities.
- Comprehensive patient pretest and posttest genetic counseling from an obstetrician–gynecologist or other health care provider with genetics expertise regarding the benefits, limitations, and results of chromosomal microarray analysis is essential. Chromosomal microarray analysis should not be ordered without informed consent, which should include discussion of the potential to identify findings of uncertain significance, nonpaternity, consanguinity, and adult-onset disease.
- The routine use of whole-genome or whole-exome sequencing for prenatal diagnosis is not recommended outside of the context of clinical trials until sufficient peer-reviewed data and validation studies are published."

ACOG and SMFM do not recommend use of CMA for evaluation of first- and second-trimester pregnancy loss due to limited clinical utility information. Additionally, they recommend pre- and post-test genetic counseling "from qualified personnel such as a genetic counselor or geneticist regarding the benefits" (ACOG, 2016). This position was reaffirmed in 2023.

A 2018 study of these guidelines analyzed 3223 prenatal samples undergoing CMA. Cases were categorized into 2 groups: those that met ACOG guidelines for CMA versus those that met ACOG guidelines for either CMA or karyotype. They found that "in patients who could have elected either CMA or karyotype, 2.5% had clinically significant chromosomal abnormalities (CSCA) that would have been missed if the patient had elected to pursue karyotype" (Hay et al., 2018).

American Society for Reproductive Medicine (ASRM)

In 2012, ASRM published a committee opinion on evaluation and treatment of recurrent pregnancy loss with clinical practice recommendations. ASRM recommended to proceed with the evaluation of recurrent pregnancy loss after two consecutive clinical pregnancy losses. This definition of recurrent pregnancy loss was reaffirmed in 2013. The recommended assessment of recurrent pregnancy loss included screening for genetic, hormonal and metabolic factors in addition to other factors. They have

stated that "karyotypic analysis of products of conception may be useful in the setting of ongoing therapy for recurrent pregnancy loss" (ASRM, 2012).

In 2020, the Practice Committee and Genetic Counseling Professional Group (GCPG) of the ASRM published their opinion on the clinical management of mosaic results from preimplantation genetic testing for aneuploidy (PGT-A) of blastocysts. Defining prenatal diagnostic testing as including chorionic villus sampling or amniocentesis, the group recommends the following:

- "Prenatal genetic counseling is strongly recommended for any pregnancy resulting from the transfer of embryos with mosaic results and should include a discussion of the risks, benefits, and limitations of CVS and amniocentesis. If prenatal diagnostic testing is performed, additional analyses beyond routine karyotyping should be considered depending on the specific PGT-A result. At the discretion of the ordering provider, these may include:
  - Chromosomal microarray, if a partial chromosome aneuploidy is involved.
  - Uniparental disomy studies (UPD), depending on the chromosome involved.
  - Additional cell counts, in an effort to identify lower-level mosaicism.
- Postnatal evaluation by peripheral blood karyotype and/or microarray should be considered, particularly if prenatal diagnostic testing is declined. Referral to a pediatric specialist in genetics is recommended in the event of an abnormal physical or developmental phenotype" (ASRM, 2020).

Society of Obstetricians and Gynaecologists of Canada (SOGC)- Canadian College of Medical Geneticists (CCMG) Joint Technical Update

The 2018 joint guideline Armour et al. (2018) supersedes the 2011 iteration.

- "Offer of chromosomal microarray analysis (in addition to any other relevant diagnostic testing) is recommended in cases with multiple fetal anomalies identified by a comprehensive obstetric ultrasound (II-1A). Other diagnostic testing may include specific single gene, multigene panels or other genetic tests if the pattern of anomalies suggests a specific genetic condition not identified by array."
- "Single structural defects in association with other abnormal ultrasound findings (e.g., intrauterine growth restriction (IUGR), oligohydramnios) should not be considered isolated, and thus array should be offered if RAD is normal."
- "In cases with a single fetal anomaly, prenatal CMA should be considered for those malformations associated with a high frequency of abnormal results. Its use in cases where the diagnostic yield is lower may be considered, if resources are available."
- "In fetuses with a nuchal translucency ≥3.5 mm, prenatal CMA should be offered."

For "analysis of fetal loss prior to 20 weeks gestation":

- "In cases of congenital anomalies and/or IUGR, in any fetal loss prior to 20 weeks gestation, if QF-PCR methodologies and/or other directed diagnostic inquiries do not provide a diagnosis and further cytogenetic analysis is intended, it is recommended that karyotype be replaced with chromosomal microarray analysis."

For fetal deaths ≥20 weeks gestation:

- "Aneuploidy is the most common abnormal chromosomal finding in stillbirths. If RAD and/or other directed diagnostic inquiries are uninformative, it is recommended that in cases complicated by congenital anomalies and/or IUGR, karyotype be replaced with CMA when further cytogenetic analysis is desired."
- "In stillbirths without structural fetal anomalies, CMA may be considered in the context of local resource availability and site-based postmortem protocol (whether complete, limited or external only)" (Armour et al., 2018).

Association for Molecular Pathology (AMP)

The MCC [maternal cell contamination] Guidelines Working Group of the AMP Clinical Practice Committee issued laboratory guidelines for detecting MCC in 2011. They state, "To determine the pure fetal origin of all prenatal specimens undergoing genetic analysis, it is recommended that MCC analysis be performed in parallel with diagnostic testing, regardless of the genetic disorder or its mode of inheritance" (Nagan et al., 2011).

The Association for Molecular Pathology Training and Education Committee published a series of quick reference cards called Molecular-in-My-Pocket™ for trainees and anyone looking to quickly reference molecular diagnostics information.

In their oncology subdivision, the AMP summarize assays and techniques for molecular biomarkers for various cancers. Below are summaries of their syntheses.

When testing for primary and recurrent tumors for the *KIT* biomarker in cutaneous melanoma, "NGS, pyrosequencing, Sanger sequencing, PCR-based assays, microarray" are applicable for therapeutic indications.

When testing for primary and recurrent tumors for 1p/19q co-deletion in tumors of the central nervous system, "FISH, array, NGS" are approved for diagnosis and prognosis. Moreover, when testing for EGFR amplification, gain of chromosome 7, and loss of chromosome 10, "FISH, array, NGS" are applicable for diagnosis.

Similarly, in their pediatric molecular pathology section, the AMP summarizes assays and techniques for pediatric brain tumors. The following table, adapted from the oncology Molecular-in-my-Pocket™ card, lists the assays and techniques mentioning CMA and their corresponding tumor types (AMP, 2023):

Tumor type	Gene/Biomarker	Significance	Primary assays
Low grade gliomas	*FGFR* mutation, fusion	Diagnosis	NGS (DNA), CMA
	NF1 loss or inactivating mutation, loss of heterozygosity	Diagnosis, familial cancer if germline	NGS (DNA), CMA
	MYB fusion, amplification	Diagnosis, prognosis	NGS (DNA), CMA
	MYBL1 fusion, amplification	Diagnosis, prognosis	NGS (DNA), CMA
High grade gliomas, IDH wildtype	*EGFR* amplification	Prognosis	NGS (DNA), CMA, FISH
	CDKN2A/B homozygous deletion	Prognosis	CMA, NGS (DNA)
	TP53 loss-of-function (LOF) and gain-of-function mutations, loss, LOH	Prognosis	NGS (DNA), CMA
	NF1 loss or inactivating mutation, loss of heterozygosity	Prognosis	NGS(DNA), CMA
	PDGFRA amplification	Prognosis	CMA, NGS (DNA)
	RB1 loss or inactivating mutation, loss of heterozygosity	Prognosis	NGS (DNA), CMA
	MDM4 amplification	Prognosis	NGS (DNA), CMA, FISH
	MET amplification, fusion, mutation	Prognosis	NGS (DNA), CMA, FISH
	Gain of chromosome 7	Prognosis	CMA
	10q loss	Prognosis	CMA
Oligodendroglioma	1p/10q codeletion	Diagnosis	CMA, FISH
	1q gain	Prognosis	CMA, FISH

Medulloblastoma, SHH-activated	*PTCH1* inactivating mutation, loss of heterozygosity	Subtype-diagnosis, familial cancer risk if germline N	NGS (DNA), CMA
	SUFU inactivating mutation, loss of heterozygosity	Subtype-diagnosis, familial cancer risk if germline N	NGS (DNA), CMA
	TP53 loss-of-function (LOF) and gain-of-function mutations, loss, loss of heterozygosity, structural alterations (rare)	Prognosis	NGS (DNA), CMA
	10q loss or loss of heterozygosity	Diagnosis	CMA, FISH
	MYCN amplification	Diagnosis, prognosis	FISH, CMA, NGS (DNA)
Medulloblastoma, Group 3	Isochromosome 17q	Subtype-diagnosis	CMA, FISH
	MYC amplification, fusion (PVT1)	Prognosis	CMA, FISH
Medulloblastoma, Group 4	Isochromosome 17q	Subtype-diagnosis	CMA, FISH
	MYCN amplification	Diagnosis, prognosis	FISH, CMA, NGS
Meningioma	*NF2* loss-of-function (LOF) and gain-of-function mutations, loss, loss of heterozygosity	Diagnosis, familial cancer risk if germline	NGS, CMA, MLPA
Choroid plexus tumors	*TP53 l*oss-of-function (LOF) and gain-of-function mutations, loss, loss of heterozygosity, structural alterations (rare)	Diagnosis, familial cancer risk if germline	NGS, CMA, MLPA
ETMR	*C19MC* amplification and gain	Diagnosis, prognosis	CMA, FISH

Regarding pediatric solid tumors, the following table, adapted from the oncology Molecular-in-my-Pocket™ card (AMP, 2023), lists the assays and techniques mentioning CMA and their corresponding tumor types:

Tissue type	Tumor type	Gene/Biomarker	Significance	Primary assays
Kidney	Wilms Tumor	1q gain, 1p/16q loss of heterozygosity	Prognosis	CMA, NGS (DNA)
	Rhabdoid Tumor	*SMARCB1 (INI1), SMARCA4 (BRG1)* loss	Diagnosis	IHC, CMA, NGS, MLPA
Eye	Retinoblastoma	*RB1* inactivating mutation, loss, or loss of heterozygosity	Diagnosis, familial cancer risk if germline	Sanger sequencing, MLPA, CMA, NGS (DNA)
Adrenal	Neuroblastoma	1p/11q LOH loss	Prognosis	CMA, NGS
Multi-system	Gorlin	*SUFU*, *PTCH1* loss-of-function sequence variants, deletion/duplication	Diagnosis, familial cancer risk	Sequencing, CMA, MLPA

Regarding pediatric soft tissue tumors, the following table, adapted from the oncology Molecular-in-my-Pocket™ card (AMP, 2023), lists the assays and techniques mentioning CMA and their corresponding tumor types:

Tissue type	Tumor type	Gene/Biomarker	Significance	Primary assays
Miscellaneous	Chordoma (Poorly differentiated)	*SMARCB1 (INI1)* loss (sequence variant, partial deletion)	Diagnosis, prognosis	IHC, CMA, NGS, MLPA
	Rhabdoid (Extra-renal)	*SMARCB1 (INI1)* and *SMARCA4 (BRG1)*	Diagnosis	IHC, CMA, NGS, MLPA
	Epithelioid sarcoma	*SMARCB1 (INI1)* and *SMARCA4 (BRG1)*	Diagnosis, treatment	IHC, CMA, NGS, MLPA
Multi-system	Gorlin	*SUFU*, *PTCH1* loss-of-function sequence variants, deletion/duplication	Diagnosis, familial cancer risk	Sequencing, CMA, MLPA

Society for Maternal-Fetal Medicine (SMFM)

The SMFM recommends chromosomal microarray for evaluation of mild fetal ventriculomegaly and nonimmune hydrops fetalis (Fox et al., 2018; Norton et al., 2015).

SMFM released guidelines on the diagnosis and management of fetal growth restriction. The following recommendations were made:

- SMFM recommends that pregnant individuals "be offered fetal diagnostic testing, including chromosomal microarray analysis, when fetal growth restriction is detected and a fetal malformation, polyhydramnios, or both are also present regardless of gestational age."
- They also recommend that pregnant individuals "be offered prenatal diagnostic testing with chromosomal microarray analysis when unexplained isolated fetal growth restriction is diagnosed at <32 weeks of gestation" (Martins et al., 2020).

International Standard Cytogenomic Array (ISCA) Consortium

The ISCA (an international group of experts in the field) assembled to "address mutual concerns about standardization and collaboration for clinical CMA testing" (Miller et al., 2010). After much research, the ISCA has stated that "Our recommendation based on current evidence is to offer CMA as the first-tier genetic test, in place of G-banded karyotype, for patients with unexplained DD/ID, ASD, or MCA [multiple congenital anomalies]" (Miller et al., 2010).

International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM), and the Perinatal Quality Foundation (PQF)

Joint guidelines on the use of genome-wide sequencing for fetal diagnosis were published by the ISPD, SMFM and PQF. However, these guidelines also mention CMA quite frequently because "The use of diagnostic sequencing is currently being introduced for evaluation of fetuses for whom standard diagnostic genetic testing, such as chromosomal microarray analysis (CMA), has already been performed and is uninformative or is offered concurrently according to accepted practice guidelines, or for whom expert genetic opinion determines that standard genetic testing is less optimal than sequencing for the presenting fetal phenotype" (ISPD, 2018).

Fetal sequencing is recommended in several scenarios, including the following which also mention CMA:

- "A current pregnancy with a fetus with a single major anomaly or with multiple organ system anomalies that are suggestive of a possible genetic etiology, but no genetic diagnosis was found after CMA; or in select situations with no CMA result,

following a multidisciplinary review and consensus, in which there is a fetus with a multiple anomaly 'pattern' that strongly suggests a single gene disorder.

- A personal (maternal or paternal) history of a prior undiagnosed fetus (or child) affected with a major single anomaly or multiple anomalies suggestive of a genetic etiology, and a recurrence of similar anomalies in the current pregnancy without a genetic diagnosis after karyotype or CMA
- In families with a history of recurrent stillbirths of unknown etiology after karyotype and/or CMA, where the fetus in the current pregnancy has a recurrent pattern of anomalies" (ISPD, 2018).

These recommendations show that CMA should be used as an initial strategy (before fetal sequencing) to determine the genetic causation during pregnancy of the aforementioned cases.

Autism Consortium Clinical Genetics/DNA Diagnostics Collaboration

These guidelines focus on clinical genetic testing for patients with autism spectrum disorders (ASDs). The authors note that "CMA had the highest detection rate among clinically available genetic tests for patients with ASD. Interpretation of microarray data is complicated by the presence of both novel and recurrent copy-number variants of unknown significance. Despite these limitations, CMA should be considered as part of the initial diagnostic evaluation of patients with ASD" (Shen et al., 2010). Further, the guidelines later state that "our results suggest that CMA with whole genome coverage should be adopted as a national standard of care for genetic testing among patients with ASDs" (Shen et al., 2010).

Endocrine Society (ES)

The Endocrine Society published guidelines on the diagnosis and treatment of children with idiopathic short stature (ISS) in 2008. These guidelines state that "In situations where a specific genetic diagnosis associated with short stature is expected (such as Noonan syndrome or GH insensitivity syndrome), the genes of interest should be examined" (Cohen et al., 2008). These guidelines do not mention CMA.

The National Fragile X Foundation

The National Fragile X Foundation's statement on genetic testing for Fragile X Syndrome and associated disorders includes within its recommendations the use of chromosomal microarray analysis and exome sequencing for the diagnosis of "the underlying cause of a child's developmental delays, autism, or intellectual disability". However, though the above are viable options for genetic testing in general, "Currently, Fragile X testing must be ordered as a separate test since expansions of the

FMR1 gene cannot be detected through microarray or exome sequencing", and as such a specific DNA test for Fragile X Syndrome via PCR and Southern Blot analysis will need to be used (National FragileX Foundation, 2024).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

In 2014 the FDA approved CytoScan® Dx Assay as a "qualitative assay intended for the postnatal detection of copy number variations (CNV) in genomic DNA obtained from peripheral whole blood in patients referred for chromosomal testing based on clinical presentation. CytoScan® Dx Assay is intended for the detection of CNVs associated with developmental delay, intellectual disability, congenital anomalies, or dysmorphic features" (FDA, 2014).

In 2017 the FDA approved GenetiSure Dx Postnatal Assay as a "qualitative assay intended for the postnatal detection of copy number variations (CNV) and copy-neutral loss of heterozygosity (cnLOH) in genomic DNA obtained from peripheral whole blood in patients referred for chromosomal testing based on clinical presentation. GenetiSure Dx Postnatal Assay is intended for the detection of CNVs and cnLOH associated with developmental delay, intellectual disability, congenital anomalies, or dysmorphic features" (FDA, 2017).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description

81228	Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of genomic regions for copy number variants, comparative genomic hybridization [CGH] microarray analysis
81229	Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants, comparative genomic hybridization (CGH) microarray analysis
81265	Comparative analysis using Short Tandem Repeat (STR) markers; patient and comparative specimen (eg, pre-transplant recipient and donor germline testing, post-transplant non-hematopoietic recipient germline [eg, buccal swab or other germline tissue sample] and donor testing, twin zygosity testing, or maternal cell contamination of fetal cells)
81349	Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of genomic regions for copy number and loss-of-heterozygosity variants, low-pass sequencing analysis
0252U	Fetal aneuploidy short tandem–repeat comparative analysis, fetal DNA from products of conception, reported as normal (euploidy), monosomy, trisomy, or partial deletion/duplications, mosaicism, and segmental aneuploidy Proprietary test: POC (Products of Conception) Lab/Manufacturer: Igenomix®
0469U	Rare diseases (constitutional/heritable disorders), whole genome sequence analysis for chromosomal abnormalities, copy number variants, duplications/deletions, inversions, unbalanced translocations, regions of homozygosity (ROH), inheritance pattern that indicate uniparental disomy (UPD), and aneuploidy, fetal sample (amniotic fluid, chorionic villus sample, or products of conception), identification and categorization of genetic variants, diagnostic report of fetal results based on phenotype with maternal sample and paternal sample, if performed, as comparators and/or maternal cell contamination Proprietary test: IriSightTM CNV Analysis Lab/Manufacturer: Variantyx Inc
S3870	Comparative genomic hybridization (CGH) microarray testing for developmental delay, autism spectrum disorder and/or intellectual disability

Current Procedural Terminology© American Medical Association. All Rights reserved.
Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

ACMG. (2021). *ACMG Technical Standards for Clinical Genetics Laboratories (2021 Revision): Clinical Molecular Genetics (Section G)*. https://www.acmg.net/PDFLibrary/ACMG%20Technical%20Lab%20Standards%20Section%20G.pdf

ACOG. (2016). Committee Opinion No.682: Microarrays and Next-Generation Sequencing Technology: The Use of Advanced Genetic Diagnostic Tools in Obstetrics and Gynecology. *Obstet Gynecol*, *128*(6), e262-e268. https://doi.org/10.1097/aog.0000000000001817

Affymetrix. (2013). CytoScan® Cytogenetics Suite. https://assets.thermofisher.com/TFS-Assets/LSG/brochures/CL00731-9-Brochure-CytoScan-Cytogenetics-Suite_final.pdf

AMP. (2023). "*Molecular in My Pocket™*" *Reference Card Series*. Association for Molecular Pathology. https://www.amp.org/education/amp-review-resources/molecular-in-my-pocket-guides/

Armour, C. M., Dougan, S. D., Brock, J. A., Chari, R., Chodirker, B. N., DeBie, I., Evans, J. A., Gibson, W. T., Kolomietz, E., Nelson, T. N., Tihy, F., Thomas, M. A., & Stavropoulos, D. J. (2018). Practice guideline: joint CCMG-SOGC recommendations for the use of chromosomal microarray analysis for prenatal diagnosis and assessment of fetal loss in Canada. *J Med Genet*, *55*(4), 215-221. https://doi.org/10.1136/jmedgenet-2017-105013

ASRM. (2012). Evaluation and treatment of recurrent pregnancy loss: a committee opinion. *Fertility and Sterility*, *98*(5), 1103-1111. https://doi.org/10.1016/j.fertnstert.2012.06.048

ASRM. (2020). Clinical management of mosaic results from preimplantation genetic testing for aneuploidy (PGT-A) of blastocysts: a committee opinion. *Fertil Steril*, *114*(2), 246-254. https://doi.org/10.1016/j.fertnstert.2020.05.014

Bajaj Lall, M., Agarwal, S., Paliwal, P., Saviour, P., Joshi, A., Joshi, A., Mahajan, S., Bijarnia-Mahay, S., Dua Puri, R., & Verma, I. C. (2021). Prenatal Diagnosis by Chromosome Microarray Analysis, An Indian Experience. *J Obstet Gynaecol India*, *71*(2), 156-167. https://doi.org/10.1007/s13224-020-01413-6

Borrell, A., Grande, M., Pauta, M., Rodriguez-Revenga, L., & Figueras, F. (2017). Chromosomal Microarray Analysis in Fetuses with Growth Restriction and Normal Karyotype: A Systematic Review and Meta-Analysis. *Fetal Diagn Ther*. https://doi.org/10.1159/000479506

Brady, P. D., Chiaie, B. D., Christenhusz, G., Dierickx, K., Bogaert, K. V. D., Menten, B., Janssens, S., Defoort, P., Roets, E., Sleurs, E., Keymolen, K., Catte, L. D., Deprest, J., Ravel, T. d., Esch, H. V., Fryns, J. P., Devriendt, K., & Vermeesch, J. R. (2013). A prospective study of the clinical utility of prenatal chromosomal microarray analysis in fetuses with ultrasound abnormalities and an exploration of a framework for reporting unclassified variants and risk factors. *Genetics in Medicine*, *16*, 469-476. https://doi.org/doi:10.1038/gim.2013.168

CentoGene. (2024). Chromosomal Microarray Analysis with CentoArrayCyto®. https://www.centogene.com/diagnostics/chromosomal-microarray-testing.html

Chau Matthew, H. W., Yunli Lai, Yanyan Zhang, Fuben Xu, Yanqing Tang, Yanfang Wang, Zihan Chen, Tak Yeung Leung, Jacqueline Pui Wah Chung, Yvonne K Kwok, Shuk Ching Chong, Kwong Wai Choy, Yuanfang Zhu, Likuan Xiong, Weihong Wei, Zirui Dong. (2020). Low-pass genome sequencing: a validated method in clinical cytogenetics. *Human Genetics*. https://doi.org/10.1007/s00439-020-02185-9

Chau, M. H. K., Wang, H., Lai, Y., Zhang, Y., Xu, F., Tang, Y., Wang, Y., Chen, Z., Leung, T. Y., Chung, J. P. W., Kwok, Y. K., Chong, S. C., Choy, K. W., Zhu, Y., Xiong, L., Wei, W., & Dong, Z. (2020). Low-pass genome sequencing: a validated method in clinical cytogenetics. *Hum Genet*, *139*(11), 1403-1415. https://doi.org/10.1007/s00439-020-02185-9

Cherry, A. M., Akkari, Y. M., Barr, K. M., Kearney, H. M., Rose, N. C., South, S. T., Tepperberg, J. H., & Meck, J. M. (2017). Diagnostic cytogenetic testing following positive noninvasive prenatal screening results: a clinical laboratory practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *19*(8), 845-850. https://doi.org/10.1038/gim.2017.91

Cohen, P., Rogol, A. D., Deal, C. L., Saenger, P., Reiter, E. O., Ross, J. L., Chernausek, S. D., Savage, M. O., & Wit, J. M. (2008). Consensus statement on the diagnosis and treatment of children with idiopathic short stature: a summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. *J Clin Endocrinol Metab*, *93*(11), 4210-4217. https://doi.org/10.1210/jc.2008-0509

Dubbs, H. (2022). NATIONAL COORDINATING CENTER for EPILEPSY Epilepsy Overview. https://www.aap.org/en/patient-care/epilepsy/diagnosing-pediatric-epilepsy/genetic-testing-for-epilepsy/

FDA. (2014). Affymetrix® CytoScan® Dx Assay. https://www.accessdata.fda.gov/cdrh_docs/reviews/K130313.pdf

FDA. (2017). GenetiSure Dx Postnatal Assay. https://www.accessdata.fda.gov/cdrh_docs/pdf16/K163367.pdf

Fox, N. S., Monteagudo, A., Kuller, J. A., Craigo, S., & Norton, M. E. (2018). Mild fetal ventriculomegaly: diagnosis, evaluation, and management. *Am J Obstet Gynecol*, *219*(1), B2-b9. https://doi.org/10.1016/j.ajog.2018.04.039

Gencove. (2023). Low-pass sequencing and imputation for evaluating genetic variation. https://gencove.com/blog/low-pass-whole-genome-sequencing

GeneDx. (2024). *Chromosomal Microarray (GenomeDx®)*. https://providers.genedx.com/tests/detail/chromosomal-microarray-718

Gregg, A. R., Skotko, B. G., Benkendorf, J. L., Monaghan, K. G., Bajaj, K., Best, R. G., Klugman, S., & Watson, M. S. (2016). Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical

Genetics and Genomics. *Genet Med*, *18*(10), 1056-1065. https://doi.org/10.1038/gim.2016.97
Hay, S. B., Sahoo, T., Travis, M. K., Hovanes, K., Dzidic, N., Doherty, C., & Strecker, M. N. (2018). ACOG and SMFM guidelines for prenatal diagnosis: Is karyotyping really sufficient? *Prenat Diagn*. https://doi.org/10.1002/pd.5212
Hyman, S. L., Levy, S. E., & Myers, S. M. (2020). Identification, Evaluation, and Management of Children With Autism Spectrum Disorder. *Pediatrics*, *145*(1). https://doi.org/10.1542/peds.2019-3447
Illumina. (2024). Coverage depth recommendations. https://www.illumina.com/science/technology/next-generation-sequencing/plan-experiments/coverage.html
Invitae. (2024). Invitae Pregnancy Loss Chromosomal Microarray Analysis for FFPE. https://www.invitae.com/en/physician/tests/81120/
ISPD. (2018). Joint Position Statement from the International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM), and the Perinatal Quality Foundation (PQF) on the use of genome-wide sequencing for fetal diagnosis. *Prenat Diagn*, *38*(1), 6-9. https://doi.org/10.1002/pd.5195
LabCorp. (2023). *SNP Microarray–Pediatric (Reveal®)*. https://www.labcorp.com/tests/510002/snp-microarray-pediatric-reveal
Li, Y., Anderson, L. A., Ginns, E. I., & Devlin, J. J. (2018). Cost Effectiveness of Karyotyping, Chromosomal Microarray Analysis, and Targeted Next-Generation Sequencing of Patients with Unexplained Global Developmental Delay or Intellectual Disability. *Mol Diagn Ther*, *22*(1), 129-138. https://doi.org/10.1007/s40291-017-0309-5
Lineagen. (2024). *FirstStepDx PLUS®*. https://lineagen.com/parents/our-testing-services/firststepdx-plus/
Lipkin, P. H., & Macias, M. M. (2020). Promoting Optimal Development: Identifying Infants and Young Children With Developmental Disorders Through Developmental Surveillance and Screening. *Pediatrics*, *145*(1). https://doi.org/10.1542/peds.2019-3449
Mandy, G. T. (2024, August 18). *Infants with fetal (intrauterine) growth restriction*. https://www.uptodate.com/contents/infants-with-fetal-intrauterine-growth-restriction
Manning, M., & Hudgins, L. (2010). Array-based technology and recommendations for utilization in medical genetics practice for detection of chromosomal abnormalities. *Genet Med*, *12*(11), 742-745. https://doi.org/10.1097/GIM.0b013e3181f8baad
Mardy, A. H., Rangwala, N., Hernandez-Cruz, Y., Gosnell, K. A., Gonzalez, J. M., Norton, M. E., & Sparks, T. N. (2020). Utility of chromosomal microarray for diagnosis in cases of nonimmune hydrops fetalis. *Prenat Diagn*. https://doi.org/10.1002/pd.5617
Martins, J. G., Biggio, J. R., & Abuhamad, A. (2020). Society for Maternal-Fetal Medicine Consult Series #52: Diagnosis and management of fetal growth restriction: (Replaces

Clinical Guideline Number 3, April 2012). *Am J Obstet Gynecol*, *223*(4), B2-b17. https://doi.org/10.1016/j.ajog.2020.05.010

Mazzonetto, P. C., Villela, D., da Costa, S. S., Krepischi, A. C. V., Milanezi, F., Migliavacca, M. P., Pierry, P. M., Bonaldi, A., Almeida, L. G. D., De Souza, C. A., Kroll, J. E., Paula, M. G., Guarischi-Sousa, R., Scapulatempo-Neto, C., & Rosenberg, C. (2024). Low-pass whole genome sequencing is a reliable and cost-effective approach for copy number variant analysis in the clinical setting. *Ann Hum Genet*, *88*(2), 113-125. https://doi.org/10.1111/ahg.12532

Mefford, H. C. (2015). Clinical Genetic Testing in Epilepsy. *Epilepsy Curr*, *15*(4), 197-201. https://doi.org/10.5698/1535-7511-15.4.197

Miller, D. (2022, July 15). *Prenatal diagnosis of chromosomal imbalance: Chromosomal microarray*. https://www.uptodate.com/contents/use-of-chromosomal-microarray-in-obstetrics

Miller, D. T., Adam, M. P., Aradhya, S., Biesecker, L. G., Brothman, A. R., Carter, N. P., Church, D. M., Crolla, J. A., Eichler, E. E., Epstein, C. J., Faucett, W. A., Feuk, L., Friedman, J. M., Hamosh, A., Jackson, L., Kaminsky, E. B., Kok, K., Krantz, I. D., Kuhn, R. M., . . . Ledbetter, D. H. (2010). Consensus Statement: Chromosomal Microarray Is a First-Tier Clinical Diagnostic Test for Individuals with Developmental Disabilities or Congenital Anomalies. In *Am J Hum Genet* (Vol. 86, pp. 749-764). https://doi.org/10.1016/j.ajhg.2010.04.006

Moeschler, J. B., & Shevell, M. (2014). Comprehensive evaluation of the child with intellectual disability or global developmental delays. *Pediatrics*, *134*(3), e903-918. https://doi.org/10.1542/peds.2014-1839

Monaghan, K. G., Leach, N. T., Pekarek, D., Prasad, P., Rose, N. C., on behalf of the, A. P. P., & Guidelines, C. (2020). The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG). *Genetics in Medicine*, *22*(4), 675-680. https://doi.org/10.1038/s41436-019-0731-7

Monier, I., Receveur, A., Houfflin-Debarge, V., Goua, V., Castaigne, V., Jouannic, J. M., Mousty, E., Saliou, A. H., Bouchghoul, H., Rousseau, T., Valat, A. S., Groussolles, M., Fuchs, F., Benoist, G., Degre, S., Massardier, J., Tsatsaris, V., Kleinfinger, P., Zeitlin, J., & Benachi, A. (2021). Should prenatal chromosomal microarray analysis be offered for isolated fetal growth restriction? A French multicenter study. *Am J Obstet Gynecol*, *225*(6), 676.e671-676.e615. https://doi.org/10.1016/j.ajog.2021.05.035

Nagan, N., Faulkner, N. E., Curtis, C., & Schrijver, I. (2011). Laboratory guidelines for detection, interpretation, and reporting of maternal cell contamination in prenatal analyses a report of the association for molecular pathology. *J Mol Diagn*, *13*(1), 7-11. https://doi.org/10.1016/j.jmoldx.2010.11.013

National FragileX Foundation. (2024). *Fragile X Syndrome Testing & Diagnosis*. https://www.fragilex.org/understanding-fragile-x/fragile-x-101/testing-diagnosis/

Norton, M. E., Chauhan, S. P., & Dashe, J. S. (2015). Society for maternal-fetal medicine (SMFM) clinical guideline #7: nonimmune hydrops fetalis. *Am J Obstet Gynecol*, *212*(2), 127-139. https://doi.org/10.1016/j.ajog.2014.12.018

Pasternak, Y., Daykan, Y., Tenne, T., Reinstein, E., Miller, N., Shechter-Maor, G., Maya, I., Biron-Shental, T., & Sukenik Halevy, R. (2020). The yield of chromosomal microarray analysis among pregnancies terminated due to fetal malformations. *J Matern Fetal Neonatal Med*, 1-5. https://doi.org/10.1080/14767058.2020.1716722

Quest. (2024). *Why ClariSure® CMA*. https://www.questdiagnostics.com/home/physicians/testing-services/by-test-name/clarisure/why-clarisure/

Robson, S. C., Chitty, L. S., Morris, S., Verhoef, T., Ambler, G., Wellesley, D. G., Graham, R., Leader, C., Fisher, J., & Crolla, J. A. (2017). Efficacy and Mechanism Evaluation. *Evaluation of Array Comparative genomic Hybridisation in prenatal diagnosis of fetal anomalies: a multicentre cohort study with cost analysis and assessment of patient, health professional and commissioner preferences for array comparative genomic hybridisation*. https://doi.org/10.3310/eme04010

Rodriguez-Revenga, L., Madrigal, I., Borrell, A., Martinez, J. M., Sabria, J., Martin, L., Jimenez, W., Mira, A., Badenas, C., & Milà, M. (2020). Chromosome microarray analysis should be offered to all invasive prenatal diagnostic testing following a normal rapid aneuploidy test result. *Clinical Genetics*, *98*(4), 379-383. https://doi.org/10.1111/cge.13810

Satya-Murti, S., Cohen, B.H., and Michelson, D. (2015). *Chromosomal microarray analysis for intellectual disabilities*. American Academy of Neurology. https://www.aan.com/siteassets/home-page/tools-and-resources/practicing-neurologist--administrators/billing-and-coding/model-coverage-policies/15microarrayanalysismodel_tr.pdf

Seaver, L. H., & Irons, M. (2009). ACMG practice guideline: genetic evaluation of short stature. *Genet Med*, *11*(6), 465-470. https://doi.org/10.1097/GIM.0b013e3181a7e8f8

Shen, Y., Dies, K. A., Holm, I. A., Bridgemohan, C., Sobeih, M. M., Caronna, E. B., Miller, K. J., Frazier, J. A., Silverstein, I., Picker, J., Weissman, L., Raffalli, P., Jeste, S., Demmer, L. A., Peters, H. K., Brewster, S. J., Kowalczyk, S. J., Rosen-Sheidley, B., McGowan, C., . . . Miller, D. T. (2010). Clinical genetic testing for patients with autism spectrum disorders. *Pediatrics*, *125*(4), e727-735. https://doi.org/10.1542/peds.2009-1684

Waggoner, D., Wain, K. E., Dubuc, A. M., Conlin, L., Hickey, S. E., Lamb, A. N., Martin, C. L., Morton, C. C., Rasmussen, K., Schuette, J. L., Schwartz, S., & Miller, D. T. (2018). Yield of additional genetic testing after chromosomal microarray for diagnosis of neurodevelopmental disability and congenital anomalies: a clinical practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *20*(10), 1105-1113. https://doi.org/10.1038/s41436-018-0040-6

Wang, H., Dong, Z., Zhang, R., Chau, M. H. K., Yang, Z., Tsang, K. Y. C., Wong, H. K., Gui, B., Meng, Z., Xiao, K., Zhu, X., Wang, Y., Chen, S., Leung, T. Y., Cheung, S. W., Kwok, Y. K., Morton, C. C., Zhu, Y., & Choy, K. W. (2020). Low-pass genome sequencing versus chromosomal microarray analysis: implementation in prenatal diagnosis. *Genet Med*, *22*(3), 500-510. https://doi.org/10.1038/s41436-019-0634-7

Zhang, Y., Zhong, M., & Zheng, D. (2021). Chromosomal mosaicism detected by karyotyping and chromosomal microarray analysis in prenatal diagnosis. *J Cell Mol Med*, *25*(1), 358-366. https://doi.org/10.1111/jcmm.16080

Esophageal Pathology Testing

Policy Number: AHS – M2171 – Esophageal Pathology Testing	Prior Policy Name and Number, as applicable:
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

The esophagus is a long tube that serves to connect the mouth to the stomach. Although the esophagus is primarily a connecting organ, it experiences significant chemical and mechanical trauma. The esophagus has mechanisms and structures to withstand this damage, but molecular injury is common (Zhang et al., 2020). Both serological and genetic markers have been suggested to identify, diagnose, or assess risk in the esophagus.

Eosinophilic esophagitis (EoE) is one such condition, as its nonspecific symptoms (pain, issues swallowing, vomiting, and so on) may be accompanied by inflammatory markers in the esophagus (Bonis & Gupta, 2023). Similarly, esophageal cancer is characterized by several nonspecific symptoms, while a predecessor condition, Barrett's esophagus (BE), may have no clinical symptoms at all (Saltzman & Gibson, 2023; Spechler, 2022).

For guidance concerning Tumor Mutational Burden Testing (TMB) and/or Microsatellite instability (MSI) analysis please refer to the AHS-M2178-Microsatellite Instability and Tumor Mutational Burden Testing policy.

II. Related Policies

Policy Number	Policy Title
AHS-M2178	Microsatellite Instability and Tumor Mutational Burden Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals who have been newly diagnosed with cancer of the esophagus or esophagogastric junction (EGJ), mismatch repair (MMR) analysis by immunohistochemistry (IHC) **MEETS COVERAGE CRITERIA**.

2) For individuals who have been diagnosed with locally advanced, recurrent, or metastatic cancer of the esophagus or EGJ and for whom PD-1 inhibitor treatment is being considered, tumor analysis of PD-L1 expression by IHC **MEETS COVERAGE CRITERIA**.

3) For individuals who have been diagnosed with inoperable locally advanced, recurrent, or metastatic adenocarcinoma of the esophagus or EGJ and for whom trastuzumab or an approved biologic or biosimilar drug to trastuzumab is being considered for first-line therapy, HER2 overexpression testing by IHC, fluorescence in situ hybridization (FISH), or other in situ hybridization (ISH) **MEETS COVERAGE CRITERIA**.

4) For individuals diagnosed with unresectable locally advanced, recurrent, or metastatic adenocarcinoma or squamous cell carcinoma of the esophagus or EGJ and for whom one of the following drugs is being considered as a second-line therapy, the corresponding gene testing **MEETS COVERAGE CRITERIA**:
   a) Larotrectinib or entrectinib: *NTRK* gene fusion.
   b) Selpercatinib: *RET* gene fusion.
   c) Dabrafenib or trametinib: *BRAF* V600E mutation.

5) The use of genetic testing (e.g., molecular panel tests, gene expression profiling) to diagnose or monitor an individual with eosinophilic esophagitis (EoE) or to assess the risk of an individual developing EoE **DOES NOT MEET COVERAGE CRITERIA**.

6) For the diagnosis and evaluation of Barrett's esophagus, low-grade esophageal dysplasia, or high-grade esophageal dysplasia, wide-area transepithelial sampling (WATS) **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

7) Assessing for risk of Barrett's esophagus and/or esophageal, including esophagogastric junction, cancer using a molecular classifier (e.g., BarreGEN test) **DOES NOT MEET COVERAGE CRITERIA**.

8) Epigenetic analysis for the likelihood for Barrett's esophagus, esophageal, or esophagogastric junction cancer (e.g., methylation analysis, EsoGuard) **DOES NOT MEET COVERAGE CRITERIA**.

9) To diagnose, assess, or monitor eosinophilic esophagitis (EoE), the Esophageal String Test **DOES NOT MEET COVERAGE CRITERIA**.

10) For esophageal and esophagogastric junction cancers, cell-free DNA/circulating tumor DNA (cfDNA/ctDNA) testing **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note: For 5 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
ACG	American College of Gastroenterology
AFS	American Foregut Society
AMACR	Alpha-methylacyl-CoA racemase
APC	*Adenomatous polyposis coli*
ARID1A	*AT-rich interactive domain-containing protein 1A*
ARID2	*AT-rich interactive domain 2*
ASGE	American Society for Gastrointestinal Endoscopy
BAT	Bethesda marker
BE	Barrett's esophagus
BLM	Bloom syndrome protein
BMJ	British Medical Journal
BS	Bloom syndrome
CAPN14	*Calpain 14*
CCL26	*C-C motif chemokine ligand 26*
CCNA1	*Cyclin A1*
cfDNA	Cell-free tumor DNA
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMM1	Familial cutaneous malignant melanoma-1
CMS	Centers For Medicare and Medicaid Services
COX2	*Cyclooxygenase 2*
CPS	Combined Positive Score
CSCO	Chinese Society of Clinical Oncology

CTCs	Circulating tumor cells
ctDNA	Circulating tumor DNA
DCC	*Deleted in colorectal carcinoma*
DNA	Deoxyribonucleic acid
DOCK2	*Dedicator of cytokinesis 2*
EAACI	European Academy of Allergy and Clinical Immunology
EAC	Esophageal adenocarcinoma
ED	Esophageal dysplasia
EDP	Eosinophilic esophagitis diagnostic panel
EGFR	*Epidermal growth factor receptor*
EGJ	Esophagogastric junction
ELISA	Enzyme-linked immunoassay
ELMO1	*Engulfment and cell motility protein 1*
EoE	Eosinophilic esophagitis
ESMO	European Society for Medical Oncology
ESPGHAN	European Society of Pediatric Gastroenterology, Hepatology And Nutrition
EST	Esophageal string test
EUREOS	European Society of Eosinophilic Oesophagitis
FA	Fanconi anemia
FANC	FA complementation group A
FB	Forceps biopsy
FBE	Familial Barrett's esophagus
FDA	Food and Drug Administration
FISH	Fluorescence in situ hybridization
GERD	Gastroesophageal reflux disease
HER2	*Human epidermal growth factor receptor 2*
HGD	High-grade dysplasia
HGD/EAC	High-grade dysplasia/esophageal adenocarcinoma
HIF1-ALPHA	Hypoxia-inducible factor 1-alpha
HoGG1	*8-oxoguanine DNA glycosylase*
ICER	Incremental cost-effectiveness ratio
IgE	Immunoglobulin E
IHC	Immunohistochemistry
IND	Indefinite for dysplasia
JSMO	Japanese Society of Medical Oncology
K20	Potassium oxide
KSMO	Korean Society of Medical Oncology

LDTs	Laboratory developed tests
LGD	Low-grade dysplasia
MBP-1	Major basic protein 1
MCC	*Colorectal mutant cancer protein*
ML	Mutational load
MMR	Mismatch repair
MSI	Microsatellite instability
MSI-H	High microsatellite instability
MXI1	*Max-interacting protein 1*
NBDE	Non-dysplastic intestinal metaplasia
NCCN	National Comprehensive Cancer Network
NDBE	Baseline nondysplastic BE
NF2	Neurofibromatosis type 2
NME1	Nucleoside Diphosphate Kinase 1
NNT	Number needed to test
NOTCH3	*Notch receptor 3*
NTRK	Neurotrophic tyrosine receptor kinase
PCR	Polymerase chain reaction
PD-1	Programmed death-1
PD-L1	Programmed death-ligand 1
PPK	Palmoplantar keratoderma
PRG2	*Proteoglycan 2, pro eosinophil major basic protein*
PSEN2	*Presenilin 2*
PTEN	*Phosphatase and TENsin homolog*
QALY	Quality-adjusted life-year
RB	*Retinoblastoma protein*
RHBDF2	*Rhomboid 5 homolog 2*
RNF43	*Ring finger protein 43*
SAGES	Society of American Gastrointestinal and Endoscopic Surgeons
SCCs	Squamous cell carcinomas
SMAD4	*SMA- and MAD-related protein 4*
SMARCA4	*Matrix associated, actin dependent regulator of chromatin, subfamily a*
SOC	Standard of care
SPG20	*Spastic paraplegia 20*
SSO	Sequence-specific oligonucleotide
STMN1	Stathmin 1
TAVAC	Technology And Value Assessment Committee
TFF1	*Trefoil factor 1*
TML	Tumor mutational load

TNFAIP8	*TNF alpha induced protein 8*
TOS	Thoracic outlet syndromes
TP53	*Tumor protein 53*
TPS	Tumor positive score
TRK	Tropomyosin receptor kinase
TSLP	*Thymic stromal lymphopoietin*
TVAC	Technology And Value Assessment Committee
UEG	United European Gastroenterology
VHL	Von hippel-lindau syndrome
VIM	Vimentin
WATS	Wide-Area Transepithelial Sampling
WATS3D	Wide-Area Transepithelial Sampling with Computer-Assisted 3-Dimensional Analysis

V. Scientific Background

The esophagus is a long tube that connects the mouth to the stomach. Its primary function is to transport food from the mouth to the stomach. However, this organ is often exposed to difficult conditions, from abrasive food to the acidic conditions of the stomach. Although mechanisms are in place to protect against injury (namely the tough squamous cells), it is common to see injury or disease in the esophagus (Zhang et al., 2020).

Many serological and genetic markers have been proposed as tools to assist in evaluation of esophageal pathology. Eosinophilic esophagitis (EoE), Barrett's esophagus (BE), and esophageal cancer are typically diagnosed with histological analysis from endoscopic biopsy (Bonis & Gupta, 2023; Saltzman & Gibson, 2023; Spechler, 2022), but biopsies frequently require careful consideration and resources to perform properly (NCCN, 2024). For these reasons, serum and genetic markers have been suggested as noninvasive markers for esophageal pathologies.

Eosinophilic Esophagitis (EoE)

Eosinophilic esophagitis (EoE) is marked by the presence of eosinophils in the esophagus. Eosinophils are typically associated with mitigating inflammation but are not normally found in the esophagus. EoE is represented by a broad set of clinical symptoms, such as difficulty swallowing, chest, or abdominal pain, and feeding dysfunction. Diagnosis is established through endoscopy with biopsies to confirm eosinophilia. The current diagnostic criteria set the cutoff for eosinophilia at ≥15 eosinophils per high power field, (60 eosinophils per mm^2) although this figure has been heavily discussed (Bonis & Gupta, 2023; Dellon et al., 2018).

Proprietary Testing- EoE

Laboratory tests have been suggested as a noninvasive adjunct for EoE. Serum IgE will be elevated in up to 60% of EoE patients, as allergy has a strong association with EoE. Many other markers, such as eotaxin-3, major basic protein-1, tryptase, chemokines, and serum eosinophil count, have all been suggested to assist in evaluation of EoE (Bonis & Gupta, 2023; Dellon et al., 2018). Immune system factors may also contribute to pathology. Since eosinophils are not normally found in the esophagus, their presence in the esophagus may suggest an underlying issue with the immune system. Various interleukins, mast cells, and T cells have all been proposed as contributing to pathogenesis, but the exact pathway and mechanisms are not completely understood (Rothenberg, 2023). Genetic features have also been used for EoE evaluation. Twin studies and family histories have indicated a role for genetics in EoE. Several genes have also been identified as potential risk factors, such as *CAPN14* (an interleukin-13 regulator), *TSLP* (a basophil regulator), and *CCL26* (promotes eosinophil movement into esophagus) (Sherrill & Rothenberg, 2014).

Wen et al. (2013) developed a diagnostic gene expression panel ("EDP") for EoE. The authors identified candidate genes using two cohorts of EoE and control patients, then validated these genes with a separate cohort of 194 patients (91 active EoE, 57 control, 34 ambiguous, 12 reflux). The panel was found to identify EoE patients at 96% sensitivity and 98% specificity. The authors also noted that the panel could separate patients in remission from unaffected patients (Wen et al., 2013).

Shoda et al. (2018) used an "EoE Diagnostic Panel" (EDP) to further classify EoE cases by histologic, endoscopic, and molecular features. The EDP consisted of 95 esophageal transcripts purported to identify EoE among both unaffected patients and patients with other conditions. 185 biopsies were studied. The authors identified three clear subtypes of EoE; subtype 1 with a normal-appearing esophagus and mild molecular changes, subtype 2 with an inflammatory and steroid-responsive phenotype, and subtype 3 with a "narrow-caliber" esophagus and severe molecular alterations. These findings were replicated in a 100-biopsy sample (Shoda et al., 2018).

Tests are commercially available for EoE. Noninvasive tests (as an alternative to endoscopy) have been recently popular. The Esophageal String Test (Testa et al.) is one such alternative. The patient swallows a gelatin-coated capsule with a string wrapped inside. Once the capsule is in the patient's stomach, the gelatin dissolves, allowing the capsule to pass through. The string itself is used to collect samples from the patient's esophagus and is easily removed from the patient. From there, the sample is analyzed for several biomarkers (major basic protein-1, eotaxins 2 and 3, and so on) to provide a

probability% (a trademarked "EoEscore") of esophageal inflammation (Ackerman et al., 2019; EnteroTrack, 2023).

Barrett's Esophagus (BE)

Barrett's esophagus (BE) is a condition in which the normal squamous tissue lining the esophagus is replaced by metaplastic columnar epithelium. This new epithelium contains gastric features and is typically caused by chronic gastroesophageal reflux disease (GERD). This condition predisposes to esophageal cancer. When noxious substances (gastric acid, bile, et al) are exposed to the squamous esophageal tissue, the damage is usually repaired through regeneration of these squamous cells. In BE cases, this damage is repaired not through creation of new squamous cells, but through metaplastic columnar cells. The exact reason for this is unknown. Although these metaplastic cells are more resistant to reflux-based damage than the normal squamous cells, these cells frequently show the oxidative DNA damage that is typical of cancer. Mutations in the p53 tumor suppressor gene appear to be the catalyst for cancers, as acquisition of this mutation in conjunction with the replication of the genome is conducive to carcinogenesis (Spechler, 2022).

Vollmer (2019) performed a review assessing incidence of adenocarcinoma detected during surveillance of BE. The author identified 55 studies encompassing 61371 total patients. Of the 61371 total patients, 1106 developed adenocarcinoma. Overall, the author found that the model created from the studies "predicted the per-person probability of developing cancer in five years of complete follow-up is approximately 0.0012." Variables affecting this probability included mean time of follow-up, definition of Barrett metaplasia, and fraction of patients followed up for at least five years (Vollmer, 2019).

Proprietary Testing- BE

Proprietary tests are commercially available for assessment of BE, usually to evaluate risk (BE progression to cancer, risk of BE itself, and such). For example, BarreGen, offered by Interpace Diagnostics, uses tumor mutational load (a measure intended to capture total genomic instability of a sample) to calculate risk of progression. Although many ways can estimate mutational load, BarreGen tests 10 key genomic loci which are as follows: "1p (*CMM1*, L-myc), 3p (*VHL*, *HoGG1*), 5q (*MCC*, *APC*), 9p (*CDKN2A*), 10q (*PTEN*, *MXI1*), 17p (*TP53*), 17q (*RNF43*, *NME1*), 18q (*SMAD4*, *DCC*), 21q (*TFF1*, *PSEN2*) and 22q (*NF2*)." These loci encompass integral tumor suppressors and are proposed to provide an accurate picture of genomic instability (Interpace, 2023; Trindade et al., 2019).

Another test, TissueCypher, also proposes to predict likelihood of progression from BE to esophageal cancer. The test measures nine protein biomarkers that represent morphological and cellular changes (p53, p16, AMACR, CD68, COX2, HER2, K20, HIF1-alpha, CD45RO). These biomarkers are quantified and converted to a risk score (1-10) and probability of progression (Castle Biosciences, 2023).

Esoguard, by Lucid Diagnostics, is an esophageal DNA test which analyzes 31 methylated biomarkers in the diagnosis of non-dysplastic Barrett's esophagus and adenocarcinoma. The assay uses next generation sequencing to examine individual DNA molecules for the presence or absence of cytosine methylation with a 90% specificity and 90% sensitivity (Lucid Diagnostics, 2024).

Finally, a proprietary imaging system, WATS3D, is commercially available. This imaging system samples from a wider area, as opposed to only taking focal samples in a traditional biopsy. This technology also provides a 3-dimensional image of the sampled area. This technology purports to provide more precise sampling than the traditional 4-quadrant biopsies, claiming an increased detection rate of BE and other dysplasias (Diagnostics, 2024).

Esophageal Cancer

Esophageal cancers are largely divided into two groups: squamous cell carcinomas (SCCs) and adenocarcinomas (EAC). SCCs usually begin in the middle of the esophagus, whereas EACs often originate near the gastroesophageal junction. Both share several risk factors, such as smoking. Due to the numerous environmental risk factors for both types of cancer, it is difficult to ascertain the true impact of genetic factors (Gibson, 2023). These cancers are primarily diagnosed through histologic examination, usually obtained through endoscopy (Saltzman & Gibson, 2023).

Advancements have been in the molecular characterization of both types of cancer. *TP53* mutations are the most common mutation seen in both types of cancer. Other frequently mutated genes in adenocarcinoma include *ELMO1* and *DOCK2* (enhance cell motility), *ARID1A*, *SMARCA4* and *ARID2* (chromatin remodelers), and *SPG20* (traffics growth factor receptors). BE, as the precursor to adenocarcinomas, includes certain similarities in genetic mutations but at a less severe rate. Further, the rate of overlap tended to increase with higher degree of dysplasia (Testa et al., 2017).

Squamous cell carcinoma mutations tend to be in genes associated with specific cellular pathways. Genes in ubiquitous pathways, such as *EGFR*, *NOTCH3*, and *RB*, are frequently mutated in SCC. The molecular profile of esophageal SCC tends to align more with other squamous cell cancers (such as head and neck cancers) rather than EAC (Testa et al., 2017). Numerous gene expression studies have been performed to

further classify molecular subtypes of esophageal cancer (Gonzaga et al., 2017; McLaren et al., 2017; Visser et al., 2017). Gene expression profiles may have utility in assessing response to treatment, prognosis, or risk assessment.

Historically, Carcinoembryonic Antigen (CEA) has been used as the serum cancer marker in the diagnosis of esophageal cancer, as CEA levels have been shown to be significantly higher in these patients. The sensitivity (8-70%), specificity (57-100%), and positive likelihood ratio (5.94) of CEA means that patients with EC have a 6-fold higher chance of having higher CEA levels. Other markers include squamous cell cancer antigen (SCC-Ag) and cytokeratin 21-1 fragment (CYFRA21-1). The sensitivity and specificity Cyfra21–1 ranged from 36% to 63% and from 89% to 100%, respectively, with patients having a 12-fold higher chance of having EC. The sensitivity and specificity of SCC-Ag ranged from 13% to 64% and from 91% to 100%, respectively, whereas its PLR was 7.66 (Visaggi et al., 2021).

Li et al. (2019) investigated potential biomarkers for lymph node metastasis for esophageal squamous cell carcinoma. Six studies encompassing 70 patients were included. The authors identified nine biomarkers and four cellular mechanisms that influence lymph node metastasis. From there, they identified three biomarkers with broader influence on prognosis of disease, *PTEN*, *STMN1*, and *TNFAIP8*. The authors suggested that those three biomarkers should be researched further (Li et al., 2019).

Plum et al. (2019) evaluated *HER2* overexpression's impact on prognosis of esophageal adenocarcinoma (EAC). 428 EAC patients that underwent a "transthoracic thoraco-abdominal esophagectomy" were included. The authors identified 44 patients with *HER2* positivity (IHC score 3+ or 2+ with gene amplification). This cohort was found to have a better overall survival (OS, 70.1 months vs 24.6 months), along with better histology, absence of lymphatic metastases, and lower tumor stages. The authors also noted a similarity in results to a large 2012 study (Plum et al., 2019).

Frankell et al. (2019) examined the molecular landscape of esophageal adenocarcinoma (EAC). The authors assessed 551 genomically characterized EACs. A total of 77 driver genes and "21 non-coding driver elements" were identified. The authors also found an average of 4.4 driver events per tumor. A three-way association was found, between hyper-mutation, *Wnt* signaling, and loss of immune signaling genes. Finally, the authors also identified "sensitizing events" (events causing a tumor to be more susceptible to a therapy) to CD4/6 inhibitors in over half of the EAC cases studied (Frankell et al., 2019).

Clinical Utility and Validity

Ackerman et al. (2019) evaluated the ability of the 1-hour Esophageal String Test (Testa et al.) to distinguish between active eosinophilic esophagitis (EoE), inactive eosinophilic esophagitis, and normal esophagi. A total of 134 patients (62 active EoE, 37 inactive EoE, 35 normal) were included. The authors found that eotaxin 3 measured from both EST samples and the control biopsy extracts to be the best marker for distinguishing active EoE from inactive EoE (by both sensitivity and specificity). Addition of major basic protein 1 (MBP-1) improved sensitivity by 0.039 (0.652 to 0.693) and specificity by 0.014 (0.261 to 0.275) across all patients (Ackerman et al., 2019).

Hao et al. (2019) performed a cost-effectiveness analysis of an "adenocarcinoma risk prediction multi-biomarker assay" (TissueCypher's Barrett's Esophagus Assay). A hypothetical cohort of 10000 patients with BE diagnoses (including non-dysplastic intestinal metaplasia [NBDE], indefinite for dysplasia [IND], and low-grade dysplasia [LGD]) was created. A Markov decision model was used to compare BE management costs between assay use and the standard of care (SOC). A surveillance interval of five years was used. Low-risk patients were found to have a 16.6% reduction in endoscopies. High-risk patients were found to have a 58.4% increase in endoscopic treatments (compared to the SOC arm), leading to a death total of 111 for the assay arm compared to 204 in the SOC arm (a 45.6% reduction). Overall, the authors calculated the incremental cost-effectiveness ratio (ICER) to be $52,483/quality-adjusted life-year (QALY), and they found that "the probability of the Assay being cost-effective compared to the SOC was 57.3% at the $100,000/QALY acceptability threshold" (Hao et al., 2019).

Eluri et al. (2018) aimed to validate a genomic panel intended to represent tumor mutational load (TML). Previously, the authors evaluated a panel of 10 genomic loci from which a TML score was calculated. This mean TML was found to be significantly higher in 23 BE patients that had progressed to high-grade dysplasia (HGD) or esophageal adenocarcinoma (EAC) as compared to 46 that had not progressed. The area under the curve in this prior study was found to be 0.95 at a mutational load (ML) cutoff of 1 (on a scale of 1-10). In the present study, 159 subjects were included. Cases had "baseline nondysplastic BE (NDBE) and developed HGD/EAC ≥ 2 years later." 58 subjects were progressors and 101 were nonprogressors. The authors identified no difference in mean ML in pre-progression tissue in both cohorts (ML = 0.73 ± 0.69 vs. ML = 0.74 ± 0.61). The area under the curve at the cutoff of ML 1 was only 0.50, and the authors concluded that the "utility of the ML to stratify BE patients for risk of progression was not confirmed in this study" (Eluri et al., 2018).

Trindade et al. (2019) evaluated tumor mutational load's (ML) ability to "risk-stratify those that may progress from non-dysplastic BE to dysplastic disease". 28 patients were included, and ML levels were compared between those that progressed to

dysplasia and those who had not. Eight total patients progressed to dysplasia (6 low-grade, 2 high-grade), and seven of these patients had "some level" of genomic stability detected (ML ≥.5 on a scale of 1 to 10). Ten of the 20 patients that did not progress to dysplasia had "no" ML level. The authors also noted that at an ML of ≥1.5, the risk of progression to high-grade dysplasia was 33%, with a sensitivity of 100% and specificity of 85%. The authors concluded "that ML may be able to risk-stratify progression to high-grade dysplasia in BE-IND. Larger studies are needed to confirm these findings" (Trindade et al., 2019).

Moinova et al. (2018) evaluated the ability of two DNA methylation signatures to detect BE. Methylation signatures of the *VIM* and *CCNA1* loci were evaluated in 173 patients with or without BE. *CCNA1* methylation was found to have an area under the curve of 0.95 for distinguishing BE-related dysplasia compared to normal esophagi. When the data for *VIM* methylation was added, the resulting sensitivity was 95%, and the resulting specificity was 91%. These findings were replicated in a validation cohort of 86 patients, with the combination of methylation markers detecting BE metaplasia at 90.3% sensitivity and 91.7% specificity (Moinova et al., 2018).

Critchley-Thorne et al. (2016) validated a pathology panel to predict progression of BE to esophageal cancer. The authors identified 15 potential biomarkers, which were evaluated in both training and validation sets. This "classifier" separated patients into three different risk classes: low, intermediate, and high in the training set of 183. The authors calculated the hazard ratio of intermediate to low risk at 4.19 and high to low at 14.73. In the validation set (n = 183), the concordance index (an estimation of area under the curve) of the 15-factor classifier was 0.772, the best of the amounts tested (3, 6, 9, 12, 15, 17). The authors also noted that this classifier provided independent prognostic information that were outperformed predictions based on other clinicopathological factors, such as segment length, age, and p53 overexpression (Critchley-Thorne et al., 2016).

Another multicenter study investigated the use of WATS^{3D} with either random or targeted FB in the detection of esophageal dysplasia (ED). A total of 12,899 patients were enrolled in the study, and WATS^{3D} detected an additional 213 cases of ED beyond the initial 88 cases identified by FB, representing an increase of 242%. Regarding screening for BE, WATS increased the overall detection by 153% (from 13.1% to 33% of the individuals enrolled). The authors noted that the order of testing (e.g., FB or WATS) did not impact the results. The authors conclude, "In this study, comprised of the largest series of patients evaluated with WATS, adjunctive use of the technique with targeted and random FB markedly improved the detection of both ED and BE. These results underscore the shortcomings of FB in detecting BE-associated neoplasia, which

can potentially impact the management and clinical outcomes of these patients" (Smith et al., 2019).

A study into the cost-effectiveness of WATS^{3D} testing as an adjunct to the standard-of-care forceps biopsy (FB) used a reference case of a 60-year-old individual with gastroesophageal reflux disease (GERD) to see the number of screens needed to avert one cancer and one cancer-related death as well as to calculate the quality-adjusted life years (QALYs) as measured in 2019 U.S. dollars. With this as a reference case, 320 – 337 individuals would need to be screened using WATS^{3D} to avert one cancer, and 328 – 367 individuals would be required to avert one death. The additional cost associated with WATS^{3D} was $1219, but an additional 0.017 QALYs were produced, resulting in an ICER of $71395/QALY. The authors concluded that screening for BE in certain GERD patients "is more cost-effective when WATS^{3D} is used adjunctively to the Seattle protocol than with the Seattle protocol alone" (Singer & Smith, 2020).

One study compared the use of the WATS^{3D} technology to standard forceps biopsy. A total of 117 individuals with a history of Barrett's esophagus with dysplasia had both techniques performed. For the biopsy, a four-quadrant biopsy quadrant protocol was performed every 1 – 2 cm. Evaluation of the biopsy and the WATS^{3D} technique was performed by separate pathologists, blinded to each other's results. Moreover, "Brush biopsy [WATS^{3D}] added an additional 16 position cases increasing the yield of dysplasia detection by 42% (95% CI: 20.7 – 72.7). The number needed to test (NNT) to detect one additional case of dysplasia was 9.4 (95% CI: 6.4 – 17.7)." The authors of the study noted that no statistical difference was evident between medical centers, the type of forceps used, or between sampling every 1 cm versus every 2 cm. They conclude, "These data suggest that computer-assisted brush biopsy is a useful adjunct to standard endoscopic surveillance regimens for the identification of dysplasia in Barrett's esophagus" (Anandasabapathy et al., 2011).

Another multicenter prospective trial of 4203 patients studied the use of WATS^{3D} as an adjunct to four-quadrant random forceps biopsy (FB) in detecting Barrett's esophagus (BE) and esophageal dysplasia (ED). FB alone detected 594 cases of BE, and the addition of WATS^{3D} detected an additional 493 cases, an increase of 83%. Likewise, WATS^{3D} detected an increase of 88.5% of low-grade dysplasia (LGD). The authors concluded that "Adjunctive use of WATS to FB significantly improves the detection of both BE and ED. Sampling effort, an inherent limitation associated with screening and surveillance, can be improved with WATS allowing better informed decisions to be made about the management and subsequent treatment of these patients" (Gross et al., 2018). These findings support the earlier study by Johanson and colleagues. In their study of 1266 patients being screened for BE and ED, they noted an overall increase of 39.8% in the detection of BE when WATS^{3D} (brush biopsy or BB) was used as an adjunct

to FB. They also report that the number of patients needed to test (NNT) to obtain a positive BE result was 8.7. Interestingly, specifically for patients with gastroesophageal reflux disease (GERD), the addition of WATS^{3D} resulted in an even higher increase in the detection of BE (by 70.5%) (Johanson et al., 2011).

Vennalaganti et al. (2018) published a randomized trial at 16 different medical centers (n = 160 patients) compared the order of testing (WATS^{3D} followed by biopsy sampling versus biopsy sampling followed by WATS^{3D}) to detect high-grade dysplasia/esophageal adenocarcinoma (HGD/EAC). The authors also stated secondary aims of determining the amount of additional time required for WATS^{3D} and the ability of each procedure to separately detect neoplasia. The order of the procedures was not statistically relevant. The use of WATS^{3D} as an adjunct to biopsy did result in a 14.4% absolute increase in the number of HGD/EAC cases detected. The authors noted that WATS^{3D}, on average, adds 4.5 minutes to the total procedure time. They conclude that "Results of this multicenter, prospective, randomized trial demonstrate that the use of WATS in a referral BE population increases the detection of HGD/EAC" (Vennalaganti et al., 2018).

Diehl et al. (2021) studied the impact of TissueCypher Barrett's esophagus (BE) assay on clinical decisions in the management of BE patients. TissueCypher was ordered for 60 patients with BE and the impact of the test was assessed. TissueCypher results impacted 55.0% of management decisions, resulting in either upstaging or downstaging of treatment. The authors note that "In 21.7% of patients, the test upstaged the management approach, resulting in endoscopic eradication therapy (Wechsler et al.) or shorter surveillance interval. The test downstaged the management approach in 33.4% of patients, leading to surveillance rather than EET. In the subset of patients whose management plan was changed, upstaging was associated with a high-risk TissueCypher result, and downstaging was associated with a low-risk result" (Diehl et al., 2021). The authors conclude that TissueCypher will help target EET for high risk patients and reduce unneeded procedures in low risk patients (Diehl et al., 2021).

Wechsler et al. (2021) studied the clinical utility of noninvasive biomarkers to identify EoE in children and predict esophageal eosinophilia. Blood/urine was collected from 183 children and several biomarkers were measured including Absolute eosinophil count (AEC), plasma eosinophil-derived neurotoxin (EDN), eosinophil cationic protein (ECP), major basic protein-1 (MBP-1), galectin-10 (CLC/GAL-10), Eotaxin-2 and Eotaxin-3, and urine osteopontin (OPN) and matrix metalloproteinase-9 (MMP-9). According to the results, all plasma and urine biomarkers were in increased in EoE. A panel that included all the other biomarkers was superior to measuring only AEC alone. AEC, CLC/GAL-10, ECP, and MBP-1 were significantly decreased in patients with esophageal eosinophil counts <15/hpf in response to treatment. AEC combined with MBP-1 best

predicted the esophageal eosinophil counts. The authors conclude that eosinophil-associated proteins along with AEC are superior to AEC alone in distinguishing EoE and predicting eosinophil counts (Wechsler et al., 2021).

VI. Guidelines and Recommendations

United European Gastroenterology (UEG), The European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), the European Academy of Allergy and Clinical Immunology (EAACI), and the European Society of Eosinophilic Oesophagitis (EUREOS)

These joint guidelines were published by a task force of 21 physicians and researchers for eosinophilic esophagitis (EoE). In it, they note that noninvasive biomarkers (inflammatory factors, total IgE, chemokines, tryptase, et al) are "not accurate" to diagnose or monitor EoE. They remark that absolute serum eosinophil count fared best in correlating with severity of disease but had a diagnostic accuracy of 0.754. The guidelines state that histology is necessary for monitoring. The String Test was also mentioned as having good preliminary results but required further corroboration (Lucendo et al., 2017).

American Gastroenterological Association (AGA) and the Joint Task Force on Allergy-Immunology Practice Parameters (JTF) guideline

Regarding allergy-based testing for the purpose of identifying food triggers in patients with Eosinophilic Esophagitis, the AGA/JTF suggest an allergy-based elimination diet over no treatment. The task force notes that "due to the potential limited accuracy of currently available, allergy-based testing for the identification of specific food triggers, patients may prefer alternative medical or dietary therapies to an exclusively testing-based elimination. An important limitation of the studies available so far "involves the degree of inconsistency due to different testing techniques (e.g., skin-prick testing, serum-specific IgE testing, patch testing, or combinations of these) used in different studies." Additionally, a "sensitivity analysis failed to show any statistically significant difference between studies that used patch testing and those that did not" (Hirano et al., 2020).

Updated International Consensus Diagnostic Criteria for Eosinophilic Esophagitis: Proceedings of the AGREE Conference

These newly published international diagnostic criteria primarily include endoscopic findings. Although the guidelines emphasize ruling out other diagnoses (in which biomarkers may be useful), it does not mention any serum or genetic factors for EoE itself (Dellon et al., 2018).

National Comprehensive Cancer Network (NCCN)

The NCCN notes four syndromes that predispose to an increased risk for esophageal and esophagogastric junction (EGJ) cancers; tylosis with non-epidermolytic palmoplantar keratoderma (PPK) with esophageal cancer (including Howel-Evans syndrome), familial Barrett esophagus (FBE), Bloom Syndrome (BS, *BLM* gene*)*, and Fanconi Anemia (FA, *FANC A-E genes*). The *RHBDF2* gene has been associated with tylosis (with non-epidermolytic palmoplantar keratosis) for genetic risk assessment.

Though FBE may be associated with "one or more autosomally inherited dominant susceptibility alleles," no gene has been validated. With regards to next-generation sequencing, the NCCN concludes that "when limited tissue is available for testing, or the patient is unable to undergo a traditional biopsy, sequential testing of single biomarkers or use of limited molecular diagnostic panels may quickly exhaust the sample. In these scenarios, comprehensive genomic profiling via a validated NGS assay performed in a CLIA-approved laboratory may be used for the identification of *HER2* amplification, MSI [microsatellite instability], MMR deficiency, TMB, *NTRK* gene fusions, *RET* gene fusions, and *BRAF v600E* mutations. The use of IHC/ISH/targeted PCR should be considered first followed by NGS testing when appropriate"(NCCN, 2024).

Under microsatellite instability (MSI) and mismatch repair testing, the NCCN recommends "universal testing for MSI by polymerase chain reaction (PCR), NGS, or MMR by IHC should be performed for all newly diagnosed esophageal and EGJ cancers."

For squamous cell carcinoma, the NCCN recommends performing universal testing for microsatellite instability (MSI) by PCR/NGS or MMR by IHC in all newly diagnosed patients. It is also recommended that PD-L1 testing (if not done previously) if advanced/metastatic cancer is suspected. They also note that NGS may be considered via validated assay (NCCN, 2024).

Liquid biopsy aids in identifying genetic mutations in solid cancers by looking at circulating tumor DNA (ctDNA) in blood and can be used in those with advanced disease who cannot undergo clinical biopsies for disease surveillance and management. Detecting mutations in DNA from esophageal and EGJ carcinomas "can identify targetable alterations or the evolution of clones with altered treatment response profiles." The NCCN has also stated that "a negative result should be interpreted with caution, as this does not exclude the presence of tumor mutations or amplifications" (NCCN, 2024).

The NCCN notes that "testing for MSI by PCR/NGS or MMR [mismatch repair] by IHC should be considered on locally advanced, recurrent, or metastatic esophageal and EGJ

cancers in patients who are candidates for treatment with programmed cell death protein 1 (PD-1) inhibitors."

The NCCN also identifies several targeted therapeutic agents currently approved by the FDA: trastuzumab, pembrolizumab/nivolumab, and entrectinib/larotrectinib. Trastuzumab is based on *HER2* overexpression (NCCN notes that an approved biologic or biosimilar drug to trastuzumab is also appropriate for use) and pembrolizumab can also be added to the regimen for treating *HER2*, notwithstanding any contraindications.

Treatment with trastuzumab is based on testing for *HER2* expression. Treatment with pembrolizumab or nivolumab is based on "testing for MSI by PCR/NGS or MMR by IHC, PD-L1 expression by IHC, or high TMB by NGS." Select TRK inhibitors have also been FDA-approved for *NTRK* gene fusion-positive tumors. Additionally, selpercatinib can be used therapeutically for *RET* gene-related tumors and dabrafenib/trametinib for tumors with *BRAF V600E* mutations (NCCN, 2024).

Genetic biomarkers such as aneuploidy and loss of p53 heterozygosity have been proposed as useful for identifying increased risk of progression in BE patients, but the NCCN remarks that these biomarkers require "further prospective evaluation as predictors of risk for the development of HGD [high-grade dysplasia] and adenocarcinoma of the esophagus in patients with Barrett esophagus" (NCCN, 2024).

The NCCN notes that wide-area transepithelial sampling (WATS) has been used to detect esophageal carcinomas in BE patients. They state, "The use of wide-area transepithelial sampling with computer-assisted 3-dimensional analysis (WATS3D), a relatively new sampling technique combining an abrasive brush biopsy of the Barrett esophagus mucosa with computer-assisted pathology analysis to highlight abnormal cells, may help increase the detection of esophageal dysplasia in patients with Barrett esophagus." They go on to cite the 2017 study by Vennalaganti and colleagues that shows a 14.4% increase in the number of additional cases of HGD/esophageal adenocarcinoma captured by using WATS. However, the NCCN remarks that the "utility and accuracy of WATS for detecting HGD/adenocarcinoma in patients with Barrett esophagus needs to be evaluated in larger phase III randomized trials" (NCCN, 2024).

American Society for Gastrointestinal Endoscopy (ASGE)

The ASGE recommends the use of WATS3D as an adjunct to "Seattle protocol biopsy sampling" in patients with known or suspected BE (conditional recommendation, low quality of evidence). The society stated that they had downrated the certainty of the recommendation due to possible risk bios, insistency, and indirectness of the studies that were available at the time of publication since some of the studies had included

LGD (whereas others had not) and many of the studies had been sponsored by the test's manufacturer. The society also had noted that, as of the date of publication, no studies addressing the cost-effectiveness of WATS-3D had been published. (Qumseya et al., 2019) It should be noted that since the publication of these guidelines the 2020 cost-effectiveness study by Singer and Smith (2020) has been published.

Society of American Gastrointestinal and Endoscopic Surgeons (SAGES) Technology and Value Assessment Committee (TAVAC)

The TAVAC of SAGES evaluated WATS^{3D} and published their findings and recommendations within the journal *Surgical Endoscopy* in 2020. They note that WATS^{3D} is not recommended "as a stand-alone substitute for cold forcep biopsies." Within their expert panel recommendation section:

- They state that no significant morbidity or mortality is associated with the testing.
- They also state that "WATS^{3D} increases diagnostic yield by 38 – 150% for Barrett's Esophagus, by 40 – 150% for Low Grade Dysplasia; and by 420% for High Grade Dysplasia; when compared to forceps biopsy alone."
- WATS^{3D} testing also "has very high inter-observer agreement for the pathological diagnosis of non-dysplastic and dysplastic Barrett's Esophagus."

Regarding value, "Increased detection of pre-malignant diseases of the esophagus by the adjunctive use of WATS^{3D} supports screening and surveillance by the adjunctive use of WATS^{3D} during upper endoscopy in appropriate patients" (Docimo et al., 2020).

American Foregut Society (AFS)

The AFS published a white paper reviewing WATS^{3D} in 2020. After reviewing the literature, they state, "The American Foregut Society (AFS) Board has concluded that there are sufficient data to support the routine use of WATS^{3D} technology in the diagnosis and ongoing evaluation of Barrett's esophagus" (AFS, 2021).

American College of Gastroenterology (ACG)

In 2022, the ACG updated the Barrett's Esophagus guideline and offered recommendations for the diagnosis, screening, surveillance, and endoscopic and medical therapy of BE. No recommendations were made regarding chemoprevention or use of "biomarkers" in routine practice due to "insufficient data."

Studies do suggest that "biomarkers may be better than routine histology alone" in helping to predict the progression of cancer. However, the ACG notes that no single prediction tool or panel to predict disease progression has been established as having

clear clinical utility. There have not been sufficient studies to evaluate the combination of clinical and biomarker variables.

The ACG could not recommend "routine use of p53 IHC or TissueCypher for risk stratification in patients with BE undergoing surveillance" due to unclear clinical validity (Shaheen et al., 2022).

However, the panel did not completely dissuade providers from the use of biomarkers under certain conditions since the predictive performance "has been shown to be better in some cases than the histologic diagnosis." In the future, and with more clinical studies, this may mean that biomarkers could have predictive value in a subset of patients with BE without dysplasia (Shaheen et al., 2022).

European Society for Medical Oncology (ESMO)

No form of molecular testing for diagnosis or risk assessment of esophageal cancer is mentioned in the ESMO 2022 guideline (Obermannová et al., 2022). The 2022 guideline does include a supplementary table listing biomarkers and molecular targets for precision medicines and corresponding scores for outcomes (ESMO, 2022):

Biomarker or genomic alteration	**Method of detection**	**Drug match**	**ESCAT score**
HER2	IHC for *HER2* protein expression or ISH for *HER2* gene amplification	Anti-*HER2* antibodies (e.g. trastuzumab)	I-A (alteration-drug match is associated with improved outcome with evidence from randomised clinical trials showing the alteration-drug match in a specific tumour type results in a clinically meaningful improvement of a survival end point)

PD-L1	Combined Positive Score (CPS) or Tumour Positive Score (TPS)	PD-1 inhibitors (e.g. pembrolizumab, nivolumab)	
MSI	High Microsatellite Instability (MSI-H)	PD-1 inhibitors (e.g. nivolumab, pembrolizumab)	I-C (alteration-drug match is associated with improved outcome with evidence from clinical trials across tumour types or basket clinical trials showing clinical benefit associated with the alteration-drug match, with similar benefit observed across tumour types)

(Obermannová et al., 2022)

Pan-Asian adapted ESMO Clinical Practice Guidelines: a JSMO-ESMO initiative endorsed by CSCO, KSMO, MOS, SSO and TOS

The only biomarker mentioned in these guidelines is *HER2*; intended "to select patients with metastatic esophageal adenocarcinoma for treatment with…trastuzumab." The guidelines go on to state that evidence for the role of other biomarkers or agents is "limited" (Muro et al., 2019).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	CPT Description
81194	NTRK (neurotrophic receptor tyrosine kinase 1, 2, and 3) (eg, solid tumors) translocation analysis
81301	Microsatellite instability analysis (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) of markers for mismatch repair deficiency (eg, BAT25, BAT26), includes comparison of neoplastic and normal tissue, if performed
81404	Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-**10** exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-**10** exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of **11**-**25** exons, regionally targeted cytogenomic array analysis)
81406	Molecular pathology procedure, Level 7 (eg, analysis of **11**-**25** exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of **26**-**50** exons)
81479	Unlisted molecular pathology procedure
88104	Cytopathology, fluids, washings or brushings, except cervical or vaginal; smears with interpretation
88112	Cytopathology, selective cellular enhancement technique with interpretation (eg, liquid based slide preparation method), except cervical or vaginal
88160	Cytopathology, smears, any other source; screening and interpretation
88271	Molecular cytogenetics; DNA probe, each (eg, FISH)
88272	Molecular cytogenetics; chromosomal in situ hybridization, analyze 3-5 cells (eg, for derivatives and markers)
88273	Molecular cytogenetics; chromosomal in situ hybridization, analyze 10-30 cells (eg, for microdeletions)
88274	Molecular cytogenetics; interphase in situ hybridization, analyze 25-99 cells

CPT	CPT Description
88275	Molecular cytogenetics; interphase in situ hybridization, analyze 100-300 cells
88305	Level IV - Surgical pathology, gross and microscopic examination
88312	Special stain including interpretation and report; Group I for microorganisms (eg, acid fast, methenamine silver)
88341	Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)
88342	Immunohistochemistry or immunocytochemistry, per specimen; initial single antibody stain procedure
88344	Immunohistochemistry or immunocytochemistry, per specimen; each multiplex antibody stain procedure
88360	Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, per specimen, each single antibody stain procedure; manual
88361	Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, per specimen, each single antibody stain procedure; using computer-assisted technology
88367	Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), using computer-assisted technology, per specimen; initial single probe stain procedure
88368	Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), manual, per specimen; initial single probe stain procedure
88369	Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), manual, per specimen; each additional single probe stain procedure (List separately in addition to code for primary procedure)
88373	Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), using computer-assisted technology, per specimen; each additional single probe stain procedure (List separately in addition to code for primary procedure)
88374	Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), using computer-assisted technology, per specimen; each multiplex probe stain procedure
88377	Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), manual, per specimen; each multiplex probe stain procedure
0095U	Inflammation (eosinophilic esophagitis), ELISA analysis of eotaxin-3 (CCL26 [C-C motif chemokine ligand 26]) and major basic protein (PRG2

CPT	CPT Description
	[proteoglycan 2, pro eosinophil major basic protein]), specimen obtained by swallowed nylon string, algorithm reported as predictive probability index for active eosinophilic esophagitis Proprietary test: Esophageal String Test™ (EST) Lab/Manufacturer: Cambridge Biomedical, Inc.
0108U	Gastroenterology (Barrett's esophagus), whole slide–digital imaging, including morphometric analysis, computer-assisted quantitative immunolabeling of 9 protein biomarkers (p16, AMACR, p53, CD68, COX-2, CD45RO, HIF1a, HER-2, K20) and morphology, formalin-fixed paraffin-embedded tissue, algorithm reported as risk of progression to high-grade dysplasia or cancer Proprietary test: TissueCypher® Barrett's Esophagus Assay Lab/Manufacturer: Cernostics
0114U	Gastroenterology (Barrett's esophagus), VIM and CCNA1 methylation analysis, esophageal cells, algorithm reported as likelihood for Barrett's esophagus Proprietary test: EsoGuard™ Lab/Manufacturer: Lucid Diagnostics
0398U	Gastroenterology (Barrett esophagus), P16, RUNX3, HPP1, and FBN1 DNA methylation analysis using PCR, formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as risk score for progression to high-grade dysplasia or cancer Proprietary test: ESOPREDICT® Barrett's Esophagus Risk Classifier Assay Lab/Manufacturer: Capsulomics, Inc d/b/a Previse

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Ackerman, S. J., Kagalwalla, A. F., Hirano, I., Gonsalves, N., Katcher, P. M., Gupta, S., Wechsler, J. B., Grozdanovic, M., Pan, Z., Masterson, J. C., Du, J., Fantus, R. J., Alumkal, P., Lee, J. J., Ochkur, S., Ahmed, F., Capocelli, K., Melin-Aldana, H., Biette, K., . . . Furuta, G. T. (2019). One-Hour Esophageal String Test: A Nonendoscopic Minimally Invasive Test That Accurately Detects Disease Activity in Eosinophilic Esophagitis. *Am J Gastroenterol*, *114*(10), 1614-1625. https://doi.org/10.14309/ajg.0000000000000371

AFS. (2021). *Wide Area Transepithelial Sampling with Computer Assisted 3D Analysis (WATS3D)*. American Foregut Society. Retrieved 07/31/2020 from https://pubmed.ncbi.nlm.nih.gov/32578042/

Anandasabapathy, S., Sontag, S., Graham, D. Y., Frist, S., Bratton, J., Harpaz, N., & Waye, J. D. (2011). Computer-assisted brush-biopsy analysis for the detection of dysplasia in a high-risk Barrett's esophagus surveillance population. *Dig Dis Sci*, *56*(3), 761-766. https://doi.org/10.1007/s10620-010-1459-z

Bonis, P. A. L., & Gupta, S. K. (2023). *Clinical manifestations and diagnosis of eosinophilic esophagitis*. https://www.uptodate.com/contents/clinical-manifestations-and-diagnosis-of-eosinophilic-esophagitis

Castle Biosciences. (2023). *What is the TissueCypher® Barrett's Esophagus Assay?* http://www.cernostics.com/products/

Costa-Barbosa, F. A., Balasubramanian, R., Keefe, K. W., Shaw, N. D., Al-Tassan, N., Plummer, L., Dwyer, A. A., Buck, C. L., Choi, J. H., Seminara, S. B., Quinton, R., Monies, D., Meyer, B., Hall, J. E., Pitteloud, N., & Crowley, W. F., Jr. (2013). Prioritizing genetic testing in patients with Kallmann syndrome using clinical phenotypes. *J Clin Endocrinol Metab*, *98*(5), E943-953. https://doi.org/10.1210/jc.2012-4116

Critchley-Thorne, R. J., Duits, L. C., Prichard, J. W., Davison, J. M., Jobe, B. A., Campbell, B. B., Zhang, Y., Repa, K. A., Reese, L. M., Li, J., Diehl, D. L., Jhala, N. C., Ginsberg, G., DeMarshall, M., Foxwell, T., Zaidi, A. H., Lansing Taylor, D., Rustgi, A. K., Bergman, J. J., & Falk, G. W. (2016). A Tissue Systems Pathology Assay for High-Risk Barrett's Esophagus. *Cancer Epidemiol Biomarkers Prev*, *25*(6), 958-968. https://doi.org/10.1158/1055-9965.Epi-15-1164

Dellon, E. S., Liacouras, C. A., Molina-Infante, J., Furuta, G. T., Spergel, J. M., Zevit, N., Spechler, S. J., Attwood, S. E., Straumann, A., Aceves, S. S., Alexander, J. A., Atkins, D., Arva, N. C., Blanchard, C., Bonis, P. A., Book, W. M., Capocelli, K. E., Chehade, M., Cheng, E., . . . Bredenoord, A. J. (2018). Updated International Consensus Diagnostic Criteria for Eosinophilic Esophagitis: Proceedings of the AGREE Conference. *Gastroenterology*, *155*(4), 1022-1033.e1010. https://doi.org/10.1053/j.gastro.2018.07.009

Diagnostics, C. (2024). *WATS3D empowers physicians to preempt cancer.* Retrieved 1/16/2024 from https://www.cdxdiagnostics.com/wats3d

Diehl, D. L., Khara, H. S., Akhtar, N., & Critchley-Thorne, R. J. (2021). TissueCypher Barrett's esophagus assay impacts clinical decisions in the management of patients with Barrett's esophagus. *Endosc Int Open*, *9*(3), E348-e355. https://doi.org/10.1055/a-1326-1533

Docimo, S., Jr., Al-Mansour, M., & Tsuda, S. (2020). SAGES TAVAC safety and efficacy analysis WATS(3D) (CDx Diagnostics, Suffern, NY). *Surg Endosc*. https://doi.org/10.1007/s00464-020-07503-w

Eluri, S., Klaver, E., Duits, L. C., Jackson, S. A., Bergman, J. J., & Shaheen, N. J. (2018). Validation of a biomarker panel in Barrett's esophagus to predict progression to esophageal adenocarcinoma. *Dis Esophagus*, *31*(11). https://doi.org/10.1093/dote/doy026

EnteroTrack. (2023). *The EnteroTracker®*. https://enterotrack.com/our-technology

ESMO. (2022). Oesophageal cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. SUPPLEMENTARY MATERIAL. https://www.annalsofoncology.org/cms/10.1016/j.annonc.2022.07.003/attachment/ec23d13e-fa60-45bc-a5fb-125278bccb51/mmc1.pdf

Frankell, A. M., Jammula, S., Li, X., Contino, G., Killcoyne, S., Abbas, S., Perner, J., Bower, L., Devonshire, G., Ococks, E., Grehan, N., Mok, J., O'Donovan, M., MacRae, S., Eldridge, M. D., Tavaré, S., & Fitzgerald, R. C. (2019). The landscape of selection in 551 esophageal adenocarcinomas defines genomic biomarkers for the clinic. *Nat Genet*, *51*(3), 506-516. https://doi.org/10.1038/s41588-018-0331-5

Gibson, M. K. (2023, February 27, 2024). *Epidemiology and risk factors for esophageal cancer*. UpToDate. https://www.uptodate.com/contents/epidemiology-and-risk-factors-for-esophageal-cancer

Gonzaga, I. M., Soares Lima, S. C., Nicolau, M. C., Nicolau-Neto, P., da Costa, N. M., de Almeida Simao, T., Hernandez-Vargas, H., Herceg, Z., & Ribeiro Pinto, L. F. (2017). TFF1 hypermethylation and decreased expression in esophageal squamous cell carcinoma and histologically normal tumor surrounding esophageal cells. *Clin Epigenetics*, *9*, 130. https://doi.org/10.1186/s13148-017-0429-0

Gross, S. A., Smith, M. S., & Kaul, V. (2018). Increased detection of Barrett's esophagus and esophageal dysplasia with adjunctive use of wide-area transepithelial sample with three-dimensional computer-assisted analysis (WATS). *United European Gastroenterol J*, *6*(4), 529-535. https://doi.org/10.1177/2050640617746298

Hao, J., Critchley-Thorne, R., Diehl, D. L., & Snyder, S. R. (2019). A Cost-Effectiveness Analysis Of An Adenocarcinoma Risk Prediction Multi-Biomarker Assay For Patients With Barrett's Esophagus. *Clinicoecon Outcomes Res*, *11*, 623-635. https://doi.org/10.2147/ceor.S221741

Interpace. (2023). *An Innovative Diagnostic Tool for Barrett's Esophagus Patients*. https://barregen.com/

Johanson, J. F., Frakes, J., & Eisen, D. (2011). Computer-assisted analysis of abrasive transepithelial brush biopsies increases the effectiveness of esophageal screening: a multicenter prospective clinical trial by the EndoCDx Collaborative Group. *Dig Dis Sci*, *56*(3), 767-772. https://doi.org/10.1007/s10620-010-1497-6

Li, J., Qi, Z., Hu, Y. P., & Wang, Y. X. (2019). Possible biomarkers for predicting lymph node metastasis of esophageal squamous cell carcinoma: a review. *J Int Med Res*, *47*(2), 544-556. https://doi.org/10.1177/0300060518819606

Lucendo, A. J., Molina-Infante, J., Arias, A., von Arnim, U., Bredenoord, A. J., Bussmann, C., Amil Dias, J., Bove, M., Gonzalez-Cervera, J., Larsson, H., Miehlke, S., Papadopoulou, A., Rodriguez-Sanchez, J., Ravelli, A., Ronkainen, J., Santander, C., Schoepfer, A. M., Storr, M. A., Terreehorst, I., . . . Attwood, S. E. (2017). Guidelines on eosinophilic esophagitis: evidence-based statements and recommendations for diagnosis and management in children and adults. *United European Gastroenterol J*, *5*(3), 335-358. https://doi.org/10.1177/2050640616689525

Lucid Diagnostics. (2024). Esoguard. https://www.luciddx.com/esoguard

McLaren, P. J., Barnes, A. P., Terrell, W. Z., Vaccaro, G. M., Wiedrick, J., Hunter, J. G., & Dolan, J. P. (2017). Specific gene expression profiles are associated with a pathologic complete response to neoadjuvant therapy in esophageal adenocarcinoma. *Am J Surg*, *213*(5), 915-920. https://doi.org/10.1016/j.amjsurg.2017.03.024

Moinova, H. R., LaFramboise, T., Lutterbaugh, J. D., Chandar, A. K., Dumot, J., Faulx, A., Brock, W., De la Cruz Cabrera, O., Guda, K., Barnholtz-Sloan, J. S., Iyer, P. G., Canto, M. I., Wang, J. S., Shaheen, N. J., Thota, P. N., Willis, J. E., Chak, A., & Markowitz, S. D. (2018). Identifying DNA methylation biomarkers for non-endoscopic detection of Barrett's esophagus. *Sci Transl Med*, *10*(424). https://doi.org/10.1126/scitranslmed.aao5848

Muro, K., Lordick, F., Tsushima, T., Pentheroudakis, G., Baba, E., Lu, Z., Cho, B. C., Nor, I. M., Ng, M., Chen, L. T., Kato, K., Li, J., Ryu, M. H., Zamaniah, W. I. W., Yong, W. P., Yeh, K. H., Nakajima, T. E., Shitara, K., Kawakami, H., . . . Douillard, J. Y. (2019). Pan-Asian adapted ESMO Clinical Practice Guidelines for the management of patients with metastatic oesophageal cancer: a JSMO-ESMO initiative endorsed by CSCO, KSMO, MOS, SSO and TOS. *Ann Oncol*, *30*(1), 34-43. https://doi.org/10.1093/annonc/mdy498

NCCN. (2024, 01/26/2024). *Esophageal and Esophagogastric Junction Cancers*. https://www.nccn.org/professionals/physician_gls/pdf/esophageal.pdf

Obermannová, R., Alsina, M., Cervantes, A., Leong, T., Lordick, F., Nilsson, M., van Grieken, N. C. T., Vogel, A., & Smyth, E. C. (2022). Oesophageal cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up[☆]. *Annals of Oncology*, *33*(10), 992-1004. https://doi.org/10.1016/j.annonc.2022.07.003

Plum, P. S., Gebauer, F., Krämer, M., Alakus, H., Berlth, F., Chon, S. H., Schiffmann, L., Zander, T., Büttner, R., Hölscher, A. H., Bruns, C. J., Quaas, A., & Loeser, H. (2019). HER2/neu (ERBB2) expression and gene amplification correlates with better survival in esophageal adenocarcinoma. *BMC Cancer*, *19*(1), 38. https://doi.org/10.1186/s12885-018-5242-4

Qumseya, B., Sultan, S., Bain, P., Jamil, L., Jacobson, B., Anandasabapathy, S., Agrawal, D., Buxbaum, J. L., Fishman, D. S., Gurudu, S. R., Jue, T. L., Kripalani, S., Lee, J. K., Khashab, M. A., Naveed, M., Thosani, N. C., Yang, J., DeWitt, J., & Wani, S. (2019). ASGE

guideline on screening and surveillance of Barrett's esophagus. *Gastrointestinal Endoscopy*, *90*(3), 335-359.e332. https://doi.org/10.1016/j.gie.2019.05.012
Rothenberg, M. E. (2023). *Eosinophilic esophagitis (EoE): Genetics and immunopathogenesis*. https://www.uptodate.com/contents/eosinophilic-esophagitis-eoe-genetics-and-immunopathogenesis
Saltzman, J. R., & Gibson, M. K. (2023). *Clinical manifestations, diagnosis, and staging of esophageal cancer*. https://www.uptodate.com/contents/clinical-manifestations-diagnosis-and-staging-of-esophageal-cancer
Shaheen, N. J., Falk, G. W., Iyer, P. G., Souza, R. F., Yadlapati, R. H., Sauer, B. G., & Wani, S. (2022). Diagnosis and Management of Barrett's Esophagus: An Updated ACG Guideline. *Am J Gastroenterol*, *117*(4), 559-587. https://doi.org/10.14309/ajg.0000000000001680
Sherrill, J. D., & Rothenberg, M. E. (2014). Genetic and epigenetic underpinnings of eosinophilic esophagitis. *Gastroenterol Clin North Am*, *43*(2), 269-280. https://doi.org/10.1016/j.gtc.2014.02.003
Shoda, T., Wen, T., Aceves, S. S., Abonia, J. P., Atkins, D., Bonis, P. A., Caldwell, J. M., Capocelli, K. E., Carpenter, C. L., Collins, M. H., Dellon, E. S., Eby, M. D., Gonsalves, N., Gupta, S. K., Falk, G. W., Hirano, I., Menard-Katcher, P., Kuhl, J. T., Krischer, J. P., . . . Rothenberg, M. E. (2018). Eosinophilic oesophagitis endotype classification by molecular, clinical, and histopathological analyses: a cross-sectional study. *Lancet Gastroenterol Hepatol*, *3*(7), 477-488. https://doi.org/10.1016/s2468-1253(18)30096-7
Singer, M. E., & Smith, M. S. (2020). Wide Area Transepithelial Sampling with Computer-Assisted Analysis (WATS(3D)) Is Cost-Effective in Barrett's Esophagus Screening. *Dig Dis Sci*. https://doi.org/10.1007/s10620-020-06412-1
Smith, M. S., Ikonomi, E., Bhuta, R., Iorio, N., Kataria, R. D., Kaul, V., & Gross, S. A. (2019). Wide-area transepithelial sampling with computer-assisted 3-dimensional analysis (WATS) markedly improves detection of esophageal dysplasia and Barrett's esophagus: analysis from a prospective multicenter community-based study. *Dis Esophagus*, *32*(3). https://doi.org/10.1093/dote/doy099
Spechler, S. (2022, September 7). *Barrett's esophagus: Epidemiology, clinical manifestations, and diagnosis*. https://www.uptodate.com/contents/barretts-esophagus-epidemiology-clinical-manifestations-and-diagnosis
Testa, U., Castelli, G., & Pelosi, E. (2017). Esophageal Cancer: Genomic and Molecular Characterization, Stem Cell Compartment and Clonal Evolution. *Medicines (Basel)*, *4*(3). https://doi.org/10.3390/medicines4030067
Trindade, A. J., McKinley, M. J., Alshelleh, M., Levi, G., Stewart, M., Quinn, K. J., & Thomas, R. M. (2019). Mutational load may predict risk of progression in patients with Barrett's oesophagus and indefinite for dysplasia: a pilot study. *BMJ Open Gastroenterology*, *6*(1), e000268. https://doi.org/10.1136/bmjgast-2018-000268

Vennalaganti, P. R., Kaul, V., Wang, K. K., Falk, G. W., Shaheen, N. J., Infantolino, A., Johnson, D. A., Eisen, G., Gerson, L. B., Smith, M. S., Iyer, P. G., Lightdale, C. J., Schnoll-Sussman, F., Gupta, N., Gross, S. A., Abrams, J., Haber, G. B., Chuttani, R., Pleskow, D. K., . . . Sharma, P. (2018). Increased detection of Barrett's esophagus-associated neoplasia using wide-area trans-epithelial sampling: a multicenter, prospective, randomized trial. *Gastrointest Endosc*, *87*(2), 348-355. https://doi.org/10.1016/j.gie.2017.07.039

Visaggi, P., Barberio, B., Ghisa, M., Ribolsi, M., Savarino, V., Fassan, M., Valmasoni, M., Marchi, S., de Bortoli, N., & Savarino, E. (2021). Modern Diagnosis of Early Esophageal Cancer: From Blood Biomarkers to Advanced Endoscopy and Artificial Intelligence. *Cancers (Basel)*, *13*(13). https://doi.org/10.3390/cancers13133162

Visser, E., Franken, I. A., Brosens, L. A., Ruurda, J. P., & van Hillegersberg, R. (2017). Prognostic gene expression profiling in esophageal cancer: a systematic review. *Oncotarget*, *8*(3), 5566-5577. https://doi.org/10.18632/oncotarget.13328

Vollmer, R. T. (2019). A review of the incidence of adenocarcinoma detected during surveillance for Barrett's esophagus. *Hum Pathol*, *84*, 150-154. https://doi.org/10.1016/j.humpath.2018.09.016

Wechsler, J. B., Ackerman, S. J., Chehade, M., Amsden, K., Riffle, M. E., Wang, M.-Y., Du, J., Kleinjan, M. L., Alumkal, P., Gray, E., Kim, K.-Y. A., Wershil, B. K., & Kagalwalla, A. F. (2021). Noninvasive biomarkers identify eosinophilic esophagitis: A prospective longitudinal study in children. *Allergy*, *76*(12), 3755-3765. https://doi.org/10.1111/all.14874

Wen, T., Stucke, E. M., Grotjan, T. M., Kemme, K. A., Abonia, J. P., Putnam, P. E., Franciosi, J. P., Garza, J. M., Kaul, A., King, E. C., Collins, M. H., Kushner, J. P., & Rothenberg, M. E. (2013). Molecular diagnosis of eosinophilic esophagitis by gene expression profiling. *Gastroenterology*, *145*(6), 1289-1299. https://doi.org/10.1053/j.gastro.2013.08.046

Zhang, X., Patil, D., Odze, R. D., Zhao, L., Lisovsky, M., Guindi, M., Riddell, R., Bellizzi, A., Yantiss, R. K., Nalbantoglu, I., & Appelman, H. D. (2020). The microscopic anatomy of the esophagus including the individual layers, specialized tissues, and unique components and their responses to injury. *Ann N Y Acad Sci*, *1434*(1), 304-318. https://doi.org/10.1111/nyas.13705

X. Revision History

Revision Date	Summary of Changes

Fibromyalgia Testing

Policy Number: AHS –M2177 – Fibromyalgia Testing	Prior Policy Name and Number, as applicable:
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Fibromyalgia is a chronic diffuse pain condition and the most common cause of chronic widespread musculoskeletal pain (Goldenberg, 2023a). It is also known by "diffuse myofascial pain syndrome," "fibromyalgia-fibromyositis syndrome," or "fibromyalgia syndrome" (Ada, 2022). FM has The etiology and pathophysiology of FM is generally unknown, but it can be found with "fatigue, cognitive disturbance psychiatric symptoms, and multiple somatic symptoms;" however, presentation may vary from patient to patient (Goldenberg, 2023a; Kimura & Walco, 2023). Controversy has arisen regarding the disease origin, as it has been considered to be psychogenic or psychosomatic, but recent research indicates it as a disorder of pain regulation, a form of "central sensitization" (Goldenberg, 2023a).

Terms such as male and female are used when necessary to refer to sex assigned at birth.

II. Related Policies

Policy Number	Policy Title
AHS-G2155	General Inflammation Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

1) For the diagnosis of fibromyalgia or chronic pain syndromes, the following tests **DO NOT MEET COVERAGE CRITERIA**:
   a) Genetic testing (e.g., mutation analysis, copy number variants, epigenetic analysis, mRNA expression, miRNA expression).
   b) Biomarker panel testing with proprietary algorithms and/or index scores (e.g., FM/a®, NutrEval FMV®).

IV. Table of Terminology

Term	Definition
5HT	Serotonin
5-HT2A	Serotonin 2A
A1AT	Alpha-1 antitrypsin
AAPT	American Pain Society Pain Taxonomy
ACE I/D	*Angiotensin-converting enzyme I/D polymorphism*
ACR	American College of Rheumatology
ACTTION	Analgesic, Anesthetic, and Addiction Clinical Trial Translations Innovations Opportunities and Networks
ADRA1A	*Adrenoreceptor alpha 1A gene*
ADRB2	*Adrenoreceptor beta 2 gene*
ADRB3	*Adrenoreceptor beta 3 gene*
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANS	Autonomic nervous system
APS	American Pain Society
BDNF	*Brain derived neurotrophic factor gene*
CB-1	Cannabinoid receptor type 1
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers for Medicare and Medicaid Services
CNR1	*Cannabinoid receptor 1 gene*
CNS	Central nervous system
CNV	Copy number variant
COMT	*Catecholamine o-methyl transferase gene*
CRA	Canadian Rheumatology Association
CRP	C-reactive protein
CSF	Cerebrospinal fluid

CWP	Chronic widespread pain
DNA	Deoxyribonucleic acid
DRD3	*Dopamine receptor D3 gene*
EDN1	*Endothelin 1 gene*
ESR	Erythrocyte sedimentation rate
FDA	Food and Drug Administration
FIQ	Fibromyalgia-Impact Questionnaire
FM	Fibromyalgia
GABRB3	*Gamma-aminobutyric acid receptor subunit beta-3 gene*
GBP1	*Guanylate binding protein 1 gene*
GCH1	*Guanosine triphosphate (GTP) cyclohydrolase 1 gene*
GERD	Gastroesophageal reflux disease
GRIA4	*Glutamate ionotropic receptor AMPA type subunit 4 gene*
HLA-DRB1	*Human leukocyte antigen DR beta 1 gene*
HTR2A	*5-Hydroxytryptamine Receptor 2A gene*
HTR3A	*5-Hydroxytryptamine Receptor 3A gene*
HTR3B	*5-Hydroxytryptamine Receptor 3B gene*
IBS	Irritable bowel syndrome
IFN-γ	Interferon-γ
IGHV1OR21-1	Immunoglobulin domain-containing like protein
IGLV3-25	Immunoglobulin lambda variable 3-25
IL-10	Interleukin-10
IL1RAP	Interleukin-1 receptor accessor protein
IL-4	*Interleukin-4 gene*
IL-5	Interleukin-5
IL-6	Interleukin-6
IL-8	Interleukin-8
LDTs	Laboratory-developed tests
MAOA	*Monoamine oxidase A gene*
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemoattractant protein-1
MEFV	*Mediterranean fever gene*
METTL18	Methyltransferase-like 18
MIP-1β	Macrophage inflammatory protein-1β
MSP	Multisite pain
MTHFR	*Methylenetetrahydrofolate reductase gene*
MYT1L	*Myelin transcription factor 1 like gene*
NA	Noradrenaline

NRXN3	*Neurexin 3 gene*
OPRM1	*Opioid receptor mu 1 gene*
PBMC	Plasma and peripheral blood mononuclear cells
PHA	Phytohemagglutinin
PMA	Phorbol-12-myristate-13-acetate
RA	Rheumatoid arthritis
RGS4	*Regulator of G protein signaling 4 gene*
SCL-90	Symptom Checklist-90 for Psychopathological Disorders
SCN9A	Sodium voltage-gated channel alpha subunit 9 gene
SLC64A4	*Solute carrier family 64 member 4 gene*
SERPINA1	*Serpin family A member 1 gene*
SLE	Systemic lupus erythematosus
SNPs	Single nucleotide polymorphisms
SS	Sjögren syndrome
TAAR1	*Trace amine-associated receptor 1 gene*
TACR1	*Tachykinin receptor 1 gene*
TMD	Temporomandibular joint disorder
TPC	Tender point count
TPQ	Tridimensional Personality Questionnaire
TRPV2	*Transient receptor potential vanilloid channel 2 gene*
TRPV3	*Transient receptor potential vanilloid channel 3 gene*
TSH	Thyroid stimulating hormone
TSPO	*Translocator protein gene*
WPI	Widespread pain index

V. Scientific Background

Fibromyalgia (FM) is a condition characterized by chronic and diffuse non-inflammatory musculoskeletal pain. FM is often accompanied by fatigue, cognitive disturbances (“fibro fog”), psychiatric symptoms, and multiple other nonspecific somatic symptoms (Galvez-Sánchez & Reyes Del Paso, 2020; Goldenberg, 2023a). Aside from the secondary symptoms, patients may at times experience abdominal and chest wall pain, irritable bowel syndrome (IBS), gastroesophageal reflux disease (GERD), and symptoms of autonomic nervous system (ANS) dysfunction. Despite this, FM is generally of unknown etiology and pathophysiology. However, FM should be considered as a diagnosis in patients experiencing idiopathic chronic pain for at least three months and is solely confirmed by a clinical, symptom-based assessment (D'Agnelli et al., 2019). What adds to the controversial and mysterious nature of the condition is that it depends on subjective symptoms. On a normal physical examination, patients often appear well and do not show abnormalities other than

"widespread soft tissue tenderness" with normal laboratory and radiologic studies, making it a common but elusive diagnosis. However, a history of other medical conditions that cause musculoskeletal pain can coexist with or mimic FM like rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and thyroid disease. Medical history can support a FM diagnosis (Goldenberg, 2023a). FM is more commonly diagnosed in women than men. The prevalence of FM in the United States and other countries is about two to three percent and incidence tends to increase with age (Bhargava & Hurley, 2020). FM can also be diagnosed in adolescents in the form of juvenile primary fibromyalgia, and it is confirmed mostly symptomatically but based on slightly different diagnostic criteria for research and epidemiological purposes (Kimura & Walco, 2023).

Fibromyalgia is categorized as a "disorder of pain regulation," under "central sensitization" (Goldenberg, 2023b). This finding is supported by biochemical abnormalities that are oftentimes present in FM patients: low concentrations of metabolites of serotonin (5HT) and noradrenaline (NA), and high concentrations of substance P and nerve growth factors (Julien et al., 2005). Patients are more sensitive to central nervous system inputs of pain, and those with FM at times can experience symptoms like those suffering from other central pain disorders, like migraines, IBS, and temporomandibular joint disorder (TMD) (Goldenberg, 2023b). Reduced 5HT and NA may actually account for the widespread pain symptoms by contributing to the dysfunction of endogenous systems that inhibit pain sensation (Julien et al., 2005).

Contrary to the established findings published about FM, recent research has demonstrated that there is a possible genetic basis to the disease progression; up to 50% of disease risk can be attributed to candidate genes (D'Agnelli et al., 2019). There also appears to be a strong familial predisposition to FM, although it is suspected to have a more polygenic mode of inheritance (Arnold et al., 2019; Buskila et al., 2007). However, there is no singular definitive gene that directly causes FM; several genes under consideration include *SLC64A4, TRPV2, MYT1L,* and *NRXN3* (D'Agnelli et al., 2019). Single nucleotide polymorphisms (SNPs) in the *SLC64A4* gene, which encodes a sodium-dependent serotonin transporter, has been associated with both FM and TMD. *SLC64A4* mutations that cause increased serotonin reuptake have been associated with high levels of depression and psychological disorders, as well as "SCL-90 [Symptom Checklist-90 for psychopathological disorders] scores for somatic awareness and anger, TPQ [tridimensional personality questionnaire] harm avoidance trait, increased salivary cortisol level, [and] increased leukocyte count" (D'Agnelli et al., 2019; Diatchenko et al., 2013). *TRPV2* (transient receptor potential vanilloid channel 2) gene is needed for not only cell cycle progression, growth, and differentiation of hematopoietic stem cells and innate immunity, but also the pain threshold due to its presence in the mechanoresponsive and thermo-responsive neurons in the dorsal root

and trigeminal ganglia (D'Agnelli et al., 2019; Mickle et al., 2015; Santoni et al., 2013). Genetic correlations in FM between mutations in *SLC6A4* and *TRPV2*, which encodes a calcium-permeable channel that is heat-activated and modulates many cellular functions, are further supported by linkage analysis done with FM to the chromosome 17p11.2-q11.2 region, which happens to house the two genes (Arnold et al., 2013; Kojima & Nagasawa, 2014).

Docampo et al. (2014) discovered that the rs11127292 polymorphism in the *MYT1L* (myelin transcription factor 1 like) gene, which is critical in the process of neuronal differentiation and has historically been associated with neuropsychiatric disorders, and an intronic CNV (copy number variant) in the *NRXN3* (neurexin 3) gene, which is involved in signal transmission by promoting synapse stability and function, affect the central nervous system (CNS) aspect of FM as well (Docampo et al., 2014).

Studies have also examined the differences in other allelic frequencies and influence of genetics on the sequelae of FM progression. Smith et al. (2012) identified significant variations in allelic frequency of *GABRB3* (rs4906902), *TAAR1* (rs8192619), and *GBP1* (rs7911) between FM patients and controls. *TAAR1* was demonstrated to alter dopamine bioavailability and function, which may cause the increased pain sensitivity seen in FM. *RGS4*, *CNR1*, and *GRIA*4 were implicated in other cohorts from the study by Smith et al. (2012), and are genes primarily involved in mechanisms of analgesia and central sensitivity as well. *RGS4*, which is expressed in the CNS regions like the dorsal horn of the spinal cord and the locus coeruleus, influences opioid receptor function when overexpressed. *CNR1*, which encodes the CB-1 cannabinoid receptor, has been indirectly utilized in FM treatment by the cannabinoid receptor agonist nabilone. Low *CNR1* expression and thus low CB-1 receptor activity may pose as a strong candidate for future testing, as several FM patients have increased circulating anandamide, an endocannabinoid, which has been hypothesized to underlie the pain in conditions like FM. Alterations to the *GRIA4* gene affect AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors, which play roles in the pain and comorbidities associated with FM (Smith et al., 2012).

Another gene commonly associated with FM is the *COMT* (catecholamine o-methyl transferase) gene. Though not directly affecting disease presentation, the *V158M* variant may contribute to subsequent depression, anxiety, and disability in many FM females (D'Agnelli et al., 2019). Mutations in the *COMT* gene are more closely linked to "pain catastrophizing, increased pain level during elevated pain attention, thermal and pressure sensitivity, psychological distress, increase number of tender points in TPC [tender point count], pain and positive affect interaction, and FIQ [fibromyalgia-impact questionnaire]-defined pain, fatigue, sleep, disturbance, morning stiffness, and disability" (Diatchenko et al., 2013). Similarly, mutations in the *HTR2A* (5-

hydroxytryptamine receptor 2A) gene, which encodes for its receptor, 5-HT2A, contributed to lower levels of total 5-HT in serum and CSF of FM patients (Park & Lee, 2017). The phenotype in FM more closely relates to psychological facets of FM – including reduced perception of environmental quality, "increased SCL-90-R total score and subscales scores for somatic awareness, anxiety, psychosis, obsessive-compulsive behavior, hostility, global severity index, interpersonal sensitivity, phobic anxiety, [and] depression" (Diatchenko et al., 2013).

Research has also demonstrated the role of epigenetics in FM phenotype and progression. Ciampi de Andrade et al. (2017) investigated DNA methylation states in samples obtained from FM cases and healthy controls, and found that the majority (1042 genes, 65%) of the differently methylated genes between the two were mostly hypomethylated. These genes were involved in "transduction and calcium signaling, MAPK signaling pathway, regulation of actin cytoskeleton endocytosis, and neuroactive ligand-receptor interaction pathways," and the sites that were identified were involved in DNA repair, immune system response and regulation, and membrane transport (D'Agnelli et al., 2019). Additionally, circulating miRNAs obtained from cerebrospinal fluid (CSF) and serum samples were found to be associated with many of the clinical symptoms of FM, including pain and fatigue, alterations in energy metabolism and growth, alterations in pain threshold, and sleep disturbance (Bjersing et al., 2015; Bjersing et al., 2013).

Proprietary Testing

The gradually progressing genetic and epigenetic findings for FM make biomarker testing attractive to researchers and physicians to solidify an alternative nonclinical diagnosis for this debilitating disease. The FM/a® Test takes advantage of the understanding of the immunological biomarkers and genetic components of FM to develop a blood test for diagnosis (FM/a, 2020). Biomarkers assayed through this test were based on a study on levels of cytokines interferon-γ (IFN-γ), interleukin-5 (IL-5), IL-6, IL-8, IL-10, macrophage inflammatory protein-1β (MIP-1β) , monocyte chemoattractant protein-1 (MCP-1), and MIP1-α obtained from samples of plasma and peripheral blood mononuclear cells (PBMC) with/without mitogenic activator treatment (Behm et al., 2012). Results identified that there was no statistically significant difference in these cytokines between FM samples and controls. However, when phytohemagglutinin (PHA), which induces PBMC proliferation and cytokine secretion, phorbol-12-myristate-13-acetate (PMA), and ionomycin, which "stimulate PBMCs and activate extracellular expressions of cytokines," were cultured with the blood samples, the concentrations of most cytokines in FM samples compared to normal samples were statistically significant, except for IL-5. This immunoassay indicated a connection to the coexisting diagnoses of FM with mental depression and

chronic pain, as both these populations tend to experience high pro-inflammatory cytokine levels as well (Behm et al., 2012).

Results supporting the FM/a® Test were further elucidated by Wallace et al. (2015), who looked into the cytokine and chemokine profiles in FM, rheumatoid arthritis, and systemic lupus erythematosus. Using the Luminex multiplex immunoassay bead array technology with mitogenic stimulation of PBMCs, cytokine concentrations for IL-6, IL-8, MIP-a, and MIP-b were recorded. Measures of analytical validity were done using a cytokine/chemokine composite test score. This measurement with all the cytokines/chemokines altogether was considered to have a sensitivity of 93%, a specificity of 89.4%, positive predictive value of 92%, and negative predictive value of 91% for the diagnosis of FM (Wallace et al., 2015).

Other researchers have opted for a proteomics approach to identify individuals with FM. Among females with FMS, Han et al. (2020) discovered dysregulated proteins and mechanisms associated with an FM diagnosis. They propose a panel of methyltransferase-like 18 (METTL18), immunoglobulin lambda variable 3-25 (IGLV3-25), interleukin-1 receptor accessor protein (IL1RAP), and putative V-set and immunoglobulin domain-containing like protein (IGHV1OR21-1) to differentiate FM from healthy controls. This conclusion was drawn from using a decision tree model that yielded an accuracy of 0.97. Collectively, they offered 100% detection sensitivity in their training cohort, and a specificity of 88% (Han et al., 2020).

Genova Diagnostics has also released the NutrEval FMV® Profile, which is a "blood and urine profile that evaluates over 125 biomarkers and assesses the body's functional need for 40 antioxidants, vitamins, minerals, essential fatty acids, amino acids, digestive support, and other select nutrients." NutrEval® aims to provide insight into conditions such as depression, anxiety, certain inflammatory conditions, and chronic pain syndromes (Genova, 2024). While there are no peer-reviewed studies published to confirm either the analytical or clinical utility of this particular proprietary test, researchers have delved into the biomarkers that comprise this assay. The aforementioned research on *COMT* proves relevant in the genomics component of this test. The clinical utility for the NutrEval® Profile is particularly pertinent to the chronic pain aspect. In patients with chronic pain, it was found that quinolinic acid, pyroglutamic acid (indicator of glutathione depletion), xanthurenic acid (indicator of vitamin B6 insufficiency), 3-hydroxyropyl mercapturic acid (acrolein metabolite), and methylmalonic acid (indicator of vitamin B12 deficiency) were all elevated to a certain degree among patients experiencing chronic pain. Seventy-seven percent of patients with chronic pain all had at least one abnormal biomarker (Gunn et al., 2020). This demonstrates that understanding the role of nutrition metabolism is necessary for

delineating the underlying processes of FM, a chronic pain condition, but additional research is needed.

Clinical Utility and Validity

Currently, most, if not all, of the FM diagnoses are solely clinical. Judgment is made subjectively and dependent on guidelines to conclude if subjective symptoms translate to FM. The sensitivity and specificity of utilizing the 2016 ACR guidelines, when compared to the 1990 criteria in a referral care setting, are 71% and 60%, respectively, with a positive predictive value of 85% and a negative predictive value of 39% (Ahmed et al., 2019). These statistical measures evidently prove that there need be more sensitive and specific measures beyond clinical symptoms for diagnosis of FM.

The clinical utility of an FM diagnosis extends beyond genetic testing and biomarkers; FM is also found concurrently among those with primary immunodeficiency and autoimmune diseases, such as RA, SLE, and Sjögren Syndrome (SS) (Han et al., 2020; Wilke, 1999). Confirming an FM diagnosis could aid in explicating the additional complications found in RA and SLE, such as "alternating constipation and diarrhea, urinary frequency, diffuse paresthesias, and cognitive difficulties." The converse also holds true – diagnoses of SS could prompt possible FM diagnoses as well, as seen in the cases of seeing sicca symptoms and tender point count of six (though not necessary with 2016 ACR guidelines) (Wilke, 1999).

Janssen et al. (2021) conducted a review that identified the polymorphisms related to FM and the respective clinical characteristics. From 27 articles, they found the relevant genes to be *MTHFR, RGS4, MYT1L, TACR1, SCN9A, DRD3, ADRB2, IL-4, HLA-DRB1, EDN1, CNR1, TAAR1, OPRM1, ADRA1A, ADRB3, BDNF, GRIA4, HTR3A, HTR3B, HTR2A, SERPINA 1 or A1AT, NRXN3, GCH1, MEFV, TRPV3, SLC6A4, ACE I/D, TSPO, COMT,* and *MAOA*. Additionally, "73.33% of the genes related to FM were also associated with some psychological disorders, such as anxiety, depression, schizophrenia, and obsessive and compulsive disorder, and 40.00% with pain sensitivity and/or migraine, besides other disorders associated (drug addiction, autoimmune disorders, circulatory problems, and metabolic alterations)." Of note, *SLC6A4, HTR3A, HTR3B,* and *HTRA* genes were associated with serotonergic regulation, chronic pain conditions, and anxiogenic situations; *COMT* was related to FM risk and increase in pain severity, increased likelihood of psychological disorders like "depression, anxiety and schizophrenia, alcoholism, opioid addiction, and eating disorders; *BDNF* was associated with hyperalgesia in FM; and *TACR1* was related to dementia and fatigue. As for treating FM, Janssen et al. (2021)also found that *TAAR1, RGS4, CNR1, GRIA4* may be considered as targets. These collective findings continue to demonstrate the

comorbidities associated with FM, and how understanding the genetic bases may aid in preventing and treating additional sequelae (Janssen et al., 2021).

Kendler et al. (2022) studied the familial genetic risk for functional somatic disorders including internalizing disorders, chronic fatigue syndrome, IBS, and FM. The study included 5,829,186 individuals in Sweden. The authors used a novel method that assessed aggregated risk in first to fifth degree relatives and adjusted for cohabitation. "Patients with FM carry substantial genetic risks not only for FM, but also for pain syndromes and internalizing, autoimmune and sleep disorders." Overall, patients with FM had a unique family risk genetic score with elevated genetic risk across other disorders; the was a similar but less marked pattern of genetic risks in patients of other functional somatic disorders. The authors suggest that the genetic risk score for FM differentiated it from classic autoimmune disorder and internalizing disorder (Kendler et al., 2022).

VI. Guidelines and Recommendations

American Pain Society

The Analgesic, Anesthetic, and Addiction Clinical Trial Translations Innovations Opportunities and Networks (ACTTION) partnership with the Food and Drug Administration (FDA) and APS created the ACTTION-APS Pain Taxonomy (AAPT) to help standardize clinically useful and consistent diagnoses of chronic pain disorders. First and foremost, all patients must have chronic pain to be diagnosed with FM. The working group members raised a concern of defining FM-pain as by the 1990 ACR criteria (CWP – chronic widespread pain) or the ACR 2010/2016 criteria of multisite pain (MSP), which were distinguished in count (MSP) versus anatomical distribution (CWP) of pain.

To facilitate identifying FM in clinical practice and in research, the AAPT core diagnostic criteria for FM are as follows:

1. "MSP defined as 6 or more pain sites from a total of 9 possible sites [head; left arm; right arm; chest; abdomen; upper back and spine; lower back and spine, including buttocks; left leg; and right leg]
2. Moderate to severe sleep problems OR fatigue
3. MSP plus fatigue or sleep problems must have been present for at least 3 months" (Arnold et al., 2019).

This guideline did not mention genetic testing for FM (Arnold et al., 2019).

American College of Rheumatology (ACR)

In 2016, the ACR published revisions to their 2010/2011 guidelines on preliminary diagnostic criteria for fibromyalgia and measurements of symptom severity. To satisfy a diagnosis of fibromyalgia, a patient must have:

1. "Widespread pain index (WPI) ≥ 7 and symptom severity (SS) scale score ≥ 5 or WPI 4-6 and SS scale score ≥ 9.
2. Generalized pain, defined as pain in at least 4 or 5 regions, must be present. Jaw, chest, and abdominal pain are not included in generalized pain definition.
3. Symptoms have been present at a similar level for at least 3 months.
4. A diagnosis of fibromyalgia is valid irrespective of other diagnoses. A diagnosis of fibromyalgia does not exclude the presence of other clinically important illnesses."

The table below obtained from the ACR's publication regarding ascertainment of WPI and SS is shown.

Ascertainment

(1) WPI: note the number of areas in which the patient has had pain over the last week. In how many areas has the patient had pain? Score will be between 0 and 19

Left upper region (Region 1)	*Right upper region (Region 2)*	*Axial region (Region 5)*
Jaw, left[a]	Jaw, right[a]	Neck
Shoulder girdle, left	Shoulder girdle, right	Upper back
Upper arm, left	Upper arm, right	Lower back
Lower arm, left	Lower arm, right	Chest[a]
		Abdomen[a]
Left lower region (region 3)	*Right lower region (Region 4)*	
Hip (buttock, trochanter), left	Hip (buttock, trochanter), right	
Upper leg, left	Upper leg, right	
Lower leg, left	Lower leg, right	

(2) Symptom severity scale (SSS) score

Fatigue
Waking unrefreshed
Cognitive symptoms

For the each of the 3 symptoms above, indicate the level of severity over the past week using the following scale:

0 = No problem
1 = Slight or mild problems, generally mild or intermittent
2 = Moderate, considerable problems, often present and/or at a moderate level
3 = Severe: pervasive, continuous, life-disturbing problems

The symptom severity scale (SSS) score: is the sum of the severity scores of the 3 symptoms (fatigue, waking unrefreshed, and cognitive symptoms) (0–9) plus the sum (0–3) of the number of the following symptoms the patient has been bothered by that occurred during the previous 6 months:

(1) Headaches (0–1)
(2) Pain or cramps in lower abdomen (0–1)
(3) And depression (0–1)

The final symptom severity score is between 0 and 12

The fibromyalgia severity (FS) scale is the sum of the WPI and SSS

The FS scale is also known as the polysymptomatic distress (PSD) scale.

[a] Not included in generalized pain definition.

Several cases previously deemed positive by the 2010/2011 criteria would be considered negative by the 2016 modified criteria, indicating a lower specificity. The current criteria also state patients can experience pain in 4-5 regions (four quadrants and axial), termed "generalized pain," to distinguish from the 1990 guideline's "widespread pain" definition. The 2016 ACR guidelines also now recognize spatial distribution of painful sites (Arnold et al., 2019). It lastly modifies the language from the 2010 guidelines that discuss other disorders serving as the etiology of experienced pain, and assumes the 1990 ACR guideline criterion of possible comorbidities with fibromyalgia (#4) (Wolfe et al., 2016).

After conducting an in-depth review of existing guidelines and literature, Heymann et al. (2017) published recommendations based on the 2010 ACR guidelines, and endorse using the 2010 ACR criteria for diagnosis of FM. They stated:

1. "The presence of widespread pain is essential for the diagnosis of patients with suspected FM.
2. Tender points may be useful in the diagnosis of fibromyalgia when evaluated in combination with other functional disorders covered in the 2010 [ACR] criteria. The tender point count may be correlated with the intensity of some symptoms, particularly emotional stress.
3. Sleep disorders and changes in cognition and fatigue should be considered in the diagnosis of FM. They should also be considered in the assessment of severity of patients with FM.

We suggest the systematic measurement of mood disorders using validated instruments suitable to the healthcare level in which they are administered because they are highly important when assessing the severity of patients with FM" (Heymann et al., 2017).

These guidelines did not mention genetic testing for FM.

Canadian Rheumatology Association (CRA)

The CRA released recommendations to the "identification, evaluation, and management" of those with FM after conducting a needs assessment. In relation to diagnosis of FM, the CRA recommends:

- "Fibromyalgia, a condition that can wax and wane over time, should be diagnosed in an individual with diffuse body pain that has been present for at least 3 months, and who may also have symptoms of fatigue, sleep disturbance, cognitive changes, mood disorder, and other somatic symptoms to variable degree, and when symptoms cannot be explained by some other illness [Level 5, Grade D].
- All patients with a symptom complaint suggesting a diagnosis of fibromyalgia should undergo a physical examination which should be within normal limits except for tenderness on pressure of soft tissues (ie. hyperalgesia which is increased pain following a painful stimulus) [Level 4, Grade D].
- Fibromyalgia should be diagnosed as a clinical construct, without any confirmatory laboratory test, and with testing limited to simple blood testing including a full blood count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), creatine kinase, and thyroid stimulating hormone (TSH). Any additional laboratory or radiographic testing should depend on the clinical

evaluation in an individual patient that may suggest some other medical condition [Level 5, Grade D]" (Fitzcharles et al., 2012).

This guideline did not mention genetic testing for FM (Fitzcharles et al., 2012).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, please visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81479	Unlisted molecular pathology procedure
81599	Unlisted multianalyte assay with algorithmic analysis
84999	Unlisted chemistry procedure

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Ada. (2022, January 28). *Fibromyalgia*. Ada Health GmbH. Retrieved January 3 from https://ada.com/conditions/fibromyalgia/

Ahmed, S., Aggarwal, A., & Lawrence, A. (2019). Performance of the American College of Rheumatology 2016 criteria for fibromyalgia in a referral care setting. *Rheumatology International*, *39*(8), 1397-1403. https://doi.org/10.1007/s00296-019-04323-7

Arnold, L. M., Bennett, R. M., Crofford, L. J., Dean, L. E., Clauw, D. J., Goldenberg, D. L., Fitzcharles, M.-A., Paiva, E. S., Staud, R., Sarzi-Puttini, P., Buskila, D., & Macfarlane, G. J. (2019). AAPT Diagnostic Criteria for Fibromyalgia. *The Journal of Pain*, *20*(6), 611-628. http://www.sciencedirect.com/science/article/pii/S1526590018308320

Arnold, L. M., Fan, J., Russell, I. J., Yunus, M. B., Khan, M. A., Kushner, I., Olson, J. M., & Iyengar, S. K. (2013). The fibromyalgia family study: a genome-wide linkage scan study. *Arthritis and rheumatism*, *65*(4), 1122-1128. https://doi.org/10.1002/art.37842

Behm, F. G., Gavin, I. M., Karpenko, O., Lindgren, V., Gaitonde, S., Gashkoff, P. A., & Gillis, B. S. (2012). Unique immunologic patterns in fibromyalgia. *BMC Clinical Pathology*, *12*(1), 25. https://doi.org/10.1186/1472-6890-12-25

Bhargava, J., & Hurley, J. A. (2020). Fibromyalgia. In *StatPearls*. StatPearls Publishing LLC. https://www.ncbi.nlm.nih.gov/books/NBK540974/

Bjersing, J. L., Bokarewa, M. I., & Mannerkorpi, K. (2015). Profile of circulating microRNAs in fibromyalgia and their relation to symptom severity: an exploratory study. *Rheumatol Int*, *35*(4), 635-642. https://doi.org/10.1007/s00296-014-3139-3

Bjersing, J. L., Lundborg, C., Bokarewa, M. I., & Mannerkorpi, K. (2013). Profile of cerebrospinal microRNAs in fibromyalgia. *PloS one*, *8*(10), e78762-e78762. https://doi.org/10.1371/journal.pone.0078762

Buskila, D., Sarzi-Puttini, P., & Ablin, J. N. (2007). The genetics of fibromyalgia syndrome. *Pharmacogenomics*, *8*(1), 67-74. https://doi.org/10.2217/14622416.8.1.67

Ciampi de Andrade, D., Maschietto, M., Galhardoni, R., Gouveia, G., Chile, T., Victorino Krepischi, A. C., Dale, C. S., Brunoni, A. R., Parravano, D. C., Cueva Moscoso, A. S., Raicher, I., Kaziyama, H. H. S., Teixeira, M. J., & Brentani, H. P. (2017). Epigenetics insights into chronic pain: DNA hypomethylation in fibromyalgia-a controlled pilot-study. *Pain*, *158*(8), 1473-1480. https://doi.org/10.1097/j.pain.0000000000000932

D'Agnelli, S., Arendt-Nielsen, L., Gerra, M. C., Zatorri, K., Boggiani, L., Baciarello, M., & Bignami, E. (2019). Fibromyalgia: Genetics and epigenetics insights may provide the basis for the development of diagnostic biomarkers. *Molecular pain*, *15*, 1744806918819944-1744806918819944. https://doi.org/10.1177/1744806918819944

Diatchenko, L., Fillingim, R. B., Smith, S. B., & Maixner, W. (2013). The phenotypic and genetic signatures of common musculoskeletal pain conditions. *Nature Reviews Rheumatology*, *9*(6), 340-350. https://doi.org/10.1038/nrrheum.2013.43

Docampo, E., Escaramís, G., Gratacòs, M., Villatoro, S., Puig, A., Kogevinas, M., Collado, A., Carbonell, J., Rivera, J., Vidal, J., Alegre, J., Estivill, X., & Rabionet, R. (2014). Genome-wide analysis of single nucleotide polymorphisms and copy number variants in fibromyalgia suggest a role for the central nervous system. *Pain*, *155*(6), 1102-1109. https://doi.org/10.1016/j.pain.2014.02.016

Fitzcharles, M. A., Ste-Marie, P. A., Goldenberg, D. L., Pereira, J. X., Abbey, S., Choinière, M., Ko, G., Moulin, D. E., Panopalis, P., Proulx, J., & Shir, Y. (2012, August 17). *Canadian Guidelines for the diagnosis and management of fibromyalgia syndrome*. Canadian Rheumatology Association. Retrieved January 3 fromhttps://rheum.ca/wpcontent/uploads/2017/11/2012CanadianFMGuidelines_17August2012.pdf

FM/a. (2020). *About FM/a*. Retrieved January 5 from https://fmtest.com/about-fma/

Galvez-Sánchez, C. M., & Reyes Del Paso, G. A. (2020). Diagnostic Criteria for Fibromyalgia: Critical Review and Future Perspectives. *Journal of clinical medicine*, *9*(4), 1219. https://doi.org/10.3390/jcm9041219

Genova. (2024). *NutrEval® FMV*. Genova Diagnostics. Retrieved January 10 from https://www.gdx.net/product/nutreval-fmv-nutritional-test-blood-urine

Goldenberg, D. L. (2023a, August 26). *Clinical manifestations and diagnosis of fibromyalgia in adults*. UpToDate. Retrieved January 3 from https://www.uptodate.com/contents/clinical-manifestations-and-diagnosis-of-fibromyalgia-in-adults

Goldenberg, D. L. (2023b, September 29). *Pathogenesis of fibromyalgia*. UpToDate. Retrieved January 4 from https://www.uptodate.com/contents/pathogenesis-of-fibromyalgia

Gunn, J., Hill, M. M., Cotten, B. M., & Deer, T. R. (2020). An Analysis of Biomarkers in Patients with Chronic Pain. *Pain Physician*, *23*(1), E41-e49.

Han, C.-L., Sheng, Y.-C., Wang, S.-Y., Chen, Y.-H., & Kang, J.-H. (2020). Serum proteome profiles revealed dysregulated proteins and mechanisms associated with fibromyalgia syndrome in women. *Scientific reports*, *10*(1), 12347-12347. https://doi.org/10.1038/s41598-020-69271-w

Heymann, R. E., Paiva, E. S., Martinez, J. E., Helfenstein, M., Rezende, M. C., Provenza, J. R., Ranzolin, A., Assis, M. R. d., Feldman, D. P., Ribeiro, L. S., & Souza, E. J. R. (2017). New guidelines for the diagnosis of fibromyalgia. *Revista Brasileira de Reumatologia (English Edition)*, *57*, 467-476. http://www.sciencedirect.com/science/article/pii/S2255502117300482

Janssen, L. P., Medeiros, L. F., Souza, A., & Silva, J. D. (2021). Fibromyalgia: A Review of Related Polymorphisms and Clinical Relevance. *An Acad Bras Cienc*, *93*(suppl 4), e20210618. https://doi.org/10.1590/0001-3765202120210618

Julien, N., Goffaux, P., Arsenault, P., & Marchand, S. (2005). Widespread pain in fibromyalgia is related to a deficit of endogenous pain inhibition. *Pain*, *114*(1), 295-302. http://www.sciencedirect.com/science/article/pii/S0304395905000023

Kendler, K. S., Rosmalen, J. G., Ohlsson, H., Sundquist, J., & Sundquist, K. (2022). A distinctive profile of family genetic risk scores in a Swedish national sample of cases of fibromyalgia, irritable bowel syndrome, and chronic fatigue syndrome compared to rheumatoid arthritis and major depression. *Psychological Medicine*, 1-8.

Kimura, Y., & Walco, G. A. (2023, May 10). *Fibromyalgia in children and adolescents: clinical manifestations and diagnosis*. UpToDate. Retrieved January 3 from https://www.uptodate.com/contents/fibromyalgia-in-children-and-adolescents-clinical-manifestations-and-diagnosis

Kojima, I., & Nagasawa, M. (2014). TRPV2. *Handb Exp Pharmacol*, *222*, 247-272. https://doi.org/10.1007/978-3-642-54215-2_10

Mickle, A. D., Shepherd, A. J., & Mohapatra, D. P. (2015). Sensory TRP channels: the key transducers of nociception and pain. *Progress in molecular biology and translational science*, *131*, 73-118. https://doi.org/10.1016/bs.pmbts.2015.01.002

Park, D.-J., & Lee, S.-S. (2017). New insights into the genetics of fibromyalgia. *The Korean journal of internal medicine*, *32*(6), 984-995. https://doi.org/10.3904/kjim.2016.207

Santoni, G., Farfariello, V., Liberati, S., Morelli, M. B., Nabissi, M., Santoni, M., & Amantini, C. (2013). The role of transient receptor potential vanilloid type-2 ion channels in innate and adaptive immune responses [Mini Review]. *Frontiers in Immunology*, *4*(34). https://doi.org/10.3389/fimmu.2013.00034

Smith, S. B., Maixner, D. W., Fillingim, R. B., Slade, G., Gracely, R. H., Ambrose, K., Zaykin, D. V., Hyde, C., John, S., Tan, K., Maixner, W., & Diatchenko, L. (2012). Large candidate gene association study reveals genetic risk factors and therapeutic targets for fibromyalgia. *Arthritis and rheumatism*, *64*(2), 584-593. https://doi.org/10.1002/art.33338

Wallace, D. J., Gavin, I. M., Karpenko, O., Barkhordar, F., & Gillis, B. S. (2015). Cytokine and chemokine profiles in fibromyalgia, rheumatoid arthritis and systemic lupus erythematosus: a potentially useful tool in differential diagnosis. *Rheumatology International*, *35*(6), 991-996. https://doi.org/10.1007/s00296-014-3172-2

Wilke, W. S. (1999). The Clinical Utility of Fibromyalgia. *JCR: Journal of Clinical Rheumatology*, *5*(2). https://journals.lww.com/jclinrheum/Fulltext/1999/04000/The_Clinical_Utility_of_Fibromyalgia.11.aspx

Wolfe, F., Clauw, D. J., Fitzcharles, M.-A., Goldenberg, D. L., Häuser, W., Katz, R. L., Mease, P. J., Russell, A. S., Russell, I. J., & Walitt, B. (2016). 2016 Revisions to the 2010/2011 fibromyalgia diagnostic criteria. *Seminars in Arthritis and Rheumatism*, *46*(3), 319-329. http://www.sciencedirect.com/science/article/pii/S0049017216302086

Gene Expression Profiling and Protein Biomarkers for Prostate Cancer

Policy Number: AHS – M2166 – Gene Expression Profiling and Protein Biomarkers for Prostate Cancer	Prior Policy Name and Number, as applicable: AHS – M2166 – Gene Expression Profiling and Protein Biomarkers for Prostate Cancer Management
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Prostate cancer is characterized by malignancy which originates in the small walnut-shaped gland that produces the seminal fluid in individuals who have a prostate. Heterogeneous in both molecular alterations and progression, clinical course ranges from a microscopic tumor that never becomes clinically significant to aggressive disease that can cause metastases, morbidity, and death (Benedettini et al., 2008; Taplin & Smith, 2024).

Gene expression assays quantify specific mRNAs being transcribed to assess the genes that are active in a particular cell or tissue. Analyses of gene expression can be clinically useful for disease classification, diagnosis, prognosis, and tailoring treatment to underlying genetic determinants of pharmacologic response (Steiling & Christenson, 2023). Protein expression-based assays measure the expression of the translation end-product(s) to assess cell-cycle progression. Similar to gene expression assays, protein biomarker-based assays can be clinically useful for disease classification and possible surveillance (Blume-Jensen et al., 2015; Ross et al., 2022).

II. Related Policies

Policy Number	Policy Title
AHS-G2007	Prostate Biopsy Specimen Analysis
AHS-G2008	Prostate Specific Antigen (PSA) Testing
AHS-G2054	Liquid Biopsy

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with low-risk or favorable intermediate-risk disease, as defined by the NCCN (see Note 1), the one-time use of Prolaris®, Oncotype DX Genomic Prostate Score®, **or** Decipher® tumor-based assays to guide the management of prostate cancer **MEETS COVERAGE CRITERIA** when **all** of the following conditions are met:
   a) When pathological examination showed localized adenocarcinoma of the prostate with no clinical evidence of metastasis or lymph node involvement;
   b) When the individual has no significant co-morbidities, including advanced age, to suggest they have an estimated life expectancy of less than 10 years.

2) For individuals with unfavorable intermediate-risk and high-risk disease, as defined by the NCCN (see Note 1), the one-time use of Prolaris® **or** Decipher® tumor-based assays to guide the management of prostate cancer **MEETS COVERAGE CRITERIA** when **all** of the following conditions are met:
   a) When pathological examination showed localized adenocarcinoma of the prostate with no clinical evidence of metastasis or lymph node involvement;
   b) When the individual has no significant co-morbidities, including advanced age, to suggest they have an estimated life expectancy of less than 10 years.

3) For individuals for whom there is a potential need for a prostate biopsy, the one-time use of the ExoDx Prostate (IntelliScore) (EPI) biomarker test (either once prior to initial biopsy or once prior to repeat biopsy) **MEETS COVERAGE CRITERIA** when **all** of the following conditions are met:
   a) The individual has confirmed (see Note 2), moderately elevated PSA levels:
      i) For individuals ages 50 – 75 years, PSA levels between 3 – 10 ng/mL
      ii) For individuals over the age of 75, PSA levels between 4 – 10 ng/mL
   b) The individual has none of the following conditions for which a prostate biopsy is already indicated:
      i) DRE suspicious for cancer.
      ii) Persistently elevated PSA.

iii) Positive multiparametric MRI, if performed.

iv) Known to have a high-penetrance prostate cancer risk gene(s) per NCCN guidelines (see Note 3).

c) The individual has no other relative contraindication for prostate biopsy, including **any** of the following:

i) A less than 10-year life expectancy.

ii) Benign disease not ruled out.

4) For individuals with a prostate, the one-time use of the 4Kscore test (either once prior to initial biopsy or once prior to repeat biopsy) **MEETS COVERAGE CRITERIA** when **all** of the following conditions are met:

a) The individual has confirmed (see Note 2), moderately elevated PSA levels:

i) For individuals ages 45 – 75 years, PSA levels greater than 3 and less than 10 ng/mL.

ii) For individuals over the age of 75, PSA levels greater than or equal to 4 and less than 10 ng/mL.

b) The individual has none of the following conditions for which a prostate biopsy is already indicated:

i) DRE suspicious for cancer.

ii) Persistently elevated PSA.

iii) Positive multiparametric MRI (if performed).

iv) Ethnicity at higher risk for prostate cancer (see Note 4)

v) First-degree relative (see Note 5) with prostate cancer.

vi) Known to have a high-penetrance prostate cancer risk gene(s) per NCCN guidelines (see Note 3).

c) The individual has no other relative contraindication for prostate biopsy, including **any** of the following:

i) A less than 10-year life expectancy.

ii) Benign disease not ruled out.

5) For individuals with a prostate, the one-time use of the IsoPSA test (either once prior to initial biopsy **or** once prior to repeat biopsy) **MEETS COVERAGE CRITERIA** when **all** of the following conditions are met:

a) For individuals 50 years of age or older who have confirmed (see Note 2) PSA levels greater than 4 and less than or equal to 25 ng/mL.

b) The individual has no other relative contraindication for prostate biopsy, including **any** of the following:

i) A less than 10-year life expectancy.

ii) Benign disease not ruled out.

6) For the assessment and/or monitoring of prostate cancer, the following tests **DO NOT MEET COVERAGE CRITERIA**:

a) Ki-67 immunohistochemistry

b) *PTEN* loss

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

7) The following tests **DO NOT MEET COVERAGE CRITERIA:**

a) All other urine testing for gene expression profile and/or protein biomarkers designed to assess prostate cancer.

b) Other screening tests for prostate cancer (e.g., alpha-methylacyl coenzyme A racemase [AMACR], ConfirmMDx, early prostate cancer antigen, endoglin, E twenty-six [ETS] gene fusions, human kallikrein 2, analysis of prostatic fluid electrolyte composition, interleukin-6, transforming growth factor-beta 1, *TMPRSS2:ERG* gene fusion, MyProstateScore, gene hypermethylation, *PCA3/KLK3* ratio, Prostate Health Index (PHI), *PCA3* score).

c) All other tests not described above that use cellular and biologic features of a tumor (e.g., those that are used to predict risk of recurrence in patients with prostate cancer).

NOTES:

Note 1: NCCN Prostate Cancer Initial Risk Stratification and Staging Workup for Clinically Localized Disease (NCCN, 2024a)

Risk Group	Clinical/Pathological Features
Very Low	Has all of the following: · cT1c; AND

	· Grade Group 1 · PSA <10 ng/mL · Fewer than 3 prostate biopsy fragments/cores positive, ≤50% cancer in each fragment/core · PSA density <0.15 ng/mL/g		
Low	Has all of the following but does not qualify for very low risk: · cT1-cT2a · Grade Group 1 · PSA <10 ng/mL		
Intermediate	Has all of the following: · No high-risk group features · No very-high-risk group features · Has one or more intermediate risk factors » cT2b-cT2c » Grade Group 2 or 3 » PSA 10-20 ng/mL	Favorable Intermediate	Has all of the following: · 1 IRF · Grade Group 1 or 2 · <50% biopsy cores positive
		Unfavorable Intermediate	Has one or more of the following: · 2 or 3 IRFs · Grade Group 3 · ≥50% biopsy cores positive
High	Has no very-high-risk features and has at least one high-risk feature: · cT3a OR · Grade Group 4 or Grade Group 5 OR · PSA >20 ng/mL		
Very High	Has at least one of the following: · T3b-T4 · Primary Gleason pattern 5 · 2 or 3 high-risk features · >4 cores with Grade Group 4 or 5		

Note 2: PSA elevation should be verified after a few weeks under standardized conditions (e.g., no ejaculation, manipulations, and urinary tract infections, no medications such as 5α-reductase) in the same laboratory or other Clinical Laboratory Improvement Amendments (CLIA) approved laboratory before considering a biopsy.

Note 3: According to the NCCN Prostate Cancer Early Detection guidelines, the main high-penetrance cancer risk genes include *BRCA1, BRCA2, ATM, MLH1, MSH2, MSH6, HOXB13, CHEK2, NBN, PALB2, RAD51D,* and *TP53* (NCCN, 2024b).

Note 4: According to the NCCN Prostate Cancer Early Detection guidelines, "Black/African American individuals have a significantly higher incidence of prostate cancer, increased prostate cancer mortality, and earlier age of diagnosis compared to white individuals" (NCCN, 2024b).

Note 5: First-degree relatives include parents, full siblings, and children of the individual.

IV. Table of Terminology

Term	Definition
ADT	Androgen deprivation therapy
AMACR	Alpha-methylacyl coenzyme A racemase
APC	*Adenomatous polyposis coli* gene
ARSI	Androgen receptor signaling inhibitor
AR-V7	Androgen receptor splice variant-7
AS	Active surveillance
ASCO	American Society of Clinical Oncology
ASTRO	American Society for Radiation Oncology
ATM	*ATM serine/threonine kinase gene*
AUA	American Urological Association
AUC	Area under the curve
BCR	Biochemical recurrence
BRCA1	*Breast cancer gene 1*
BRCA2	*Breast cancer gene 2*
CAPRA	Cancer of the prostate risk assessment
CCP	Cell-cycle progression
CCR	Cell-cycle risk
CDK12	*Cyclin dependent kinase 12 gene*
CDx	Companion diagnostic
CHEK2	*Checkpoint kinase 2 gene*
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CNAs	Copy number alterations
CTCs	Circulating tumor cells
DDR	DNA damage and repair
DLX1	*Distal-less homeobox 1 gene*

DRE	Digital rectal examination
DX	Diagnosis
EANM	European Association of Nuclear Medicine
EAU	European Association of Urology
EBRT	External beam radiation therapy
EDTA	Ethylenediaminetetraacetic acid
EPI	ExoDx Prostate (IntelliScore)
ERG	*ETS Transcription Factor ERG*
ESMO	European Society for Medical Oncology
ESTRO	European Society for Radiotherapy and Oncology
ESUR	European Society of Urogenital Radiology
ETS	E-twenty-six
FANCA	*Fanconi anemia complementation group A gene*
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
FISH	Fluorescence *in situ* hybridization
GEC	Gene/genomic expression classifiers
GPS	Genomic prostate score
GSTP1	*Glutathione S-transferase pi 1 gene*
HGPC	High-grade prostate cancer
hK2	Human kallikrein-2
HOXB13	*Homeobox B13 gene*
HOXC6	*Homeobox C6 gene*
HT	Hormonal therapy
IHC	Immunohistochemistry
indels	Insertion and deletion alterations
IRF	Intermediate-risk factor
ISUP	International Society of Urological Pathology
KLK3	*Kallikrein related peptidase 3 gene*
LDTs	Laboratory-developed tests
mCRPC	Metastatic castration-resistant prostate cancer
MLH1	*MutL homolog 1 gene*
MRI	Magnetic resonance imaging
MSH2	*MutS homolog 2 gene*
MSH6	*MutS homolog 6 gene*
NBN	*Nibrin gene*
NCCN	National Comprehensive Cancer Network
NPV	Negative predictive value

PALB2	*Partner and localizer of BRCA2 gene*
PARP	Poly (ADP-ribose) polymerase
PCa	Prostate cancer
PCA3	Prostate cancer gene 3
PPCR	Polymerase chain reaction
PCRMP	Prostate Cancer Risk Management Programme
PHI	Prostate health index
PLA	Proprietary laboratory analyses
PPV	Positive predictive value
PSA	Prostate specific antigen
PTEN	*Phosphatase and tensin homolog gene*
QALY	Quality adjusted life-years
RAD51D	*RAD51 poaralog d gene*
RASSF1	*Ras association domain family 1 gene*
RNA	Ribonucleic acid
RP	Radical prostatectomy
RT	Radiation therapy
RT-PCR	Reverse transcription-polymerase chain reaction
TMPRSS2	*Transmembrane serine protease 2*
TP53	*Tumor protein 53 gene*
TRUS	Transrectal ultrasound guided biopsy

V. Scientific Background

Prostate cancer (PCa) is the most common cancer in American individuals who have a prostate and the second leading cause of death in the same group. In 2024, the American Cancer Society estimates that approximately 299,010new prostate cancer diagnoses and approximately 35.250prostate cancer deaths will occur; although, the five-year survival rate between 2012-2018 was 97%. About one individual in eight amongst those who have a prostate will be diagnosed with prostate cancer during their lifetime in the United States (ACS, 2024a, 2024b).

Many cases of prostate cancer do not become clinically evident, as indicated in autopsy studies, where prostate cancer is detected in approximately 30 percent of individuals with a prostate aged 55 or older and approximately 60 percent of individuals with a prostate by age 80 (Bell et al., 2015). These data suggest that prostate cancer often grows so slowly that most individuals die of other causes before the disease becomes clinically advanced (Hoffman, 2023).

Prostate cancer survival is related to many factors, especially the extent of tumor at the time of diagnosis. The five-year relative survival among individuals with cancer

localized to the prostate or with regional spread is 100%, compared with 31% among those diagnosed with distant metastases (Hoffman, 2023). Gene expression profiling has been proposed as a method of risk stratification for prostate cancer. Several tests evaluating the expression levels of various genes have been produced to be used in conjunction with other tools such as Gleason score and prostate-specific antigen (PSA) assessment. The Gleason score is a scoring system used to categorize a prostate cancer biopsy based on risk assessment.

Tissue-based gene expression classifiers (GEC) are now widely used to assist in prostate cancer prognosis. These tests are RNA-based prognostic biomarkers that analyze a distinct multigene panel to predict cancer progression, from the chance of having the disease to the probability of death at ten years due to prostate cancer. Genomic tests can predict prostate cancer aggressiveness, detect potentially dangerous prostate cancer-related genomic activity, and utilize biopsy samples to deliver prognostic information via immunofluorescence imaging. Additionally, researchers have identified the potential of microRNAs as human prostate cancer biomarkers (Song et al., 2018). While several types of biomarker tests exist, the NCCN specifically recommends Prolaris, Oncotype DX Genomic Prostate Score® (GPS™), and Decipher as tumor-based molecular assays to consider during initial risk stratification (NCCN, 2024a). Ki-67 and PTEN are also listed in NCCN guidelines, but are not recommended (NCCN, 2024a).

Proprietary Testing, Clinical Utility, and Analytical Validity

Hu et al. (2018) evaluated the utility of three genomic expression classifiers (GEC), including Decipher, Oncotype Dx, and Prolaris. A total of 747 patients underwent GEC testing. The authors found that "Among patients with clinical favorable risk of cancer, the rate of active surveillance (AS) differed significantly among patients with a GEC result above the threshold (46.2%), those with a GEC result below the threshold (75.9%), and those who did not undergo GEC (57.9%)." The authors further estimated that for every nine individuals "with favorable risk of cancer who undergo GEC testing, one additional patient may have their disease initially managed with AS" (Hu et al., 2018).

Prolaris

The test "Prolaris" (created by Myriad Genetics) has been used to inform decision making on AS and whether to proceed to a treatment option, such as radiation or surgery. Prolaris is an assessment of the average expression of 31 cell-cycle progression (CCP) genes compared to 15 reference genes. This score is combined with the patient's age, PSA, percent positive cores, clinical stage, Gleason score, and American Urological Association (AUA) risk category; it is intended to provide a 10-year prostate cancer-specific mortality risk. Scores range from zero to ten, with each

unit increase representing a doubling of disease-risk progression. Prolaris may also be used to assess risk post-prostatectomy, and the same scale of zero to ten is used. Each unit increase represents a doubling of risk of biochemical recurrence (BCR) (Alford et al., 2017).

Cell cycle progression expression has been found to correlate with mortality rate of prostate cancer and can provide important pretreatment prognostic information. Cuzick et al. (2015) found that not only was there a relationship between CCP expression and mortality rate, the increased expression of CCP was predictive of BCR after 10 years. Even after adjusting for factors such as PSA and Gleason score, the CCP was both "highly significant" and "independent" of prostate cancer mortality rate. The authors noted that the CCP score could be created from minimal tumor mass (as little as 0.5 mm), with a 90% success rate with >0.5 mm visible tumor, as well as Prolaris' objective criteria compared to the Gleason score (Cuzick et al., 2015).

Prolaris may be used to lower unnecessary treatment by providing a molecular indication of the disease's progression. Radical treatments, such as prostatectomies, are often unnecessary, and there is utility in a biomarker metric than can reliably inform providers of a course of treatment or condition. An AS status is preferable to treatment. Hu et al. (2018) used data provided by the CCP score (along with two other biomarker tests) to perform risk stratification and assess whether further treatment was needed or if the condition could be managed by active surveillance. Lin et al. (2018) clearly separated high- and low-risk patients using the CCP score. The study combined the CCP score as well as a clinical assessment from the Cancer of the Prostate Risk Assessment (CAPRA) into a cell-cycle risk (CCR) score. This CCR score was used to select patients for an AS status. The threshold created from both the molecular measures and the clinical measures has the advantage of including higher-risk patients whose clinical features may be lower-risk. Furthermore, the patients that fell below the threshold were found to have a mortality risk of 2.5%, and the probability of survival of patients with scores under the threshold was 100% (Hu et al., 2018; Lin et al., 2018). Finally, Prolaris has been used by providers to inform clinician decision making. A survey by Carneiro et al. (2018) found that the course of treatment for prostate cancer patients was influenced by Prolaris' results. About 65% of cases were reported to have shifted in the intended treatment based on the test results, and about 40% were reported to have opted for the AS choice (a "decrease" in treatment) (Carneiro et al., 2018).

Tward et al. (2020) studied the ability of CCR to predict prostate cancer metastasis using Prolaris. According to a CCR threshold of 2.112, 29.5% patients were hypothesized to be high risk metastasis (CCR>2.112) and 70.5% were unfavorable intermediate risk patients (CCR < 2.112). Patients were followed five years later to

determine if CCR accurately predicted metastasis in those undergoing multimodality therapy (androgen deprivation with surgery) or radiation therapy. According to the results, the CCR score does provide a clinically meaningful different risk of metastasis for patients receiving multimodality therapy or radiation therapy. Multimodality therapy reduced patients' risk of metastasis and treatment benefit can be evaluated as a function of the CCR score. For those with CCR scores below the threshold of 2.112 (27% of high-risk group and 73% of the unfavorable intermediate group), radiation therapy was considered after assessing the difference in the risk of metastasis (Tward et al., 2020).

Oncotype DX Genomic Prostate Score® (GPS™) is similar to Prolaris in that it assesses levels of gene expression, should be used for lower-risk patients, and can inform clinicians about the possible course of treatment. The primary difference is that Oncotype DX only tests 12 genes, with five reference genes (compared to 31 and 15, respectively, for Prolaris)(Exact Sciences, 2021). These expression levels are combined into an algorithm to produce a genomic prostate score (GPS) of 0-100. This GPS score correlated with prediction of cancer aggression (outcomes such as death or recurrence) (Cullen et al., 2015).

Cullen et al. (2015) found that the GPS score correlated well with BCR. The researchers noted that OncoType DX is a good predictor of both early and late BCR and is validated for adverse pathology whereas Prolaris is validated for 10-year mortality or BCR after radical prostatectomy (Alford et al., 2017; Cullen et al., 2015; Davis, 2014; NCCN, 2024a). Oncotype DX was recently validated in a group of individuals separated by race, showing that this tool is an independent predictor of adverse pathology with similar predictive accuracy in both African American (n=96) and European American (n=76) populations (Murphy et al., 2020).

AR-V7 Nucleus Detect test

The AR-V7 Nucleus Detect test is available through Epic Sciences. This test evaluates the Androgen Receptor Splice Variant-7 (AR-V7) protein in the nucleus of circulating tumor cells and is intended to identify metastatic castration-resistant prostate cancer patients who will not respond to androgen-receptor targeted therapies (Epic Sciences, 2023).

Scher et al. (2016) examined 161 patients with progressive metastatic castration-resistant prostate cancer (mCRPC) to assess its association with AR-V7. Out of 191 samples (128 pre-ARS inhibitor and 63 pretaxane), the investigators found AR-V7-positive circulating tumor cells in 34 samples, and those samples were found to have worse clinical outcomes and overall survival than those without AR-V7. Scher et al. (2016) concluded that "the results validate CTC nuclear expression of AR-V7 protein in

[individuals] with mCRPC as a treatment-specific biomarker that is associated with superior survival on taxane therapy over ARS-directed therapy in a clinical practice setting" (Scher et al., 2016).

Further, Chen et al. (2018) studied the overexpression of the nuclear AR-V7 protein in prostate cancer cases. A total of 401 individuals participated in this study. Participants were split into two cohorts: cohort I included those who were high-risk (n=238), and cohort II included those who were not considered high-risk (n=238). Analyses showed that high nuclear AR-V7 protein expression was detected in approximately 30-40% of participants, and a "High baseline expression of nuclear AR-V7 protein was associated with an unfavorable BCR-free survival in the high-risk patient cohort I but not in the unselected consecutive cohort II. Remarkably, AR-V7 was an independent negative prognostic factor in high-risk prostate cancer patients of cohort I who were selected to receive adjuvant treatment" (Chen et al., 2018).

Graf et al. (2020) studied the clinical utility of AR-V7 as a biomarker for patients with progressing metastatic castration-resistant prostate cancer (mCRPC). The results were used by physicians to make a second line of therapy choice of either an androgen receptor signaling inhibitor (ARSI) or taxane chemotherapy. There were 255 samples of circulating tumor cells (CTCs) tested for AR-V7. Patients with detectable AR-V7 in the CTCs had superior survival with taxane treatment over ARSIs and patients who were AR-V7- negative had superior survival on ARSIs over taxanes. These results showed that individuals who tested AR-V7- positive were more likely to survive longer on taxane chemotherapy. Overall, the authors suggest that the use of AR-V7 CTC test "to inform treatment choice can improve patient outcomes relative to decisions based solely on standard-of-care measures" (Graf et al., 2020).

Decipher

Decipher is a genomic prognostic test that is used to predict cancer outcomes in patients that have undergone a radical prostatectomy (RP), which is the removal of the prostate gland and surrounding tissues. Decipher relies on the expression levels of 22 RNA markers in the RP specimen and is primarily used to predict likeliness of metastases or mortality. The algorithm score ranges from zero to one, where a higher score corresponds with higher chance of metastasis. This algorithm was shown to have outperformed the traditional assessment of clinical and pathological features in predicting metastasis (0.75 accuracy compared to 0.69) as well as 17 other genetic tests (0.54 to 0.68 accuracy) (Alford et al., 2017; Dalela et al., 2016).

Van den Broeck et al. (2019) aimed to validate the Decipher test in the prediction of distant metastatic recurrence in individuals with high-risk nonmetastatic prostate cancer 10 years after the surgery was completed. A total of 298 people participated in

this study. Results showed that "the median Decipher scores were higher in the population that developed metastases" suggesting that this study "validates Decipher as a predictor for metastatic recurrence even in patients with high-risk, nonmetastatic PC [prostate cancer] within 10-yr follow-up" (Van den Broeck et al., 2019). Specifically, the data showed that each 10% increase in Decipher score resulted in an increased risk of distant metastatic prostate cancer recurrence.

In a prospective trial by Marascio et al. (2020), the clinical utility of the Decipher tumor test on postoperative management of prostate cancer post prostatectomy was discussed. There were 3,455 individuals with prostates enrolled in the study and the change in treatment decision-making was recorded. In the cohort, 61% of the patients had high-risk tumors with a two-year prostate cancer reoccurrence. As a result of genome classifier testing, providers' recommendations changed for 39% of the patients, translating to a number needed to test of three to change one treatment decision. This study demonstrated that genome classifier testing favorably impacts treatment decision making post radical prostatectomy, promoting more post-operative radiotherapy. This translated to improved patient reported quality of life (Marascio et al., 2020).

Nguyen et al. (2023) published a meta-analysis examining the prognostic ability of the Decipher Genomic Classifier for distant metastases, prostate cancer-specific mortality, and overall survival within the context of three randomized phase three high-risk definitive radiation therapy trials. The total cohort consisted of 265 individuals whose median age was 69 years and median pretreatment PSA of 25.8 ng/mL. The authors report that upon meta-analysis, the Decipher Genomic Classifier score was statistically significantly associated with time to distant metastasis, prostate cancer–specific mortality, and overall survival (Nguyen et al., 2023).

Spratt et al. (2023) reported on the Decipher Genomic Classifier's performance in the context of intermediate-risk prostate cancer. Through multivariable analysis of 215 individual samples, it was determined that the test was "independently prognostic for disease progression (subdistribution hazard ratio [sHR], 1.12; 95% confidence interval [CI], 1.00-1.26; P = .04), biochemical failure (sHR, 1.22; 95% CI, 1.10-1.37; P < .001), distant metastasis (sHR, 1.28; 95% CI, 1.06-1.55; P = .01), and prostate cancer-specific mortality (sHR, 1.45; 95% CI, 1.20-1.76; P < .001)." Beyond prognostic utility, the authors argue that the data herein support the predictive value of the Decipher Genomic Classifier for individuals with intermediate risk prostate cancer; among individuals with a test score of intermediate-high, radiation dose-escalation showed greater absolute benefit, with 10-year metastasis-free survival of 75% (95% CI, 55-95) compared with 54% (95% CI, 31-77) for standard dose (Spratt et al., 2023).

ExoDX Prostate IntelliScore (EPI)

ExoDX is a urinary test that detects the expression level of three genetic biomarkers (ERG, PCA3, and SPDEF) (ExoSome, 2024a, 2024b). This test integrates the expression levels of these three biomarkers and assigns an individualized risk score to predict the risk of high-grade prostate cancer (Gleason score ≥ seven). This test is intended for individuals 50 or over with a PSA level of two to ten ng/mL presenting for an initial biopsy (prior to a DRE) and as a useful test in the post-biopsy setting for patients thought to be higher risk despite a negative prostate biopsy(ExoSome, 2024a, 2024b; NCCN, 2024b).

McKiernan et al. (2016) used ExoDX to discriminate between benign prostate cancer (Gleason score 6 and under) and high-risk cancer (Gleason score ≥7). The prognostic score was derived from a sample of 499 patients with PSA levels of two to 20 ng/mL; it was then validated in a sample of 1064 patients and evaluated in a population of 255. The test was compared to the standard of care practices (SOC), and the area under the curve (AUC) of the test was 0.77 compared to the SOC's 0.66. An independent validation found the AUC of the test to be 0.73 compared to the SOC's 0.63. The authors calculated that 138 of 519 biopsies (27%) would have been avoided and that the test only missed five percent of patients with high-risk disease (McKiernan et al., 2016). Within a second phase of the long-term study, McKiernan and colleagues report that using the EPI validated cut-point of 15.6 results in avoiding 26% of unnecessary prostate biopsies and a 20% decrease in all biopsies. If the EPI cut-point is raised to 20, then 31% of total biopsies would be avoided, including 40% of unnecessary biopsies (McKiernan et al., 2018).

A study published in 2018 did a cost-effectiveness analysis and comparison of not only ExoDx (EPI), but also Prostate Health Index (PHI), 4Kscore, and SelectMDx to current standard care of care. Using 2017 US dollars for their calculations, the cost and quality adjusted life-years (QALY) for the current standard of care—transrectal ultrasound guided biopsy (TRUS biopsy)—was $3,863 and 18.0865, respectively. The authors of the study note that EPI, PHI, and SelectMDx cost less than performing TRUS biopsy. They note, "The EPI provided the highest QALY with an incremental cost-effectiveness ratio of $58,404 per QALY. The use of biomarkers could reduce the number of unnecessary biopsies by 24% to 34% compared to the current standard of care… Using SelectMDx or the EPI following elevated prostate specific antigen but before proceeding to biopsy is a cost-effective strategy in this setting" (Sathianathen Niranjan et al., 2018).

A randomized, blinded, two-armed clinical utility study was published in 2020 using ExoDx (EPI) in individuals presenting for initial biopsy with PSA values in the

intermediate range (two to ten ng/mL). This large study (n = 1,094) included 72 urologists from 24 different practices. All patients had an EPI test performed, but the patients were divided into two different groups (control and experimental) where only the experimental group received results prior to their biopsy decision. Of the individuals within the experimental group who received negative EPI scores, 74% deferred biopsy. For individuals within the experimental group who received positive EPI scores, 87% were recommended by their urologists to undergo the biopsy, and ultimately 72% did. As compared to the control arm of the study, there is a 30% increase in the detection of high-grade prostate cancer [HGPC], and the authors "estimate that 49% fewer HGPC were missed due to deferrals compared to standard of care (SOC). Overall, 68% of urologists reported that the EPI test influenced their biopsy decision" (Tutrone et al., 2020).

McKiernan et al. (2020) investigated the use of the EPI test in a prospective clinical validation study of 229 individuals who were undergoing repeat biopsy. The EPI test demonstrated an NPV of 92% and results evidenced avoidance of 26% of unnecessary biopsies while missing 2.1% of the incidences of high-grade prostate cancer (a total of five patients) (McKiernan et al., 2020).

4Kscore

4Kscore is intended to assess the risk for "aggressive" prostate cancer. The test incorporates total PSA, free PSA, "intact" PSA, and "hk2" [human kallikrein 2] (NCCN, 2024a; OPKO, 2021). These biomarkers, along with other patient clinical information (such as age and prior biopsy status) are evaluated by the 4Kscore algorithm, which generates a risk score for aggressive cancer (percent risk of Gleason seven or higher, if a biopsy were to be performed).

Zappala et al. (2017) performed a meta-analysis of 4kScore validation studies. A total of 12 studies encompassing 11134 patients were included, and the pooled area under curve (AUC) for the test to "discriminate for high-grade PCa [prostate cancer] was found to be 0.81" (Zappala et al., 2017).

Two key prospective and blinded investigations were completed in 2015 and 2018, attempting to validate 4Kscore in a total of 937 patients, defined as the "intended use" population. The test demonstrated an overall sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV) of 96.9%, 27.4%, 95.9%, and 33.7%, respectively. These metrics showed little variation between African American and non-African American individuals, with the exception of PPV (46.7% compared to 28.1%, respectively) (Parekh et al., 2015; Punnen et al., 2018).

Wysock et al. (2020) compared the performance of 4K score to SelectMDx in detecting prostate cancer in 114 patients who received both tests. These tests were analyzed to provide guidance on whether to perform biopsy. Based on the results, the two scores lead to different biopsy recommendations. A total of 50 out of 144 patients underwent biopsy based on the test results. Of the 50 patients, 22 (44%) were found to have clinically significant prostate cancer. In addition, the specificity of 4K score was significantly greater compared to SelectMDx while sensitivity was similar. The area under the curve for 4K score was 0.830 and SelectMDx was 0.672. The authors state that "the 4Kscore when combined with magnetic resonance imaging was superior to the SelectMDx" in detecting prostate cancer (Wysock et al., 2020).

Mi et al. (2021) completed a meta-analysis to help inform the diagnostic accuracy of 4Kscore in detecting high-grade prostate cancer, covering a total of nine studies and 1,689 patients. The investigators reported a pooled sensitivity, specificity, and AUC of 0.90 (95%CI: 0.86-0.92), 0.44 (95%CI: 0.36-0.52), and 0.81 (95%CI: 0.77-0.84), respectively, and concluded that "4Kscore can be used as a model for the diagnosis of high-grade CaP [prostate cancer]. However, we detected significant heterogeneity among studies that was not explained by subgroup or meta-regression analysis, thus lowering our confidence in these results."

Further validation of the test will be useful; however, to date, 4Kscore has demonstrated relatively high sensitivity and AUC compared to other molecular testing for the assessment of high-grade prostate cancer risk.

ConfirmMDX

ConfirmMDX uses methylation-specific polymerase chain reaction (PCR) to identify methylation of three genes (*GSTP1*, *APC*, and *RASSF1*), and determine whether a patient with a previously negative prostate biopsy should undergo a repeat biopsy (MDxHealth, 2023a). This test has been evaluated by Van Neste, Partin, et al. (2016) and was found to have an NPV of 96% for high-grade prostate cancer. A total of 7899 prostate core biopsies from 803 patients were assessed, and the NPV of finding low levels of DNA methylation was 89.2% for all cancers. The PPV of the genetic assay was found to be 28.2% (for detection of any cancer on a repeat biopsy), and this was calculated to be "significantly higher" than the PPV of standard of care practices. The final algorithm was optimized to a maximum of 0.742 AUC (Van Neste, Partin, et al., 2016). Wojno et al. (2014) evaluated the utility of this test and found that out of 138 patients that the test had been performed on, only six with a negative result had undergone a repeat biopsy.

SelectMDX

SelectMDX evaluates two mRNA cancer-related biomarkers (HOXC6 and DLX1 with *KLK3* as a reference gene) to assist a clinician in deciding to continue routine screening or to order a prostate biopsy. This test is considered a "non-invasive urine test" and reports a binary result of "increased risk" or "very low risk" (MDxHealth, 2023b). Van Neste, Hendriks, et al. (2016) evaluated this test at a 0.90 AUC in a validation cohort. The authors concluded that the mRNA signature was one of the most significant components of the validation results (Van Neste, Hendriks, et al., 2016). Shore (2018) assessed the effect of SelectMDX results on clinical decision making, and found that out of 253 patients that SelectMDX evaluated as "negative," only 12% underwent a biopsy (Shore, 2018).

IsoPSA®

IsoPSA® is a blood test indicated for use in individuals with a prostate who are over 50 years of age with elevated PSA, to help inform the likelihood of having high-grade prostate cancer. Utilizing a proprietary, 2-phase aqueous polymer and salt mixture, PSA isoforms separate between the two aqueous phases, where the discriminatory power between benign and cancerous clinical phenotypes purportedly resides primarily in the top phase. The PSA isoform content in the top layer is then measured with conventional, FDA-approved PSA ELISA immunoassays, and a single numerical score (IsoPSA Index) that is either above or below an established cutoff is generated, providing a binary positive or negative result.

The clinical validity of IsoPSA® was demonstrated in several studies. Stovsky et al. (2019) performed a multicenter, prospective validation in 271 individuals scheduled for prostate biopsy, and found that the test yielded an area under the receiver operating characteristic curve of 0.784 for high grade cancer. Klein et al. (2022) completed an additional multicenter study of 888 individuals scheduled for prostate biopsy and found similar results, establishing an AUC of 0.783 for IsoPSA®. These investigators further reported a sensitivity, specificity, NPV, and PPV of 0.902, 0.455, 0.893, and 0.477, respectively.

To investigate the clinical utility of IsoPSA®, Scovell et al. (2022) performed a "real-world" observational study engaging 38 providers across the Cleveland Clinic health system. The authors examined whether an IsoPSA® result changed the number of biopsy and magnetic resonance imaging recommendations for a cohort of 734 individuals with total serum prostate specific antigen [PSA] ≥ four and <100 ng/ml and no history of prostate cancer. The authors determined that "IsoPSA testing resulted in a 55% (284 vs 638) net reduction in recommendations for prostate biopsy" for those "with total PSA ≥ four ng/ml."

Progensa PCA3

Progensa *PCA3* is an FDA-approved assay that examines the concentration of the prostate cancer gene three (*PCA3*) and compares it to the amount of prostate-specific antigen RNA. This test is intended for assistance in decision making for a repeat biopsy in individuals with a prostate who are 50 years or older, and a *PCA3* score under 25 was associated with a decreased likelihood of a positive biopsy. However, the manufacturer states this test should not be used for those with atypical small acinar proliferation on their most recent biopsy (Hologic, 2024). A total of 466 samples were provided, and 102 of these samples were evaluated to require a repeat biopsy. This assay was evaluated at a 77.5% sensitivity, a 57.1% specificity, a 33.6% positive predictive value, and a 90.0% negative predictive value (Gittelman et al., 2013).

Rodríguez and García-Perdomo (2020) performed a systematic review and meta-analysis of the diagnostic accuracy of PCA3 prior to a patient's first prostate biopsy. They found that with a cutoff of 35, the sensitivity of the diagnostic tests was 0.69 (95% confidence interval 0.61-0.75), specificity was 0.65 (95% confidence interval 0.553-0.733), the diagnostic odds ratio was 4.244 (95% confidence interval 3.487-5.166), and the AUC was 0.734 (95% confidence interval 0.674-0.805). This study suggests that there may be a greater clinical utility with 35 as the cutoff as opposed to the 25 approved by the FDA, and ultimately urinary PCA3 can "be used as a guide for directing the performance of the first prostate biopsy and decreasing unnecessary biopsies" (Matuszczak et al., 2021; Rodríguez & García-Perdomo, 2020).

MyProstateScore

MyProstateScore is a panel that measures urinary prostate cancer antigen three (PCA3), urinary TMPRSS2:ERG gene fusion (T2:ERG), and serum PSA, to predict the likelihood of prostate cancer in biopsy-naïve patients. Validating the test in a cohort of 1225 patients, Tomlins et al. (2016) found that MyProstateScore was superior to PSA alone, yielding an AUC of 0.693 (compared to 0.585 for PSA). Tosoian et al. (2021) aimed to validate an optimal MyProstateScore threshold for ruling out clinically significant (grade group ≥ two) cancer, finding that a threshold of 10 resulted in 97% sensitivity and 98% NPV. The investigators further concluded that use of the test could have prevented about one out of three of the biopsies that patients received.

ProMark

Another test that may have utility is ProMark. It measures the levels of eight proteins through the quantitative immunofluorescence of a biopsy specimen. ProMark is used to predict cancer aggression in patients with a Gleason score of 3+3 or 3+4. The proteins chosen have roles in cell proliferation, signaling, or stress response, and the score is reported from one to 100. This score represents individualized risk. Blume-Jensen et al. (2015) narrowed down the eight primary protein biomarkers used (down

from the 12 proposed by an earlier study) as well as assessed its ability to predict clinical endpoints of favorable and nonfavorable disease. They recommended a cutoff of 0.33 (on a scale of zero to one) for "nonfavorable" pathology (83.6% of patients with favorable disease fell below this cutoff). Conversely, a cutoff of 0.8 was recommended for favorable pathology as 76.9% of patients with nonfavorable pathology were above this cutoff. The authors concluded that this assay provided useful information, especially when differentiating between Gleason scores (Alford et al., 2017; Blume-Jensen et al., 2015).

Prostate Health Index

Prostate Health Index (PHI) measures total PSA, fPSA (free non-protein bound PSA), and p2PSA (an isoform of fPSA). Levels of these three proteins are combined and calculated, implying that individuals with a higher total PSA and p2PSA and a lower fPSA have a higher risk of presenting with prostate cancer (Couñago et al., 2020). PHI is clinically used to reduce the number of unnecessary biopsies in those with border-line PSA levels, predict biochemical recurrence after radical prostatectomy, and enhance the predictive value of multi-parametric MRI. PHI is not recommended in primary screening for prostate cancer (Duffy, 2020).

Jia et al. (2020) compared the diagnostic value of PCA3 and PHI for detection of prostate cancer at initial biopsy in a meta-analysis of 10,376 patients from 20 studies. The pooled sensitivity for PCA3 and PHI was 0.55 and 0.88, respectively. The pooled specificity for PCA3 and PHI was 0.74 and 0.36. The area under the curve, measuring overall quality of the diagnostic test, was 0.72 for PCA3 and 0.76 for PHI. The combination use of PCA3 and PHI resulted in a higher area under the curve of 0.79. Overall, this study suggests that both PCA3 and PHI show acceptable results and a "combination of these two diagnostic tests may be more helpful than the use of either test alone in prostate cancer management" (Jia et al., 2020).

White et al. (2018) evaluated the clinical utility of the PHI on "biopsy decision management" among patients with "non-suspicious DRE findings and tPSA in the four to ten ng/mL range" in an observational study at several large urology group practices. They found that there was a "significant reduction in biopsy procedures performed" in individuals receiving a PHI test when comparing to the control group (36.4% biopsy vs 60.3% biopsy), and that the "PHI score impacted physician's patient management plan in 73% of cases, including biopsy deferrals when the PHI score was low, and decisions to perform biopsies when the PHI score indicated an intermediate or high probability of prostate cancer," defined as a score greater than or equal to 36. This altogether conveyed the importance of the PHI score in clinical decision making in terms of how

to proceed with individual patient circumstances (Matuszczak et al., 2021; White et al., 2018)

Ki-67 and PTEN

Finally, the NCCN specifically recommends *against* two particular tests in assessment of prostate cancer; Ki-67 staining and phosphatase and tensin homolog (*PTEN*) loss (NCCN, 2024a).

Ki-67 is a nuclear protein involved in cell cycle proliferation and is intended to provide prognostic information on metastasis and prostate cancer-specific mortality (NCCN, 2024a; Ross et al., 2022). Ki-67 staining has shown some promising results. However, the primary limitation with these studies is that most active surveillance populations will have a Gleason Score of 6 or less, which is considered "low-risk." This population will most likely have low Ki-67 levels, clouding its utility in populations trying to decide between immediate and deferred treatment (Ross et al., 2022).

PTEN loss is a relatively early event in the course of prostate cancer. *PTEN* is a tumor suppressor gene on chromosome 10q and is involved in cell cycle regulation. PTEN is intended to provide prognostic information on prostate cancer-specific mortality, biochemical recurrence, and cancer progression (NCCN, 2024a; Ross et al., 2022). Data on prognostic value of PTEN loss post-treatment have been conflicting. It is possible that active treatments contribute to the disruption of the PTEN pathway or the high correlation between PTEN loss and clinicopathologic factors. Lotan et al. (2011) found that when clinicopathologic factors, such as Gleason Score and surgical margin status, were included in their multivariable analysis, PTEN's association with metastasis and prostate cancer-specific mortality decreased significantly.

ArteraAI Prostate Cancer

ArteraAI Prostate Cancer is a multimodal artificial intelligence (MMAI) digital pathology-based post-radical prostatectomy biomarker test that stratifies the risk of metastasis as well as identifies the potential benefits of additional hormone therapy. It is for patients who have been diagnosed with localized prostate cancer who have not yet received radiation therapy (RT) or androgen-deprivation therapy (ADT) before getting a biopsy. The current standard for individuals with intermediate-risk localized prostate cancer includes treatment with ST-ADT in combination with radiation therapy, but a clinical study of the predictive biomarker used in the ArteraAI Prostate test showed that only 34% of patients require radiation therapy. The AI portion of the test "learns" from digital pathology images and clinical data inputs to then predict the likelihood of additional therapy being beneficial, as well provides details as to the

prognosis of the patient. Test results do not require tissue, but rather a validated algorithm is used to assess the "digital image" from a patient's biopsy and clinical data.

The development of the ArteraAI model relied on two trials and utilized clinical data and digital pathology. The 5-year DM area under the curve was 0.83 for ArteraAI, compared with 0.72 for NCCN risk group (P < .001). Results show promising risk stratification for hormonal therapy (Results identified 40% of biochemical recurrence (BCR) patients who benefited from therapy intensification vs. 60% of patients who did not) indicating potential benefits for high-risk patients; this tool is purported to be of use with both newly-diagnosed patients and those that show biochemical recurrence (ArteraAI, 2024; Hannah Clarke, 2024).

Esteva et al. (2022) performed a study to demonstrate the usage of multimodal deep learning architecture to personalize therapy for prostate cancer by predicting long-term outcomes using clinical data and digital histopathology from prostate biopsies. The models, trained and validated on data from the five phase III randomized trials, outperformed the National Cancer Center Network (NCCN) risk groups in terms of discriminatory performance on all endpoints. The AI-based tool (ArteraAI) provided superior prognostication with a relative improvement over these current risk group tools (based on digital rectal exam, serum prostate-specific antigen (PSA) level, and tumor biopsy grade) of between 9.2% to 14.6% in a held-out validation set (Esteva et al., 2022).

VI. Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

Patients with low or favorable intermediate-risk disease and life expectancy ≥10 y may consider the use of the following tumor-based molecular assays: Decipher, Oncotype DX Prostate, and Prolaris. Patients with unfavorable intermediate- and high-risk disease and life expectancy ≥10 y may consider the use of Decipher and Prolaris tumor-based molecular assays. Retrospective studies have shown that molecular assays performed on prostate biopsy or RP specimens provide prognostic information independent of NCCN or CAPRA risk groups. These include, but are not limited to, "likelihood of death with conservative management, likelihood of biochemical recurrence after radical prostatectomy or EBRT, likelihood of adverse pathologic features after radical prostatectomy, and likelihood of developing metastasis after operation, definitive EBRT, or post-recurrence EBRT" (NCCN, 2024a). Furthermore, they note that clinicians may consider testing patients with metastatic prostate cancer and regional prostate cancer for alterations in homologous recombination DNA repair genes *BRCA1*, *BRCA2*, *ATM, PALB2, FANCA, RAD51D*, *CHEK2,* and *CDK12*; "Post-test genetic counseling is recommended if pathogenic/likely pathogenic somatic mutations in any gene that has

clinical implications if also identified in germline (e.g., *BRCA2*, *BRCA1*, *ATM, PALB2, CHEK2, HOXB13, MLH1, MSH2, MSH6, PMS2)"* (NCCN, 2024a). The NCCN noted that somatic tumor testing of the aforementioned genes has potential for early use of platinum chemotherapy, use of PARP inhibitors, or eligibility for clinical trials. Lastly, they recommend that individuals with regional disease, metastatic castration-resistant disease, or castration-naïve metastatic disease should additionally consider tumor testing for microsatellite instability or mismatch repair deficiency. The NCCN also specifically does not recommend either Ki-67 or *PTEN* testing (NCCN, 2024a).

The NCCN does include available tissue-based tests for prostate cancer risk stratification/prognosis within their table of possible testing as indicated below. Regarding Decipher testing, NCCN states that Decipher "may be considered to inform adjuvant treatment if adverse events are found after radical prostatectomy and during workup for radical prostatectomy PSA persistence or recurrence." NCCN discourages repeat molecular tumor analysis (NCCN, 2024a, 2024b):

Test	Platform	Recommendation
Decipher	Whole-Transcriptome 1.4M RNA expression (46,050 genes and non-coding RNA), oligonucleotide microarray optimized for FFPE tissue	Cover post-biopsy for NCCN very-low-, low-risk, favorable intermediate, and unfavorable intermediate risk prostate cancer in patients with at least 10 years life expectancy who have not yet received treatment for prostate cancer and are candidates for active surveillance or definitive therapy. Cover post-RP for 1) pT2 with positive margins; 2) any pT3 disease; 3) rising PSA (above nadir)
KI-67	IHC	Not recommended
Oncotype DX Prostate	Quantitative RT-PCR for 12 prostate cancer-related genes and 5 housekeeping controls	Cover post-biopsy for NCCN very-low-, low-risk, and favorable intermediate-risk prostate cancer in patients with at least 10 years life expectancy who have not yet received treatment for prostate cancer and are candidates for active surveillance or definitive therapy.
Prolaris	Quantitative RT-PCR for 31	Cover post-biopsy for NCCN very-

	cell cycle-related genes and 15 housekeeping controls	low-, low-risk, and favorable intermediate-risk prostate cancer in patients with at least 10 years life expectancy who have not yet received treatment for prostate cancer and are candidates for active surveillance or definitive therapy.
PTEN	Fluorescence in situ hybridization or IHC	Not recommended

The NCCN, within the algorithm for the indications for prostate biopsy, says to "consider biomarkers that improve the specificity of screening" for individuals who have had elevated levels of PSA (above three ng/mL for those ages 45 – 75 years or four ng/mL or higher for those individuals over the age of 75 years. The NCCN goes on to state, "Biomarkers that improve the specificity of detection are not, as yet, mandated as first-line screening tests in conjunction with serum PSA. However, there may be some patients who meet PSA standards for consideration of prostate biopsy, but for whom the patient and/or the physician wish to further define risk. Lower percent-free PSA and/or higher PSA density are associated with a greater risk of high-grade prostate cancer. The probability of high-grade cancer (Gleason score ≥ 3+4, Grade Group two or higher) may be further defined utilizing the Prostate Health Index (PHI), SelectMDx, 4Kscore, and ExoDx Prostate Test, MyProstateScore (MPS), and IsoPSA. Extent of validation of these tests across diverse populations is variable. It is not yet known how such tests could be applied in optimal combination with MRI" (NCCN, 2024b).

The NCCN notes that "Tests that improve specificity in the post-biopsy setting—including percent-free PSA, 4Kscore, PHI, PCA3, ConfirmMDx, ExoDx Prostate Test, MPS, and IsoPSA—should be considered in patients thought to be higher risk despite a negative prostate biopsy" (NCCN, 2024b).The NCCN panel remarks that 4Kscore "can be considered for patients prior to biopsy and for those with a prior negative biopsy who are thought to be at higher risk for clinically significant prostate cancer" The NCCN further remarks that SelectMDx is "potentially informative" in patients who have never undergone biopsy and can therefore be "considered" in these patients. The NCCN also acknowledged that ConfirmMDX can be considered an option for individuals contemplating repeat biopsy and is approved for limited coverage by MolDX to reduce unnecessary repeat biopsies. Further, ExoDx Prostate (IntelliScore), also called EPI, "can be considered as an option for individuals contemplating initial or repeat biopsy because the assay may identify individuals at higher risk of prostate

cancer diagnosis on repeat biopsy." IsoPSA and MyProstrateScore "can be considered for patients prior to biopsy." Lastly, the PCA3 assay can be used to help "decide, along with other factors, whether a repeat biopsy in individuals aged ≥50 years with one or more previous negative prostate biopsies is necessary" (NCCN, 2024b).

The NCCN also lists ArteraAI Prostate Cancer in "Table 2: Risk Stratification, Selected Advanced Tools for Localized Prostate Cancer." The category of the listing is "AI Pathology" and it is listed as "predictive" and "prognostic" for "biochemical recurrence," "distant metastases," and "prostate cancer specific mortality" with a IB category for predictive and IB for prognostic (IB meaning: "Prospective clinical trial(s) using archived samples with design that accommodates tumor marker utility, ≥1 validation study available with consistent results"), it also falls under "**Category 2A:** Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate."(NCCN, 2024a).

American Society of Clinical Oncology (ASCO)

In 2020, an ASCO multidisciplinary panel published guidelines on molecular biomarkers in localized prostate cancer. These guidelines are below.

- "Commercially available molecular biomarkers (i.e., Oncotype Dx Prostate, Prolaris, Decipher, and ProMark) may be offered in situations in which the assay result, when considered as a whole with routine clinical factors, is likely to affect management. Routine ordering of molecular biomarkers is not recommended."
- "Any additional molecular biomarkers evaluated do not have sufficient data to be clinically actionable or are not commercially available and thus should not be offered"
- "The Expert Panel recommends consideration of a commercially available molecular biomarker (e.g., Decipher Genomic Classifier) in situations in which the assay result, when considered as a whole with routine clinical factors, is likely to affect management. In the absence of prospective clinical trial data, routine use of genomic biomarkers in the postprostatectomy setting to determine adjuvant versus salvage radiation or to initiate systemic therapies should not be offered."
- In individuals "with newly diagnosed prostate cancer who are eligible for active surveillance, both genomics and MRI intend to identify clinically significant cancers. The Expert Panel endorses their use only in situations in which the result, when considered as a whole with routine clinical factors, is likely to have an impact on patient management" (Eggener et al., 2020).

In 2020, an ASCO panel published guidelines on the use of molecular biomarkers in localized prostate cancer. In concordance with the 2018 and 2019, ASCO recommends

the use of commercially available tests (Oncotype Dx Prostate, Prolaris, Decipher, and ProMark) when the assay result "is likely to have an impact on patient management. Examples include select individuals with high-volume low-risk or favorable intermediate-risk prostate cancer who are considering active surveillance or in individuals with high-risk features for treatment intensification. While testing may influence management decisions, there is no high-level evidence that the results from these panels will improve quality of life or cancer-specific outcomes" (Eggener et al., 2020).

European Association of Urology (EAU), European Association of Nuclear Medicine (EANM), European Society for Radiotherapy and Oncology (ESTRO), European Society of Urogenital Radiology (ESUR), International Society of Urological Pathology (ISUP), and the International Society of Geriatric Oncology (SIOG)

In 2023, the EAU, EANM, ESTRO, ESUR, ISUP and SIOG released joint guidelines on prostate cancer. These guidelines state that asymptomatic individuals with a prostate-specific antigen level between three and ten ng/mL and a normal digital rectal examination, use one of the following tools for biopsy indication:

- "risk-calculator, provided it is correctly calibrated to the population prevalence(strong);
- magnetic resonance imaging of the prostate (strong).
- An additional serum, urine biomarker test (weak)."

These joint guidelines acknowledged PHI, Progensa *PCA3*, and SelectMDX as tests used to select for repeat biopsies, but stated that "given the limited available data and the fact that the role of MRI in tumour detection was not accounted for, no recommendation can be made regarding the routine application of ConfirmMDX, in particular in light of current use of MRI before biopsy." They also noted that the "clinically added value of SelectMDX in the era of upfront MRI and targeted biopsies remains unclear."

Other tests recognized as having use in the evaluation of prostate cancer included Oncotype Dx, Prolaris, Decipher, Decipher PORTOS and Promark. These five commercially available tests have "extensive validation in large retrospective studies and evidence that their tests results might actually impact clinical decision-taking." However, "since the long-term impact of the use of these commercially available tests on oncological outcome remains unproven and prospective trials are largely lacking, the Panel concluded that these tests should not be offered routinely but only in subsets of patients where the test result provides clinically actionable information." They provide the examples of an individual with favorable intermediate-risk PCa who

decides to continue with active surveillance or an individual with unfavorable intermediate-risk PCa who opts for radiotherapy (RT) to consider treatment intensification with hormonal therapy" (Mottet et al., 2023).

European Society for Medical Oncology (ESMO)

European Society for Medical Oncology (ESMO) provided recommendations on the use of precision medicine in providing prognostic information for prostate cancer. These are the following recommendations provided:

- ESMO does not recommend the use of AR-V7 testing, stating that the test is of limited value in therapy selection.
- Other tissue-based molecular assays may be used on conjunction with clinicopathological factors to make treatment decision.
- Germline testing for *BRCA2* and other DDR [DNA damage and repair] genes is recommended in patients with a family history of cancer and should be considered in patients with metastatic cancer (Parker et al., 2020).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, please visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81313	PCA3/KLK3 (prostate cancer antigen 3 [non-protein coding]/kallikrein-related peptidase 3 [prostate specific antigen]) ratio (eg, prostate cancer)

CPT	Code Description
81479	Unlisted molecular pathology procedure
81539	Oncology (high-grade prostate cancer), biochemical assay of four proteins (Total PSA, Free PSA, Intact PSA, and human kallikrein-2 [hK2]), utilizing plasma or serum, prognostic algorithm reported as a probability score.
81541	Oncology (prostate), mRNA gene expression profiling by real-time RT-PCR of 46 genes (31 content and 15 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a disease-specific mortality risk score
81542	Oncology (prostate), mRNA, microarray gene expression profiling of 22 content genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as metastasis risk score
81551	Oncology (prostate), promoter methylation profiling by real-time PCR of 3 genes (GSTP1, APC, RASSF1), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a likelihood of prostate cancer detection on repeat biopsy
0005U	Oncology (prostate) gene expression profile by real-time RT-PCR of 3 genes (ERG, PCA3, and SPDEF), urine, algorithm reported as risk score Proprietary test: ExoDx® Prostate (IntelliScore) Lab/manufacturer: Exosome Diagnostics, Inc.
0113U	Oncology (prostate), measurement of PCA3 and TMPRSS2-ERG in urine and PSA in serum following prostatic massage, by RNA amplification and fluorescence-based detection, algorithm reported as risk score Proprietary test: MyProstateScore Lab/Manufacturer: Lynx DX
0021U	Oncology (prostate), detection of 8 autoantibodies (ARF 6, NKX3-1, 5'-UTR-BMI1, CEP 164, 3'-UTR-Ropporin, Desmocollin, AURKAIP-1, CSNK2A2), multiplexed immunoassay and flow cytometry serum, algorithm reported as risk score Proprietary test: Apifiny® Lab/Manufacturer: Armune BioScience, Inc.
0047U	Oncology (prostate), mRNA, gene expression profiling by real-time RT-PCR of 17 genes (12 content and 5 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a risk score Proprietary test: Genomic Prostate Score® (GPS) Test Lab/manufacturer: MDxHealth, Inc
0053U	Oncology (prostate cancer), FISH analysis of 4 genes (ASAP1, HDAC9, CHD1 and PTEN), needle biopsy specimen, algorithm reported as probability of higher tumor grade

CPT	Code Description
	Proprietary test: Prostate Cancer Risk Panel Lab/Manufacturer: Mayo Clinic Laboratory
0228U	Oncology (prostate), multianalyte molecular profile by photometric detection of macromolecules adsorbed on nanosponge array slides with machine learning, utilizing first morning voided urine, algorithm reported as likelihood of prostate cancer Proprietary test: PanGIA Prostate Lab/Manufacturer: Genetics Institute of America/Entopsis, LLC
0339U	Oncology (prostate), mRNA expression profiling of HOXC6 and DLX1, reverse transcription polymerase chain reaction (RT-PCR), first-void urine following digital rectal examination, algorithm reported as probability of high-grade cancer Protietary test: SelectMDx® for Prostate Cancer Lab/Manufacturer: MDxHealth®, Inc
0359U	Oncology (prostate cancer), analysis of all prostate-specific antigen (PSA) structural isoforms by phase separation and immunoassay, plasma, algorithm reports risk of cancer Proprietary test: IsoPSA® Lab/Manufacturer: Cleveland Diagnostics, Inc
0403U	Oncology (prostate), mRNA, gene expression profiling of 18 genes, first-catch post-digital rectal examination urine (or processed first-catch urine), algorithm reported as percentage of likelihood of detecting clinically significant prostate cancer Proprietary test: MyProstateScore 2.0 Lab/Manufacturer: LynxDX
0424U	Oncology (prostate), exosome- based analysis of 53 small noncoding RNAs (sncRNAs) by quantitative reverse transcription polymerase chain reaction (RT-qPCR), urine, reported as no molecular evidence, low-, moderate- or elevated-risk of prostate cancer. Proprietary test: miR Sentinel™ Prostate Cancer Test Lab/Manufacturer: miR Scientific®, LLC
0433U	Oncology (prostate), 5 DNA regulatory markers by quantitative PCR, whole blood, algorithm, including prostate-specific antigen, reported as likelihood of cancer. Proprietary test: EpiSwitch® Prostate Screening Test (PSE) Lab/Manufacturer: Oxford BioDynamics PLC

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

ACS. (2024a). *Key Statistics for Prostate Cancer*. Retrieved 5/10/2024 from https://www.cancer.org/cancer/types/prostate-cancer/about/key-statistics.html

ACS. (2024b). *Survival Rates for Prostate Cancer*. https://www.cancer.org/cancer/types/prostate-cancer/detection-diagnosis-staging/survival-rates.html

Alford, A. V., Brito, J. M., Yadav, K. K., Yadav, S. S., Tewari, A. K., & Renzulli, J. (2017). The Use of Biomarkers in Prostate Cancer Screening and Treatment. *Rev Urol*, *19*(4), 221-234. https://pubmed.ncbi.nlm.nih.gov/29472826/

ArteraAI. (2024). *New Data Validating the First AI-Based Biomarker to Stratify Risk of Metastasis in Radical Prostatectomy Patients with Biochemical Recurrence*. https://www.businesswire.com/news/home/20240506052855/en/New-Data-Validating-the-First-AI-Based-Biomarker-to-Stratify-Risk-of-Metastasis-in-Radical-Prostatectomy-Patients-with-Biochemical-Recurrence

Bell, K. J., Del Mar, C., Wright, G., Dickinson, J., & Glasziou, P. (2015). Prevalence of incidental prostate cancer: A systematic review of autopsy studies. *Int J Cancer*, *137*(7), 1749-1757. https://doi.org/10.1002/ijc.29538

Benedettini, E., Nguyen, P., & Loda, M. (2008). The pathogenesis of prostate cancer: from molecular to metabolic alterations. *Diagn Histopathol (Oxf)*, *14*(5), 195-201. https://doi.org/10.1016/j.mpdhp.2008.03.001

Blume-Jensen, P., Berman, D. M., Rimm, D. L., Shipitsin, M., Putzi, M., Nifong, T. P., Small, C., Choudhury, S., Capela, T., Coupal, L., Ernst, C., Hurley, A., Kaprelyants, A., Chang, H., Giladi, E., Nardone, J., Dunyak, J., Loda, M., Klein, E. A., . . . Saad, F. (2015). Development and clinical validation of an in situ biopsy-based multimarker assay for risk stratification in prostate cancer. *Clin Cancer Res*, *21*(11), 2591-2600. https://doi.org/10.1158/1078-0432.Ccr-14-2603

Carneiro, A., Priante Kayano, P., Gomes Barbosa, Á. R., Langer Wroclawski, M., Ko Chen, C., Cavlini, G. C., Reche, G. J., Sanchez-Salas, R., Tobias-Machado, M., Sowalsky, A. G., & Bianco, B. (2018). Are localized prostate cancer biomarkers useful in the clinical practice? *Tumor Biology*, *40*(9), 1010428318799255. https://doi.org/10.1177/1010428318799255

Chen, X., Bernemann, C., Tolkach, Y., Heller, M., Nientiedt, C., Falkenstein, M., Herpel, E., Jenzer, M., Grullich, C., Jager, D., Sultmann, H., Duensing, A., Perner, S., Cronauer, M. V., Stephan, C., Debus, J., Schrader, A. J., Kristiansen, G., Hohenfellner, M., & Duensing, S. (2018). Overexpression of nuclear AR-V7 protein in primary prostate cancer is an independent negative prognostic marker in men with high-risk disease

receiving adjuvant therapy. *Urol Oncol*, *36*(4), 161.e119-161.e130. https://doi.org/10.1016/j.urolonc.2017.11.001

Couñago, F., López-Campos, F., Díaz-Gavela, A. A., Almagro, E., Fenández-Pascual, E., Henríquez, I., Lozano, R., Linares Espinós, E., Gómez-Iturriaga, A., de Velasco, G., Quintana Franco, L. M., Rodríguez-Melcón, I., López-Torrecilla, J., Spratt, D. E., Guerrero, L. L., Martínez-Salamanca, J. I., & Del Cerro, E. (2020). Clinical Applications of Molecular Biomarkers in Prostate Cancer. *Cancers (Basel)*, *12*(6). https://doi.org/10.3390/cancers12061550

Cullen, J., Rosner, I. L., Brand, T. C., Zhang, N., Tsiatis, A. C., Moncur, J., Ali, A., Chen, Y., Knezevic, D., Maddala, T., Lawrence, H. J., Febbo, P. G., Srivastava, S., Sesterhenn, I. A., & McLeod, D. G. (2015). A Biopsy-based 17-gene Genomic Prostate Score Predicts Recurrence After Radical Prostatectomy and Adverse Surgical Pathology in a Racially Diverse Population of Men with Clinically Low- and Intermediate-risk Prostate Cancer. *Eur Urol*, *68*(1), 123-131. https://doi.org/10.1016/j.eururo.2014.11.030

Cuzick, J., Stone, S., Fisher, G., Yang, Z. H., North, B. V., Berney, D. M., Beltran, L., Greenberg, D., Moller, H., Reid, J. E., Gutin, A., Lanchbury, J. S., Brawer, M., & Scardino, P. (2015). Validation of an RNA cell cycle progression score for predicting death from prostate cancer in a conservatively managed needle biopsy cohort. *Br J Cancer*, *113*(3), 382-389. https://doi.org/10.1038/bjc.2015.223

Dalela, D., Löppenberg, B., Sood, A., Sammon, J., & Abdollah, F. (2016). Contemporary Role of the Decipher® Test in Prostate Cancer Management: Current Practice and Future Perspectives. *Rev Urol*, *18*(1), 1-9. https://pubmed.ncbi.nlm.nih.gov/27162506/

Davis, J. W. (2014). Novel commercially available genomic tests for prostate cancer: a roadmap to understanding their clinical impact. *BJU International*, *114*(3), 320-322. https://doi.org/10.1111/bju.12695

Duffy, M. J. (2020). Biomarkers for prostate cancer: prostate-specific antigen and beyond. *Clin Chem Lab Med*, *58*(3), 326-339. https://doi.org/10.1515/cclm-2019-0693

Eggener, S. E., Rumble, R. B., Armstrong, A. J., Morgan, T. M., Crispino, T., Cornford, P., van der Kwast, T., Grignon, D. J., Rai, A. J., Agarwal, N., Klein, E. A., Den, R. B., & Beltran, H. (2020). Molecular Biomarkers in Localized Prostate Cancer: ASCO Guideline. *J Clin Oncol*, Jco1902768. https://doi.org/10.1200/jco.19.02768

Epic Sciences. (2023). *AR-V7 by Epic Sciences*. https://www.epicsciences.com/ar-v7-test/

Esteva, A., Feng, J., van der Wal, D., Huang, S.-C., Simko, J. P., DeVries, S., Chen, E., Schaeffer, E. M., Morgan, T. M., Sun, Y., Ghorbani, A., Naik, N., Nathawani, D., Socher, R., Michalski, J. M., Roach, M., Pisansky, T. M., Monson, J. M., Naz, F., . . . Consortium, N. R. G. P. C. A. (2022). Prostate cancer therapy personalization via multi-modal deep learning on randomized phase III clinical trials. *npj Digital Medicine*, *5*(1), 71. https://doi.org/10.1038/s41746-022-00613-w

Exact Sciences. (2021). *A Tailored Report for Clinically Low Risk Prostate Cancer Patients* https://d2ft3j3kbsqj8w.cloudfront.net/-

/media/Project/PrecisionOncology/PrecisionOncology/OIQ-Reports/Prostate/GPS-Interactive-Report-Tool/GPSRE_GPS_Annotated_Report-2-Pager_M_US_GPS_00374.pdf?rev=50c0c67b355241178c9deba9609ded65

ExoSome. (2024a). *ExoDx™ Prostate Test*. Retrieved 2/8/21 from http://www.exosomedx.com/physicians/exodx-prostate-test

ExoSome. (2024b). *Prostate Cancer*. http://www.exosomedx.com/prostate-cancer-0

Gittelman, M. C., Hertzman, B., Bailen, J., Williams, T., Koziol, I., Henderson, R. J., Efros, M., Bidair, M., & Ward, J. F. (2013). PCA3 molecular urine test as a predictor of repeat prostate biopsy outcome in men with previous negative biopsies: a prospective multicenter clinical study. *J Urol*, *190*(1), 64-69. https://doi.org/10.1016/j.juro.2013.02.018

Graf, R. P., Hullings, M., Barnett, E. S., Carbone, E., Dittamore, R., & Scher, H. I. (2020). Clinical Utility of the Nuclear-localized AR-V7 Biomarker in Circulating Tumor Cells in Improving Physician Treatment Choice in Castration-resistant Prostate Cancer. *Eur Urol*, *77*(2), 170-177. https://doi.org/10.1016/j.eururo.2019.08.020

Hannah Clarke. (2024). *ArteraAI Prostate Test included in NCCN guidelines*. Urology Times. https://www.urologytimes.com/view/arteraai-prostate-cancer-test-included-in-nccn-guidelines

Hoffman, R. (2023). *Screening for prostate cancer*. https://www.uptodate.com/contents/screening-for-prostate-cancer

Hologic. (2024). *Progensa Brochure*. https://stage.hologic.com/sites/default/files/package-insert/Progensa%20PCA3%20Physician%20Brochure-USA.pdf

Hu, J. C., Tosoian, J. J., Qi, J., Kaye, D., Johnson, A., Linsell, S., Montie, J. E., Ghani, K. R., Miller, D. C., Wojno, K., Burks, F. N., Spratt, D. E., & Morgan, T. M. (2018). Clinical Utility of Gene Expression Classifiers in Men With Newly Diagnosed Prostate Cancer. (2), 1-15. https://doi.org/10.1200/po.18.00163

Jia, W., Wu, B., Shao, Y., Cao, X., & Wang, D. (2020). Diagnostic performance of prostate cancer antigen 3 and the Prostate Health Index in detecting overall and clinically significant prostate cancer in men at first biopsy: A meta-analysis. *International Journal of Urology*, *n/a*(n/a). https://doi.org/10.1111/iju.14464

Klein, E. A., Partin, A., Lotan, Y., Baniel, J., Dineen, M., Hafron, J., Manickam, K., Pliskin, M., Wagner, M., Kestranek, A., & Stovsky, M. (2022). Clinical validation of IsoPSA, a single parameter, structure-focused assay for improved detection of prostate cancer: A prospective, multicenter study. *Urol Oncol*, *40*(9), 408 e409-408 e418. https://doi.org/10.1016/j.urolonc.2022.06.002

Lin, D. W., Crawford, E. D., Keane, T., Evans, B., Reid, J., Rajamani, S., Brown, K., Gutin, A., Tward, J., Scardino, P., Brawer, M., Stone, S., & Cuzick, J. (2018). Identification of men with low-risk biopsy-confirmed prostate cancer as candidates for active surveillance.

Urologic Oncology: Seminars and Original Investigations, *36*(6), 310.e317-310.e313. https://doi.org/10.1016/j.urolonc.2018.03.011

Lotan, T. L., Gurel, B., Sutcliffe, S., Esopi, D., Liu, W., Xu, J., Hicks, J. L., Park, B. H., Humphreys, E., Partin, A. W., Han, M., Netto, G. J., Isaacs, W. B., & De Marzo, A. M. (2011). PTEN protein loss by immunostaining: analytic validation and prognostic indicator for a high risk surgical cohort of prostate cancer patients. *Clin Cancer Res*, *17*(20), 6563-6573. https://doi.org/10.1158/1078-0432.Ccr-11-1244

Marascio, J., Spratt, D. E., Zhang, J., Trabulsi, E. J., Le, T., Sedzorme, W. S., Beeler, W. H., Davicioni, E., Dabbas, B., Lin, D. W., Gore, J. L., Bloom, M., Mann, M., Mark, J. R., Calvaresi, A., Godwin, J. L., McCue, P., Hurwitz, M. D., Kelly, W. K., . . . Den, R. B. (2020). Prospective study to define the clinical utility and benefit of Decipher testing in men following prostatectomy. *Prostate Cancer and Prostatic Diseases*, *23*(2), 295-302. https://doi.org/10.1038/s41391-019-0185-7

Matuszczak, M., Schalken, J. A., & Salagierski, M. (2021). Prostate Cancer Liquid Biopsy Biomarkers' Clinical Utility in Diagnosis and Prognosis. *Cancers (Basel)*, *13*(13). https://doi.org/10.3390/cancers13133373

McKiernan, J., Donovan, M. J., Margolis, E., Partin, A., Carter, B., Brown, G., Torkler, P., Noerholm, M., Skog, J., Shore, N., Andriole, G., Thompson, I., & Carroll, P. (2018). A Prospective Adaptive Utility Trial to Validate Performance of a Novel Urine Exosome Gene Expression Assay to Predict High-grade Prostate Cancer in Patients with Prostate-specific Antigen 2-10ng/ml at Initial Biopsy. *Eur Urol*, *74*(6), 731-738. https://doi.org/10.1016/j.eururo.2018.08.019

McKiernan, J., Donovan, M. J., O'Neill, V., Bentink, S., Noerholm, M., Belzer, S., Skog, J., Kattan, M. W., Partin, A., Andriole, G., Brown, G., Wei, J. T., Thompson, I. M., Jr., & Carroll, P. (2016). A Novel Urine Exosome Gene Expression Assay to Predict High-grade Prostate Cancer at Initial BiopsyUrine Exosome Signature to Predict High-Grade Prostate CancerUrine Exosome Signature to Predict High-Grade Prostate Cancer. *JAMA Oncology*, *2*(7), 882-889. https://doi.org/10.1001/jamaoncol.2016.0097

McKiernan, J., Noerholm, M., Tadigotla, V., Kumar, S., Torkler, P., Sant, G., Alter, J., Donovan, M. J., & Skog, J. (2020). A urine-based Exosomal gene expression test stratifies risk of high-grade prostate Cancer in men with prior negative prostate biopsy undergoing repeat biopsy. *BMC Urol*, *20*(1), 138. https://doi.org/10.1186/s12894-020-00712-4

MDxHealth. (2023a). *ConfirmMDx for Prostate Cancer*. https://mdxhealth.com/confirm-mdx-for-physicians/

MDxHealth. (2023b). *SelectMDx for Prostate Cancer*. https://mdxhealth.com/select-mdx-for-physicians/

Mi, C., Bai, L., Yang, Y., Duan, J., & Gao, L. (2021). 4Kscore diagnostic value in patients with high-grade prostate cancer using cutoff values of 7.5% to 10%: A meta-analysis. *Urol Oncol*, *39*(6), 366 e361-366 e310. https://doi.org/10.1016/j.urolonc.2020.11.001

Mottet, N., Cornford, P., van den Bergh, R. C. N., Briers, E., Eberli, D., De Meerleer, G., De Santis, M., Gillessen, S., Grummet, J., Henry, A. M., van der Kwast, T. H., van Leenders, G. J. L. H., Mason, M. D., O'Hanlon, S., van Oort, I. M., Oprea-Lager, D. E., Ploussard, G., Rouvière, O., Schoots, I. G., . . . Willemse, P.-P. (2023). Guidelines on Prostate Cancer. https://d56bochluxqnz.cloudfront.net/documents/full-guideline/EAU-EANM-ESTRO-ESUR-ISUP-SIOG-Guidelines-on-Prostate-Cancer-2023_2023-06-13-141145.pdf

Murphy, A. B., Carbunaru, S., Nettey, O. S., Gornbein, C., Macias, V., Sharifi, R., Kittles, R. A., Yang, X., Kajdacsy-Balla, A., & Gann, P. (2020). A 17-Gene Panel Genomic Prostate Score has Similar Predictive Accuracy for Adverse Pathology at Radical Prostatectomy in African American and European American Men. *Urology*. https://doi.org/10.1016/j.urology.2020.01.052

NCCN. (2024a). *NCCN Clinical Practice Guidelines in Oncology: Prostate Cancer Version 4.2024 — May 17, 2024*. https://www.nccn.org/professionals/physician_gls/pdf/prostate.pdf

NCCN. (2024b). *Prostate Cancer Early Detection Version Version 2.2024 — March 6, 2024*. https://www.nccn.org/professionals/physician_gls/pdf/prostate_detection.pdf

Nguyen, P. L., Huang, H. R., Spratt, D. E., Davicioni, E., Sandler, H. M., Shipley, W. U., Efstathiou, J. A., Simko, J. P., Pollack, A., Dicker, A. P., Roach, M., Rosenthal, S. A., Zeitzer, K. L., Mendez, L. C., Hartford, A. C., Hall, W. A., Desai, A. B., Rabinovitch, R. A., Peters, C. A., . . . Feng, F. Y. (2023). Analysis of a Biopsy-Based Genomic Classifier in High-Risk Prostate Cancer: Meta-Analysis of the NRG Oncology/Radiation Therapy Oncology Group 9202, 9413, and 9902 Phase 3 Randomized Trials. *Int J Radiat Oncol Biol Phys*, *116*(3), 521-529. https://doi.org/10.1016/j.ijrobp.2022.12.035

OPKO. (2021). *OPKO Health Receives U.S. FDA Approval for the 4Kscore® Test*. https://www.opko.com/news-media/press-releases/detail/454/opko-health-receives-u-s-fda-approval-for-the-4kscore

Parekh, D. J., Punnen, S., Sjoberg, D. D., Asroff, S. W., Bailen, J. L., Cochran, J. S., Concepcion, R., David, R. D., Deck, K. B., Dumbadze, I., Gambla, M., Grable, M. S., Henderson, R. J., Karsh, L., Krisch, E. B., Langford, T. D., Lin, D. W., McGee, S. M., Munoz, J. J., . . . Zappala, S. M. (2015). A multi-institutional prospective trial in the USA confirms that the 4Kscore accurately identifies men with high-grade prostate cancer. *Eur Urol*, *68*(3), 464-470. https://doi.org/10.1016/j.eururo.2014.10.021

Parker, C., Castro, E., Fizazi, K., Heidenreich, A., Ost, P., Procopio, G., Tombal, B., & Gillessen, S. (2020). Prostate cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*, *31*(9), 1119-1134. https://doi.org/10.1016/j.annonc.2020.06.011

Punnen, S., Freedland, S. J., Polascik, T. J., Loeb, S., Risk, M. C., Savage, S., Mathur, S. C., Uchio, E., Dong, Y., & Silberstein, J. L. (2018). A Multi-Institutional Prospective Trial

Confirms Noninvasive Blood Test Maintains Predictive Value in African American Men. *J Urol*, *199*(6), 1459-1463. https://doi.org/10.1016/j.juro.2017.11.113

Rodríguez, S. V. M., & García-Perdomo, H. A. (2020). Diagnostic accuracy of prostate cancer antigen 3 (PCA3) prior to first prostate biopsy: A systematic review and meta-analysis. *Can Urol Assoc J*, *14*(5), E214-e219. https://doi.org/10.5489/cuaj.6008

Ross, A. E., D'Amico, A. V., & Freedland, S. J. (2022). *Molecular prognostic tests for prostate cancer*. Wolters Kluwer. Retrieved 10/10/2023 from https://www.uptodate.com/contents/molecular-prognostic-tests-for-prostate-cancer

Sathianathen Niranjan, J., Kuntz Karen, M., Alarid-Escudero, F., Lawrentschuk Nathan, L., Bolton Damien, M., Murphy Declan, G., Weight Christopher, J., & Konety Badrinath, R. (2018). Incorporating Biomarkers into the Primary Prostate Biopsy Setting: A Cost-Effectiveness Analysis. *Journal of Urology*, *200*(6), 1215-1220. https://doi.org/10.1016/j.juro.2018.06.016

Scher, H. I., Lu, D., Schreiber, N. A., Louw, J., Graf, R. P., Vargas, H. A., Johnson, A., Jendrisak, A., Bambury, R., Danila, D., McLaughlin, B., Wahl, J., Greene, S. B., Heller, G., Marrinucci, D., Fleisher, M., & Dittamore, R. (2016). Association of AR-V7 on Circulating Tumor Cells as a Treatment-Specific Biomarker With Outcomes and Survival in Castration-Resistant Prostate CancerAR-V7 Expression in Circulating Tumor Cells and Castration-Resistant Prostate Cancer OutcomesAR-V7 Expression in Circulating Tumor Cells and Castration-Resistant Prostate Cancer Outcomes. *JAMA Oncology*, *2*(11), 1441-1449. https://doi.org/10.1001/jamaoncol.2016.1828

Scovell, J. M., Hettel, D., Abouassaly, R., Almassi, N., Berglund, R., Breaux, T., Weight, C., Isac, W., Zampini, A., Stark, E., Rochelle, R., Kestranek, A., Stovsky, M., & Klein, E. (2022). IsoPSA® Reduces Provider Recommendations for Biopsy and Magnetic Resonance Imaging in Men with Total Prostate Specific Antigen ≥4 ng/ml: A Real-World Observational Clinical Utility Study. https://doi.org/10.1097/UPJ.0000000000000291

Shore, N. (2018). SelectMDx Impacts Prostate Biopsy Decision-making in Routine Clinical Practice. https://abstracts.mirrorsmed.org/abstracts/selectmdx-impacts-prostate-biopsy-decision-making-routine-clinical-practice

Song, C. J., Chen, H., Chen, L. Z., Ru, G. M., Guo, J. J., & Ding, Q. N. (2018). The potential of microRNAs as human prostate cancer biomarkers: A meta-analysis of related studies. *J Cell Biochem*, *119*(3), 2763-2786. https://doi.org/10.1002/jcb.26445

Spratt, D. E., Liu, V. Y. T., Michalski, J., Davicioni, E., Berlin, A., Simko, J. P., Efstathiou, J. A., Tran, P. T., Sandler, H. M., Hall, W. A., Thompson, D. J. S., Parliament, M. B., Dayes, I. S., Correa, R. J. M., Robertson, J. M., Gore, E. M., Doncals, D. E., Vigneault, E., Souhami, L., . . . Feng, F. Y. (2023). Genomic Classifier Performance in Intermediate-Risk Prostate Cancer: Results From NRG Oncology/RTOG 0126 Randomized Phase 3 Trial. *International Journal of Radiation Oncology, Biology, Physics*, *117*(2), 370-377. https://doi.org/10.1016/j.ijrobp.2023.04.010

Steiling, K., & Christenson, S. (2023, 10/10/2023). *Tools for genetics and genomics: Gene expression profiling*. https://www.uptodate.com/contents/tools-for-genetics-and-genomics-gene-expression-profiling

Stovsky, M., Klein, E. A., Chait, A., Manickam, K., Stephenson, A. J., Wagner, M., Dineen, M., Lotan, Y., Partin, A., Baniel, J., Kestranek, A., Gawande, P., & Boris, Z. (2019). Clinical Validation of IsoPSA, a Single Parameter, Structure Based Assay for Improved Detection of High Grade Prostate Cancer. *J Urol*, *201*(6), 1115-1120. https://doi.org/10.1097/JU.0000000000000185

Taplin, M.-E., & Smith, J. A. (2024, 03/22/2024). *Clinical presentation and diagnosis of prostate cancer*. https://www.uptodate.com/contents/clinical-presentation-and-diagnosis-of-prostate-cancer

Tomlins, S. A., Day, J. R., Lonigro, R. J., Hovelson, D. H., Siddiqui, J., Kunju, L. P., Dunn, R. L., Meyer, S., Hodge, P., Groskopf, J., Wei, J. T., & Chinnaiyan, A. M. (2016). Urine TMPRSS2:ERG Plus PCA3 for Individualized Prostate Cancer Risk Assessment. *Eur Urol*, *70*(1), 45-53. https://doi.org/10.1016/j.eururo.2015.04.039

Tosoian, J. J., Trock, B. J., Morgan, T. M., Salami, S. S., Tomlins, S. A., Spratt, D. E., Siddiqui, J., Kunju, L. P., Botbyl, R., Chopra, Z., Pandian, B., Eyrich, N. W., Longton, G., Zheng, Y., Palapattu, G. S., Wei, J. T., Niknafs, Y. S., & Chinnaiyan, A. M. (2021). Use of the MyProstateScore Test to Rule Out Clinically Significant Cancer: Validation of a Straightforward Clinical Testing Approach. *J Urol*, *205*(3), 732-739. https://doi.org/10.1097/JU.0000000000001430

Tutrone, R., Donovan, M. J., Torkler, P., Tadigotla, V., McLain, T., Noerholm, M., Skog, J., & McKiernan, J. (2020). Clinical utility of the exosome based ExoDx Prostate(IntelliScore) EPI test in men presenting for initial Biopsy with a PSA 2-10 ng/mL. *Prostate Cancer Prostatic Dis*. https://doi.org/10.1038/s41391-020-0237-z

Tward, J. D., Schlomm, T., Bardot, S., Freedland, S. J., Lenz, L., Cohen, T., Stone, S., & Bishoff, J. (2020). Ability of the combined clinical cell-cycle risk score to identify patients that benefit from multi versus single modality therapy in NCCN intermediate and high-risk prostate cancer. *Journal of Clinical Oncology*, *38*(6_suppl), 346-346. https://doi.org/10.1200/JCO.2020.38.6_suppl.346

Van den Broeck, T., Moris, L., Gevaert, T., Tosco, L., Smeets, E., Fishbane, N., Liu, Y., Helsen, C., Margrave, J., Buerki, C., Davicioni, E., Van Poppel, H., Everaerts, W., Weinmann, S., Den, R., Davis, J., Schaeffer, E., Karnes, R. J., Claessens, F., & Joniau, S. (2019). Validation of the Decipher Test for Predicting Distant Metastatic Recurrence in Men with High-risk Nonmetastatic Prostate Cancer 10 Years After Surgery. *Eur Urol Oncol*, *2*(5), 589-596. https://doi.org/10.1016/j.euo.2018.12.007

Van Neste, L., Hendriks, R. J., Dijkstra, S., Trooskens, G., Cornel, E. B., Jannink, S. A., de Jong, H., Hessels, D., Smit, F. P., Melchers, W. J., Leyten, G. H., de Reijke, T. M., Vergunst, H., Kil, P., Knipscheer, B. C., Hulsbergen-van de Kaa, C. A., Mulders, P. F., van Oort, I. M., Van Criekinge, W., & Schalken, J. A. (2016). Detection of High-grade

Prostate Cancer Using a Urinary Molecular Biomarker-Based Risk Score. *Eur Urol*, *70*(5), 740-748. https://doi.org/10.1016/j.eururo.2016.04.012

Van Neste, L., Partin, A. W., Stewart, G. D., Epstein, J. I., Harrison, D. J., & Van Criekinge, W. (2016). Risk score predicts high-grade prostate cancer in DNA-methylation positive, histopathologically negative biopsies. *Prostate*, *76*(12), 1078-1087. https://doi.org/10.1002/pros.23191

White, J., Shenoy, B. V., Tutrone, R. F., Karsh, L. I., Saltzstein, D. R., Harmon, W. J., Broyles, D. L., Roddy, T. E., Lofaro, L. R., Paoli, C. J., Denham, D., & Reynolds, M. A. (2018). Clinical utility of the Prostate Health Index (phi) for biopsy decision management in a large group urology practice setting. *Prostate Cancer Prostatic Dis*, *21*(1), 78-84. https://doi.org/10.1038/s41391-017-0008-7

Wojno, K. J., Costa, F. J., Cornell, R. J., Small, J. D., Pasin, E., Van Criekinge, W., Bigley, J. W., & Van Neste, L. (2014). Reduced Rate of Repeated Prostate Biopsies Observed in ConfirmMDx Clinical Utility Field Study. *Am Health Drug Benefits*, *7*(3), 129-134. https://pubmed.ncbi.nlm.nih.gov/24991397/

Wysock, J. S., Becher, E., Persily, J., Loeb, S., & Lepor, H. (2020). Concordance and Performance of 4Kscore and SelectMDx for Informing Decision to Perform Prostate Biopsy and Detection of Prostate Cancer. *Urology*, *141*, 119-124. https://doi.org/10.1016/j.urology.2020.02.032

Zappala, S. M., Scardino, P. T., Okrongly, D., Linder, V., & Dong, Y. (2017). Clinical performance of the 4Kscore Test to predict high-grade prostate cancer at biopsy: A meta-analysis of us and European clinical validation study results. *Rev Urol*, *19*(3), 149-155. https://pubmed.ncbi.nlm.nih.gov/29302237/

General Genetic Testing, Germline Disorders

Policy Number: AHS – M2145 – General Genetic Testing Germline Disorders	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUNDU | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Germline variants or mutations are defined as genetic alterations that occur within the germ cells (egg or sperm), such that the alteration becomes incorporated into the DNA of every cell in the body of the offspring. It may also be called a hereditary mutation (Li et al., 2017; NCI, 2024).

Genetic testing refers to the use of technologies that identify genetic variation, which include genomic, transcriptional, proteomic, and epigenetic alterations, for the prevention, diagnosis, and treatment of disease (Kohlmann & Slavotinek, 2024; Li et al., 2017).

II. Related Policies

Policy Number	Policy Title
AHS-M2066	Genetic Cancer Susceptibility Using Next Generation Sequencing
AHS-M2146	General Genetic Testing, Somatic Disorders

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Single gene or multi-gene panel testing (see Note 1 and Note 2) for inherited diseases **MEETS COVERAGE CRITERIA** (once per patient lifetime) when **one** of the following criteria are met:

   a) The individual is currently symptomatic with the suspicion of a known genetic disease in which knowledge of the likely pathogenic or pathogenic variant will assist in the diagnosis, treatment, or procreative management.

b) For asymptomatic individuals who are judged to be at significant risk (based on family history and/or ethnicity) for an inherited disorder or an inherited cancer risk factor, **and** meet one of the following conditions:

   i) The individual is being tested for their risk of an adult-onset condition and is at or above the age of majority, (e.g., 18 years).

   ii) An individual not at or above the age of majority is being tested for their risk of an adult-onset condition for which there is documented evidence that early intervention during childhood may prevent disease severity or time of disease onset.

c) For asymptomatic individuals who are **both**:

   i) judged to be at risk as a carrier of an inherited disorder or cancer risk factor based on family history and/or ethnicity;

   ii) would benefit from procreative management.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

2) The following genetic tests for inherited diseases **DO NOT MEET COVERAGE CRITERIA**:

   a) Tests for genes that do not meet the above criteria.

   b) Inherited disease diagnosis or carrier assessment using panels of genes that include genes outside of those specifically related to the disease being investigated.

   c) Repeat germline testing of a unique gene using the identical method of gene analysis.

   d) Testing as a screening tool for the general population.

   e) Direct-to-consumer genetic testing (e.g., mail order, online ordering, pharmacy, retail).

NOTES:

Note 1: Genetic tests being considered must meet all of the following conditions:

a) Scientific literature shows that a specific a gene likely pathogenic or pathogenic variant (or variants) is associated with the disease in question and that

identification of the variant is clinically actionable (there is clinical utility) with a non-investigational treatment;

b) When confirmation of a gene likely pathogenic or pathogenic variant is standard of care for the disease state and other testing for the disease is either equivocal or does not exist;
c) The disease in question is associated with significant morbidity and/or mortality;
d) The results of testing can impact clinical management (via surveillance or treatment strategies) and will guide decisions on healthcare management to mitigate symptoms or progression of the disorder.

Note 2: For two or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
ACMG	American College of Medical Genetics and Genomics
AMP	Association For Molecular Pathology
APC	*Adenomatous polyposis coli*
ASCO	American Society of Clinical Oncology
ATM	*Ataxia telangiectasia mutated*
BRCA1/2	*Breast cancer gene 1/2*
CAP	College of American Pathologists
CDH1	*Cadherin-1*
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CNV	Copy number variant
CSG	Cancer susceptibility gene
ctDNA	Circular tumor deoxyribonucleic acid
FDA	Food and Drug Administration
EMSO	European Society for Medical Oncology
LDTs	Laboratory-developed tests
LOF	Loss-of-function
MGPT	Multi-gene panel testing
NCCN	National Comprehensive Cancer Network
NGS	Next-generation sequencing
PALB2	*Partner and localizer of BRCA2*
PCR	Polymerase chain reaction
PTEN	*Phosphatase and tensin homolog*
PVG	Pathogenic germline variants

smMIPS	Single molecule molecular inversion probes
SNPs	Single nucleotide polymorphisms
TP53	*Tumor protein P53*
VUS	Variant of uncertain significance

V. Scientific Background

Gene mutations are referred to as "germline" if they are within gametes (ova and sperm). Therefore, these mutations may be passed on from parent to offspring (Raby & Blank, 2024). There are many different types of germline mutations, such as single nucleotide polymorphisms (SNPs), structural variations such as deletions, inversions, or translocations, as well as smaller chromosomal abnormalities such as short tandem repeats, or gene fusions. Mutations may not necessarily result in disease (Christensen & Hulick, 2024).

Single nucleotide polymorphisms are the most common type of genetic mutation, such as missense mutations. These mutations are single base-pair changes where one nucleotide is replaced with a different nucleotide. Millions of SNPs have been identified through genome-wide association studies, approximately 4000 SNPs have a potential association with disease (Attia, 2024). Insertion/deletion (indel) polymorphisms are often a single nucleotide but may be up to four nucleotides. SNPs often lead to frameshift mutations, which can cause premature stop codons and the failure of the allele (Kohlmann & Slavotinek, 2024).

Structural variations are usually classified as larger than 1000 base pairs. These include deletions, duplications, inversions, translocations, or ring chromosome formation. Due to the large number of bases affected, these variations may lead to severe genetic abnormalities. For example, a major cause of Duchenne muscular dystrophy is the deletion of large portions of exons (coding portions of genes). The most common structural variation is the copy number variant (CNV), which refers to differing amounts of DNA segments in different individuals. For example, one person may have three copies of a specific segment whereas another may only have two. These variations may lead to dysregulation, gain-of-function, or loss-of-function of the affected genes. The sensitive genes that require or produce precise amounts of a protein product tend to suffer more from these variations (Bacino, 2023).

Germline mutations are unique in that the risk for certain conditions, including many forms of cancer, may be passed from parent to offspring. Testing for these conditions will often involve testing entire families if one member is found to have a germline

mutation; for example, the National Comprehensive Cancer Network (NCCN) guidelines for hereditary breast cancer recommend testing for high-penetrance breast cancer susceptibility genes such as *BRCA1/2, CDH1, PALB2, PTEN, STK*, and *TP53* mutations if any blood relative has a known or likely pathogenic variant in a cancer susceptibility gene (NCCN, 2024a). Wilson et al. (2020) estimate that 21,800 adult survivors of childhood cancer in the United States carry a pathogenic or likely pathogenic variant in one of 156 cancer predisposition genes.

Some types of mutations are unique to germline mutations. Errors in chromosome number (aneuploidy) are typically caused by nondisjunctions in meiosis, causing either a monosomic (one chromosome) or a trisomic (three chromosomes) set of chromosomes. Some aneuploidies, trisomy 21, or Down Syndrome, being most notable, are compatible with life. Aneuploidies may also result with sex chromosomes, resulting in conditions such as Turner's Syndrome (one X chromosome) or Klinefelter's Syndrome (XXY) (Bacino, 2024; Schrijver & Zehnder, 2024).

Any size mutation may be pathogenic and must be classified as to how likely they are to cause disease. The American College of Medical Genetics and Genomics (ACMG) has classified mutations in five categories, which are as follows: pathogenic, likely pathogenic, uncertain significance, likely benign, and benign. The "likely pathogenic" and "likely benign" refer to weaker evidence than their respective pathogenic and benign categories, and "uncertain significance" refers to evidence that does not meet criteria for benignity or pathogenicity or has conflicting evidence from both sides (Christensen & Hulick, 2024). Prediction algorithms have been used to interpret variants and to predict whether a variant will affect the gene function or splicing of the gene. These algorithms are publicly available but have a tendency of predicting harmful impact of a variant. The specificity of these databases has been estimated at 60-80% (Li et al., 2017).

Due to the enormous number of variants, as well as the rate that variants are discovered, comprehensive databases of genetic variants have been published and are easily available. For example, the Haplotype Reference Consortium contains over 40 million identified SNPs (Christensen & Hulick, 2024). Databases focusing on cancer-specific variants, reference sequences, and the general population are all available publicly (Li et al., 2017).

For many years, single-gene testing was the standard approach for germline mutation testing. In recent years, multi-gene panel testing (MGPT) has been introduced and

widely accepted as the first-tier test. MGPT increases the probability of identifying pathogenic mutations and represents an affordable application of next-generation sequencing (NGS) into clinical practice. However, the clinical utility of MGPT is not well established, especially in cases where more than one pathogenic variant is identified. The risk for a specific malignancy is complex and if a gene panel discovers a mutation incidentally, management can be difficult. Many guidelines call for radical procedures for these disease states and it may cause unnecessary harm for the patient concerned about predisposition to the disease. Additionally, a combination of mutations may interact to alter the profile of the disease. For instance, certain combinations of mutations may be detrimental and increase the overall risk of cancer malignancy, while other combinations may reduce overall risk of malignancy. In this regard, identifying clinically actionable mutations may be unclear with MGPT (Slaught et al., 2021).

Clinical Utility and Validity

Genetic testing for germline mutations "can be conducted on virtually any tissue type," although many laboratories prefer blood samples, check swabs or saliva samples (Kohlmann & Slavotinek, 2024). Advancements in technology and availability of sequencing, previously constrained by limitations of sequential single-gene testing on limited patient samples, have led to significant strides in the understanding of the genetic basis of inherited and somatic conditions.

Variants detected by genetic testing include inherited germline variants and somatic mutations; NGS has allowed for superior detection for these mutations (Konnick & Pritchard, 2016). The accuracy of NGS varies depending on how many genes are sequenced; fewer genes tend to result in higher accuracy since there will be more "probe-template overlap." Although Sanger sequencing remains the most accurate at >99.99% accuracy, it cannot sequence a large quantity of genes in a timely fashion and is best used for sequencing of a specific gene (Hulick, 2024). Pogoda et al. (2019) identified rare variants in the *ATM* gene by using single molecule molecular inversion probes (smMIPSs), an NGS-based screening method. A total of 373 patients with dystonia and six positive controls with previously identified *ATM* variants participated in this study. Results generated by the smMIPs "produced similar results as routinely used NGS-based approaches" (Pogoda et al., 2019). This suggests that *ATM* screening should be routinely used when genetic testing dystonia patients. Further, smMIPs may be an important technique for the germline screening for all rare neurodegenerative disorders.

The clinical validity of a genetic test depends primarily on the expressivity and penetrance of a given phenotype. Penetrance refers to the likelihood of developing a disease when the pathogenic mutation is present, and expressivity refers to the variations in the way the disease is expressed. For example, virtually any mutation in the *APC* gene will cause symptoms of familial adenomatous polyposis, thereby increasing the clinical validity of an *APC* assessment while other conditions may not clinically manifest at all despite a mutated genotype (Kohlmann & Slavotinek, 2024).

The clinical utility of a genetic test generally relies on available treatments for a condition. Conditions such as Huntington Disease that do not have many options for treatment will have limited clinical utility compared to another condition even though the actual test is highly valid. Factors, such as severity of the disease and management options, affect the clinical utility of a genetic test (Kohlmann & Slavotinek, 2024).

Lincoln et al. (2020) performed a retrospective study to investigate the yield and utility of germline testing on cancer patients following tumor DNA sequencing. The authors calculated the prevalence of pathogenic germline variants (PVG) and the potential actionability of the PVGs in 2023 cancer patients. A total of 30.5% (n=617) of participants had PVGs. Participants with PVGs spanned all ages and cancer types. Tumor DNA sequencing missed 8.1% of PGVs. A total of 11.2% of missed PVGs were only detected after developing a second primary cancer. The results suggest that missed PVGs could have been detected earlier and the second cancer could have been treated earlier or prevented. The authors concluded that germline testing following tumor DNA sequencing can result in important findings that can impact patient care (Lincoln et al., 2020).

There is an ethical concern associated with genetic testing for germline disorders, and patients can have mixed preferences about receiving their results. Although the information can be clinically useful, it can also be burdensome knowledge on patients and their families. Best et al. (2022) studied the preferences on receiving results in patients who have undergone germline genome sequencing. The study included 335 cancer patients and 199 of their relatives, all of whom were undergoing germline genome sequencing. "A significantly higher percentage of probands thought people would like to be informed about genetic conditions for which there is prevention or treatment that can change cancer risk compared to conditions for which there is no prevention or treatment (93% [311] versus 65% [216]; $p < 0.001$). Similar results were obtained for relatives (91% [180] versus 61% [121]; $p < 0.001$)." The authors also conducted interviews with 40 participants and identified four themes: "1) Recognised benefits of GS, (2) Balancing benefits with risks, (3) Uncertain results are perceived as unhelpful and (4) Competing obligations." The authors conclude by noting the

importance in ensuring patient understanding of the relevant test validity and consent options (Best et al., 2022).

VI. Guidelines and Recommendations

American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP)

The ACMG and AMP released criteria on the types and severity of mutations, which are as follows:

- **Very strong evidence of pathogenicity**: Null variants (nonsense, frameshifts, canonical +/- one to two splice sites, initiation codon, exon deletions) in a gene where loss-of-function (LOF) is a known mechanism of disease. The guidelines note to use caution in genes where LOF is not a mechanism, if LOF variants are at the 3' end, if exon skipping occurs, and if multiple transcripts are present.
- **Strong:** Amino acid change to a pathogenic version, de novo mutations, established studies supporting a damaging gene or gene product, or if the prevalence of the variant is increased in affected individuals compared to healthy controls. The guidelines note to be careful of changes impacting splicing and if only the paternity has been confirmed.
- **Moderate:** Located in a mutational hot spot or well-established functional domain (e.g., active site of an enzyme) without a benign variation, absent from controls in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium, detected in *trans* with pathogenic variants for a recessive disorder, protein length changes, novel missense changes where a different missense change has been pathogenic before, and a possible de novo mutation.
- **Supporting:** Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease, missense variant in a gene with low rate of benign missense variation, if the mutation has evidence that it is deleterious, or if the patient's phenotype is highly specific for disease with a single genetic cause.

The guidelines also list criteria for benign gene variants.

- **Stand-alone evidence of benignity:** Allele frequency is > five percent in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium.

- **Strong:** Allele frequency is greater than expected for disorder, observed in healthy adult with full penetrance at early age, lack of segregation in affected family members (although pathogenic variants may masquerade as nonsegregated), or well-established studies that show no damaging effect on protein production.
- **Supporting:** Missense variant of a gene for which truncating mutations are pathogenic, indels in repetitive region of unknown function, silent variants, variants of unknown significance, or a *trans* version of a *cis* mutation (Richards et al., 2015).

National Comprehensive Cancer Network (NCCN)

Germline mutations have been incorporated into the diagnostic workups recommended by the NCCN. Furthermore, the NCCN has several guidelines which recommend that gene expression profiling, or multiple gene testing, may be helpful, more efficient and/or cost effective for selected patients (NCCN, 2024b).

The NCCN includes some general testing criteria. They believe that testing is indicated in the following scenarios:

- "Individuals with any blood relative with a known P/LP variant in a cancer susceptibility gene"
- "Individuals meeting the criteria below but who tested negative with previous limited testing (eg, single gene and/or absent deletion duplication analysis) and are interested in pursuing multi-gene testing"
- "A P/LP variant identified on tumor genomic testing that has clinical implications if also identified in the germline"
- "To aid in systemic therapy and surgical decision-making" (NCCN, 2024a).

It is also notes that testing may be considered in the following scenario with appropriate pre-test education and access to post-test management: an individual of Ashkenazi Jewish ancestry without additional risk factors and with a personal history of serous endometrial cancer (NCCN, 2024a).

Regarding the choice of multi-gene testing, the NCCN states that:

- "The introduction of multi-gene testing for hereditary forms of cancer has rapidly altered the clinical approach to hereditary cancer testing of patients at increased risk of inherited susceptibility to cancer and their families. Based on next-

generation sequencing (NGS) technology, these tests simultaneously analyze a set of genes that are associated with a specific family cancer phenotype or multiple phenotypes."

- "An individual's personal and/or family history may be explained by more than one inherited cancer syndrome; thus, phenotype-directed testing based on personal and family history through a tailored multi-gene panel test is often more efficient and cost effective and increases the yield of detecting a P/LP variant in a gene that will impact medical management for the individual or their family members with increased risk."
    - The NCCN explains here that 'tailored' is "defined as a disease-focused multi-gene panel of clinically actionable cancer susceptibility genes, in contrast to large multi-gene panels of uncertain or unknown clinical relevance."
- "There may also be a role for multi-gene testing in individuals who have tested negative for a single syndrome, but whose personal or family history remains suggestive of an inherited susceptibility."
- "Some individuals may carry P/LP germline variants in more than one cancer susceptibility gene; thus, consideration of a multi-gene panel for individuals already known to carry a single P/ LP germline variant from phenotype-directed testing may be considered on a case-by-case basis, based on the degree of suspicion for there being additional variants."
- "Because commercially available tests differ in the specific genes analyzed, variant classification, and other factors (eg, methods of DNA/RNA analysis or option to reflex from a narrow to a larger panel; provision of financial assistance for cascade testing of relatives), it is important to consider the indication for testing and expertise of the laboratory when choosing the specific laboratory and test panel."
- "Multi-gene testing can include "intermediate" penetrant (moderate-risk) genes." This is because "For many of these genes, there are limited data on the degree of cancer risk, and there may currently be no clear guidelines on risk management for carriers of P/LP variants. Not all genes included on available multi-gene tests will change risk management compared to that based on other risk factors such as family history."
- "It may be possible to refine risks associated with both moderate and high penetrance genes, taking into account the influence of gene/gene or gene/ environment interactions. In addition, certain P/LP variants in a gene may pose higher or lower risk than other P/LP variants in that same gene. This information

should be taken into consideration when assigning risks and management recommendations for individuals and their relatives who also have increased risk."
- "P/LP variants in many breast, ovarian, pancreatic, and prostate cancer susceptibility genes involved in DNA repair may be associated with rare autosomal recessive conditions, thus posing risks to offspring if the partner is also a carrier."
- "As more genes are tested, there is an increased likelihood of finding VUS, mosaicism, and clonal hematopoiesis of indeterminate potential."
- "When a P/LP variant with clinical implications for the patient and/or their family members is found on tumor genomic testing, germline confirmatory testing should be recommended."
- "There are significant limitations in interpretation of polygenic risk scores (PRS). PRS should not be used for clinical management at this time and use is recommended in the context of a clinical trial, ideally including diverse populations" (NCCN, 2024a).

For more information, please see the individual policies.

Association for Molecular Pathology (AMP), American Society of Clinical Oncology (ASCO), and College of American Pathologists (CAP)

The Joint Commission noted that germline variants should focus on the pathogenicity of a given variant rather than their impact on clinical care. The guidelines recommend reporting germline variants with known clinical impact, such as *BRCA1* or *2*. A genetic counseling recommendation should also be provided if a pathogenic germline mutation is found (Li et al., 2017).

The guidelines note that it is critical to identify a somatic vs a germline mutation as the type of mutation may have significant clinical consequences (Li et al., 2017).

American Society of Clinical Oncology (ASCO)

The ASCO published guidelines regarding genetic and genomic testing for cancer susceptibility. These guidelines state that the "ASCO recognizes that concurrent multigene testing (i.e., panel testing) may be efficient in circumstances that require evaluation of multiple high-penetrance genes of established clinical utility as possible explanations for a patient's personal or family history of cancer. Depending on the specific genes included on the panel employed, panel testing may also identify mutations in genes associated with moderate or low cancer risks and mutations in

high-penetrance genes that would not have been evaluated on the basis of the presenting personal or family history… ASCO affirms that it is sufficient for cancer risk assessment to evaluate genes of established clinical utility that are suggested by the patient's personal and/or family history" (Robson et al., 2015).

The ASCO released guidelines regarding germline testing for epithelial ovarian cancer. ASCO recommends that "all [individuals] diagnosed with epithelial ovarian cancer should be offered germline genetic testing for *BRCA1, BRCA2*, and other ovarian cancer susceptibility genes, irrespective of their clinical features or family cancer history." In addition, "first- or second-degree blood relatives of a patient with ovarian cancer with a known germline pathogenic cancer susceptibility gene mutation or variant should be offered individualized genetic risk evaluation, counseling, and genetic testing." Lastly, "clinical decisions should not be based on a variant of uncertain significance (VUS)." In this case, the patient's clinical features and family history should be taken into consideration and should inform clinical decision making" (Konstantinopoulos et al., 2020).

The ASCO released guidelines for the selection of germline genetic testing panels in patients with cancer. ASCO recommends "all patients should have a family history taken and recorded that includes details of cancers in first- and second-degree relatives and the patient's ethnicity." The guidelines went on to add that "when germline genetic testing is indicated for a patient with cancer, multi-gene panel testing should be offered if more than one gene is relevant" (Tung et al., 2024).

European Society for Medical Oncology (EMSO)

The EMSO published recommendations on the use of circular tumor DNA (ctDNA) assays in patients with cancer. Regarding germline disorders, the authors report that "Pathogenic germline variants in cancer susceptibility genes may be detected in ctDNA (such as BRCA1, BRCA2, PALB2), and detection of such variants requires reflex germline testing with a validated assay to confirm somatic versus germline nature." They also note that "Caution should be carried out in interpretation of pathogenic variants in high penetrance cancer susceptibility genes (such as BRCA1, BRCA2, PALB2); validated germline testing should be carried out to confirm germline or somatic nature" (Pascual et al., 2022).

The ESMO reports that ctDNA assays are validated and sensitive enough to "genotype advanced cancers and select patients for targeted therapies." They note that ctDNA assay results are limited by false-negative results and lower sensitivity for fusion and

copy number changes, and ctDNA should not be used to detect molecular residual disease (Pascual et al., 2022).

The ESMO released recommendations for germline-focussed analysis of tumor-only sequencing:

1. Germline-focussed tumour analysis should be carried out in all laboratories as part of routine analysis of a large tumour panel.
2. Germline-focussed tumour analysis can be delivered via an automated pipeline so as not to add substantial additional manual work, cost or delay to tumour analysis.
3. Variants in should be flagged which are (i) predicted to result in protein truncation in genes acting through LOF and/or (ii) classified as Pathogenic/Likely Pathogenic via a well-maintained, comprehensive and curated clinical resource (ClinVar is recommended).
4. Germline-focussed tumour analysis can be restricted to variants of VAF >30% (SNVs) or >20% (small insertions/deletions). Local validation will be required to confirm the accuracy of tumour VAF estimates, especially for PCR-based NGS methodologies.
5. Samples known or suspected to be hypermutated should be included for germline-focussed tumour analysis.
6. Germline-focussed tumour analysis in the off-tumour context should be restricted to 'High Actionability- [cancer susceptibility genes] CSGs'.
7. Recessively acting 'High Actionability-CSGs' (currently *MUTYH* alone) should be included for germline-focussed tumour analysis but reporting and germline follow-up testing should be undertaken only on detection of two pathogenic variants.
8. Germline-focussed tumour analysis of 'standard actionability'-CSGs should be restricted to the on-tumour setting.
9. 'Standard actionability'-CSGs included for germline-focussed tumour analysis can be restricted to genes of high penetrance.
10. Germline-focussed tumour analysis can be restricted to gene-scenarios for which the germline conversion rate is >10%. For selected genes, it may therefore be appropriate to restrict germline-focussed tumour analysis to just those tumours arising age <30 years.
11. Formal variant review and classification should be undertaken by an experienced clinical scientist before initiation of patient re-contact and/or germline testing.
12. Before analysis of their germline sample for the pathogenic variant, adequate information should be provided to the patient regarding the implications of germline testing, along with documentation of their consent.

13. The tumour-observed pathogenic variant should be analyzed in an appropriate germline sample (lymphocytes, saliva/buccal swab, normal tissue) in a laboratory accredited for germline analysis.
14. A patient in whom a germline pathogenic variant is detected should be referred to a specialist genetics service for long term follow-up and management of the family.
15. A normal/negative tumour sequencing result should not be taken as equivalent to a normal/negative germline result unless robust analysis of dosage has been carried out. This distinction is particularly important for genes such as *BRCA1* and *MSH2*, for which whole exon deletion/duplications constitute a substantial proportion of pathogenic variants.
16. Re-evaluation of this workflow, revised analyses and update of these recommendations should be undertaken at least 2-yearly. Reanalysis should include updated data regarding pathogenicity of variants and penetrance of CSGs, along with review of thresholds for 'germline conversion rates' and VAF cut-offs (Mandelker et al., 2019).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81105	Human Platelet Antigen 1 genotyping (HPA-1), ITGB3 (integrin, beta 3 [platelet glycoprotein IIIa], antigen CD61 [GPIIIa]) (eg, neonatal alloimmune

	thrombocytopenia [NAIT], post-transfusion purpura), gene analysis, common variant, HPA-1a/b (L33P)
81106	Human Platelet Antigen 2 genotyping (HPA-2), GP1BA (glycoprotein Ib [platelet], alpha polypeptide [GPIba]) (eg, neonatal alloimmune thrombocytopenia [NAIT], post-transfusion purpura), gene analysis, common variant, HPA-2a/b (T145M)
81107	Human Platelet Antigen 3 genotyping (HPA-3), ITGA2B (integrin, alpha 2b [platelet glycoprotein IIb of IIb/IIIa complex], antigen CD41 [GPIIb]) (eg, neonatal alloimmune thrombocytopenia [NAIT], post-transfusion purpura), gene analysis, common variant, HPA-3a/b (I843S)
81108	Human Platelet Antigen 4 genotyping (HPA-4), ITGB3 (integrin, beta 3 [platelet glycoprotein IIIa], antigen CD61 [GPIIIa]) (eg, neonatal alloimmune thrombocytopenia [NAIT], post-transfusion purpura), gene analysis, common variant, HPA-4a/b (R143Q)
81109	Human Platelet Antigen 5 genotyping (HPA-5), ITGA2 (integrin, alpha 2 [CD49B, alpha 2 subunit of VLA-2 receptor] [GPIa]) (eg, neonatal alloimmune thrombocytopenia [NAIT], post-transfusion purpura), gene analysis, common variant (eg, HPA-5a/b [K505E])
81110	Human Platelet Antigen 6 genotyping (HPA-6w), ITGB3 (integrin, beta 3 [platelet glycoprotein IIIa, antigen CD61] [GPIIIa]) (eg, neonatal alloimmune thrombocytopenia [NAIT], post-transfusion purpura), gene analysis, common variant, HPA-6a/b (R489Q)
81111	Human Platelet Antigen 9 genotyping (HPA-9w), ITGA2B (integrin, alpha 2b [platelet glycoprotein IIb of IIb/IIIa complex, antigen CD41] [GPIIb]) (eg, neonatal alloimmune thrombocytopenia [NAIT], post-transfusion purpura), gene analysis, common variant, HPA-9a/b (V837M)
81112	Human Platelet Antigen 15 genotyping (HPA-15), CD109 (CD109 molecule) (eg, neonatal alloimmune thrombocytopenia [NAIT], post-transfusion purpura), gene analysis, common variant, HPA-15a/b (S682Y)
81161	DMD (dystrophin) (eg, Duchenne/Becker muscular dystrophy) deletion analysis, and duplication analysis, if performed
81173	AR (androgen receptor) (eg, spinal and bulbar muscular atrophy, Kennedy disease, X chromosome inactivation) gene analysis; full gene sequence
81174	AR (androgen receptor) (eg, spinal and bulbar muscular atrophy, Kennedy disease, X chromosome inactivation) gene analysis; known familial variant
81177	ATN1 (atrophin 1) (eg, dentatorubral-pallidoluysian atrophy) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81178	ATXN1 (ataxin 1) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg, expanded) alleles

81179	ATXN2 (ataxin 2) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81180	ATXN3 (ataxin 3) (eg, spinocerebellar ataxia, Machado-Joseph disease) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81181	ATXN7 (ataxin 7) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81182	ATXN8OS (ATXN8 opposite strand [non-protein coding]) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81183	ATXN10 (ataxin 10) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81187	CNBP (CCHC-type zinc finger nucleic acid binding protein) (eg, myotonic dystrophy type 2) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81188	CSTB (cystatin B) (eg, Unverricht-Lundborg disease) gene analysis; evaluation to detect abnormal (eg, expanded) alleles
81189	CSTB (cystatin B) (eg, Unverricht-Lundborg disease) gene analysis; full gene sequence
81190	CSTB (cystatin B) (eg, Unverricht-Lundborg disease) gene analysis; known familial variant(s)
81204	AR (androgen receptor) (eg, spinal and bulbar muscular atrophy, Kennedy disease, X chromosome inactivation) gene analysis; characterization of alleles (eg, expanded size or methylation status)
81228	Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number variants (eg, bacterial artificial chromosome [BAC] or oligo-based comparative genomic hybridization [CGH] microarray analysis)
81229	Cytogenomic constitutional (genome-wide) microarray analysis; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants for chromosomal abnormalities
81233	BTK (Bruton's tyrosine kinase) (eg, chronic lymphocytic leukemia) gene analysis, common variants (eg, C481S, C481R, C481F)
81234	DMPK (DM1 protein kinase) (eg, myotonic dystrophy type 1) gene analysis; evaluation to detect abnormal (expanded) alleles
81236	EZH2 (enhancer of zeste 2 polycomb repressive complex 2 subunit) (eg, myelodysplastic syndrome, myeloproliferative neoplasms) gene analysis, full gene sequence
81237	EZH2 (enhancer of zeste 2 polycomb repressive complex 2 subunit) (eg, diffuse large B-cell lymphoma) gene analysis, common variant(s) (eg, codon 646)

81238	F9 (coagulation factor IX) (eg, hemophilia B), full gene sequence
81239	DMPK (DM1 protein kinase) (eg, myotonic dystrophy type 1) gene analysis; characterization of alleles (eg, expanded size)
81247	G6PD (glucose-6-phosphate dehydrogenase) (eg, hemolytic anemia, jaundice), gene analysis; common variant(s) (eg, A, A-)
81248	G6PD (glucose-6-phosphate dehydrogenase) (eg, hemolytic anemia, jaundice), gene analysis; known familial variant(s)
81249	G6PD (glucose-6-phosphate dehydrogenase) (eg, hemolytic anemia, jaundice), gene analysis; full gene sequence
81252	GJB2 (gap junction protein, beta 2, 26kDa, connexin 26) (eg, nonsyndromic hearing loss) gene analysis; full gene sequence
81260	IKBKAP (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein) (eg, familial dysautonomia) gene analysis, common variants (eg, 2507+6T>C, R696P)
81271	HTT (huntingtin) (eg, Huntington disease) gene analysis; evaluation to detect abnormal (eg, expanded) alleles
81274	HTT (huntingtin) (eg, Huntington disease) gene analysis; characterization of alleles (eg, expanded size)
81283	IFNL3 (interferon, lambda 3) (eg, drug response), gene analysis, rs12979860 variant
81284	FXN (frataxin) (eg, Friedreich ataxia) gene analysis; evaluation to detect abnormal (expanded) alleles
81285	FXN (frataxin) (eg, Friedreich ataxia) gene analysis; characterization of alleles (eg, expanded size)
81286	FXN (frataxin) (eg, Friedreich ataxia) gene analysis; full gene sequence
81289	FXN (frataxin) (eg, Friedreich ataxia) gene analysis; known familial variant(s)
81305	MYD88 (myeloid differentiation primary response 88) (eg, Waldenstrom's macroglobulinemia, lymphoplasmacytic leukemia) gene analysis, p.Leu265Pro (L265P) variant
81307	PALB2 (partner and localizer of BRCA2) (eg, breast and pancreatic cancer) gene analysis; full gene sequence
81308	PALB2 (partner and localizer of BRCA2) (eg, breast and pancreatic cancer) gene analysis; known familial variant
81312	PABPN1 (poly[A] binding protein nuclear 1) (eg, oculopharyngeal muscular dystrophy) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81320	PLCG2 (phospholipase C gamma 2) (eg, chronic lymphocytic leukemia) gene analysis, common variants (eg, R665W, S707F, L845F)

81329	SMN1 (survival of motor neuron 1, telomeric) (eg, spinal muscular atrophy) gene analysis; dosage/deletion analysis (eg, carrier testing), includes SMN2 (survival of motor neuron 2, centromeric) analysis, if performed
81333	TGFBI (transforming growth factor beta-induced) (eg, corneal dystrophy) gene analysis, common variants (eg, R124H, R124C, R124L, R555W, R555Q)
81336	SMN1 (survival of motor neuron 1, telomeric) (eg, spinal muscular atrophy) gene analysis; full gene sequence
81337	SMN1 (survival of motor neuron 1, telomeric) (eg, spinal muscular atrophy) gene analysis; known familial sequence variant(s)
81343	PPP2R2B (protein phosphatase 2 regulatory subunit Bbeta) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg,
81344	TBP (TATA box binding protein) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81400	Molecular pathology procedure, Level 1 (eg, identification of single germline variant [eg, SNP] by techniques such as restriction enzyme digestion or melt curve analysis)
81401	Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)
81402	Molecular pathology procedure, Level 3 (eg, >10 SNPs, 2-10 methylated variants, or 2-10 somatic variants [typically using non-sequencing target variant analysis], immunoglobulin and T-cell receptor gene rearrangements, duplication/deletion variants of 1 exon, loss of heterozygosity [LOH], uniparental disomy [UPD])
81403	Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)
81404	Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons, cytogenomic array analysis for neoplasia)

81407	Molecular pathology procedure, Level 8 (eg, analysis of 26-50 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of >50 exons, sequence analysis of multiple genes on one platform)
81408	Molecular pathology procedure, Level 9 (eg, analysis of >50 exons in a single gene by DNA sequence analysis)
81437	Hereditary neuroendocrine tumor-related disorders (eg, medullary thyroid carcinoma, parathyroid carcinoma, malignant pheochromocytoma or paraganglioma), genomic sequence analysis panel, 5 or more genes, interrogation for sequence variants and copy number variants
81438	Hereditary neuroendocrine tumor disorders (eg, medullary thyroid carcinoma, parathyroid carcinoma, malignant pheochromocytoma or paraganglioma); duplication/deletion analysis panel, must include analyses for SDHB, SDHC, SDHD, and VHL
81441	Inherited bone marrow failure syndromes (IBMFS) (eg, Fanconi anemia, dyskeratosis congenita, Diamond-Blackfan anemia, Shwachman-Diamond syndrome, GATA2 deficiency syndrome, congenital amegakaryocytic thrombocytopenia) sequence analysis panel, must include sequencing of at least 30 genes, including BRCA2, BRIP1, DKC1, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, GATA1, GATA2, MPL, NHP2, NOP10, PALB2, RAD51C, RPL11, RPL35A, RPL5, RPS10, RPS19, RPS24, RPS26, RPS7, SBDS, TERT, and TINF2
81442	Noonan spectrum disorders (eg, Noonan syndrome, cardio-facio-cutaneous syndrome, Costello syndrome, LEOPARD syndrome, Noonan-like syndrome), genomic sequence analysis panel, must include sequencing of at least 12 genes, including BRAF, CBL, HRAS, KRAS, MAP2K1, MAP2K2, NRAS, PTPN11, RAF1, RIT1, SHOC2, and SOS1
81443	Genetic testing for severe inherited conditions (eg, cystic fibrosis, Ashkenazi Jewish-associated disorders [eg, Bloom syndrome, Canavan disease, Fanconi anemia type C, mucolipidosis type VI, Gaucher disease, Tay-Sachs disease], beta hemoglobinopathies, phenylketonuria, galactosemia), genomic sequence analysis panel, must include sequencing of at least 15 genes (eg, ACADM, ARSA, ASPA, ATP7B, BCKDHA, BCKDHB, BLM, CFTR, DHCR7, FANCC, G6PC, GAA, GALT, GBA, GBE1, HBB, HEXA, IKBKAP, MCOLN1, PAH)
81470	X-linked intellectual disability (XLID) (eg, syndromic and non-syndromic XLID); genomic sequence analysis panel, must include sequencing of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL, RPS6KA3, and SLC16A2

81471	X-linked intellectual disability (XLID) (eg, syndromic and non-syndromic XLID); duplication/deletion gene analysis, must include analysis of at least 60 genes, including ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL, RPS6KA3, and SLC16A2
81479	Unlisted molecular pathology procedure
0130U	Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis), targeted mRNA sequence analysis panel (APC, CDH1, CHEK2, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, and TP53) (List separately in addition to code for primary procedure - 81435, 0101U) Proprietary test: RNAinsight™ for ColoNext® Lab/Manufacturer: Ambry Genetics
0138U	BRCA1 (BRCA1, DNA repair associated), BRCA2 (BRCA2, DNA repair associated) (eg, hereditary breast and ovarian cancer) mRNA sequence analysis (List separately in addition to code for primary procedure) Proprietary test: RNAinsight™ for BRCA1/2 Lab/Manufacturer: Ambry Genetics
0230U	AR (androgen receptor) (eg, spinal and bulbar muscular atrophy, Kennedy disease, X chromosome inactivation), full sequence analysis, including small sequence changes in exonic and intronic regions, deletions, duplications, short tandem repeat (STR) expansions, mobile element insertions, and variants in non-uniquely mappable regions Proprietary test: Genomic Unity® AR Analysis Lab/Manufacturer: Variantyx Inc
0232U	CSTB (cystatin B) (eg, progressive myoclonic epilepsy type 1A, Unverricht-Lundborg disease), full gene analysis, including small sequence changes in exonic and intronic regions, deletions, duplications, short tandem repeat (STR) expansions, mobile element insertions, and variants in non-uniquely mappable regions Proprietary test: Genomic Unity® CSTB Analysis Lab/Manufacturer: Variantyx Inc
0236U	SMN1 (survival of motor neuron 1, telomeric) and SMN2 (survival of motor neuron 2, centromeric) (eg, spinal muscular atrophy) full gene analysis, including small sequence changes in exonic and intronic regions, duplications and deletions, and mobile element insertions Proprietary test: Genomic Unity® SMN1/2 Analysis Lab/Manufacturer: Variantyx Inc
0269U	Hematology (autosomal dominant congenital thrombocytopenia), genomic sequence analysis of 14 genes, blood, buccal swab, or amniotic fluid

	Proprietary test: Versiti™ Autosomal Dominant Thrombocytopenia Panel Lab/Manufacturer: Versiti™ Diagnostic Laboratories/Versiti™
0270U	Hematology (congenital coagulation disorders), genomic sequence analysis of 20 genes, blood, buccal swab, or amniotic fluid Proprietary test: Versiti™ Coagulation Disorder Panel Lab/Manufacturer: Versiti™ Diagnostic Laboratories/Versiti™
0271U	Hematology (congenital neutropenia), genomic sequence analysis of 23 genes, blood, buccal swab, or amniotic fluid Proprietary test: Versiti™ Congenital Neutropenia Panel Lab/Manufacturer: Versiti™ Diagnostic Laboratories/Versiti™
0272U	Hematology (genetic bleeding disorders), genomic sequence analysis of 51 genes, blood, buccal swab, or amniotic fluid, comprehensive Proprietary test: Versiti™ Comprehensive Bleeding Disorder Panel Lab/Manufacturer: Versiti™ Diagnostic Laboratories/Versiti™
0273U	Hematology (genetic hyperfibrinolysis, delayed bleeding), genomic sequence analysis of 8 genes (F13A1, F13B, FGA, FGB, FGG, SERPINA1, SERPINE1, SERPINF2, PLAU), blood, buccal swab, or amniotic fluid Proprietary test: Versiti™ Fibrinolytic Disorder Panel Lab/Manufacturer: Versiti™ Diagnostic Laboratories/Versiti™
0274U	Hematology (genetic platelet disorders), genomic sequence analysis of 43 genes, blood, buccal swab, or amniotic fluid Proprietary test: Versiti™ Comprehensive Platelet Disorder Panel Lab/Manufacturer: Versiti™ Diagnostic Laboratories/Versiti™
0276U	Hematology (inherited thrombocytopenia), genomic sequence analysis of 23 genes, blood, buccal swab, or amniotic fluid Proprietary test: Versiti™ Inherited Thrombocytopenia Panel Lab/Manufacturer: Versiti™ Comprehensive Bleeding Disorder Panel
0277U	Hematology (genetic platelet function disorder), genomic sequence analysis of 31 genes, blood, buccal swab, or amniotic fluid Proprietary test: Versiti™ Platelet Function Disorder Panel Lab/Manufacturer: Versiti™ Comprehensive Bleeding Disorder Panel
0318U	Pediatrics (congenital epigenetic disorders), whole genome methylation analysis by microarray for 50 or more genes, blood Proprietary test: EpiSign Complete Lab/Manufacturer: Greenwood Genetic Center
S3840	DNA analysis for germline mutations of the RET proto-oncogene for susceptibility to multiple endocrine neoplasia type 2

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Attia, J. (2024, March 20). *Genetic association and GWAS studies: Principles and applications*. https://www.uptodate.com/contents/genetic-association-and-gwas-studies-principles-and-applications

Bacino, C. (2023, December 14, 2023). *Genomic disorders: An overview*. https://www.uptodate.com/contents/genomic-disorders-an-overview

Bacino, C. (2024, April 22). *Sex chromosome abnormalities*. https://www.uptodate.com/contents/sex-chromosome-abnormalities

Best, M. C., Butow, P., Savard, J., Jacobs, C., Bartley, N., Davies, G., Napier, C. E., Ballinger, M. L., Thomas, D. M., & Biesecker, B. (2022). Preferences for return of germline genome sequencing results for cancer patients and their genetic relatives in a research setting. *European Journal of Human Genetics*, 1-8. https://doi.org/10.1038/s41431-022-01069-y

Christensen, K., & Hulick, P. J. (2024, March 26). *Basic genetics concepts: DNA regulation and gene expression*. https://www.uptodate.com/contents/basic-genetics-concepts-dna-regulation-and-gene-expression

Hulick, P. J. (2024, 02/07/2024). *Next-generation DNA sequencing (NGS): Principles and clinical applications*. https://www.uptodate.com/contents/next-generation-dna-sequencing-ngs-principles-and-clinical-applications

Kohlmann, W., & Slavotinek, A. (2024, July 22). *Genetic testing*. https://www.uptodate.com/contents/genetic-testing

Konnick, E. Q., & Pritchard, C. C. (2016). Germline, hematopoietic, mosaic, and somatic variation: interplay between inherited and acquired genetic alterations in disease assessment. *Genome Med*, *8*(1), 100. https://doi.org/10.1186/s13073-016-0350-8

Konstantinopoulos, P. A., Norquist, B., Lacchetti, C., Armstrong, D., Grisham, R. N., Goodfellow, P. J., Kohn, E. C., Levine, D. A., Liu, J. F., Lu, K. H., Sparacio, D., & Annunziata, C. M. (2020). Germline and Somatic Tumor Testing in Epithelial Ovarian Cancer: ASCO Guideline. *Journal of Clinical Oncology*, *38*(11), 1222-1245. https://doi.org/10.1200/jco.19.02960

Li, M. M., Datto, M., Duncavage, E. J., Kulkarni, S., Lindeman, N. I., Roy, S., Tsimberidou, A. M., Vnencak-Jones, C. L., Wolff, D. J., Younes, A., & Nikiforova, M. N. (2017). Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn*, *19*(1), 4-23. https://doi.org/10.1016/j.jmoldx.2016.10.002

Lincoln, S. E., Nussbaum, R. L., Kurian, A. W., Nielsen, S. M., Das, K., Michalski, S., Yang, S., Ngo, N., Blanco, A., & Esplin, E. D. (2020). Yield and Utility of Germline Testing

Following Tumor Sequencing in Patients With Cancer. *JAMA Netw Open*, *3*(10), e2019452. https://doi.org/10.1001/jamanetworkopen.2020.19452

Mandelker, D., Donoghue, M., Talukdar, S., Bandlamudi, C., Srinivasan, P., Vivek, M., Jezdic, S., Hanson, H., Snape, K., Kulkarni, A., Hawkes, L., Douillard, J. Y., Wallace, S. E., Rial-Sebbag, E., Meric-Bersntam, F., George, A., Chubb, D., Loveday, C., Ladanyi, M., . . . Turnbull, C. (2019). Germline-focussed analysis of tumour-only sequencing: recommendations from the ESMO Precision Medicine Working Group. *Ann Oncol*, *30*(8), 1221-1231. https://doi.org/10.1093/annonc/mdz136

NCCN. (2024a, September 11). *Genetic/Familial High-Risk Assessment: Breast, Ovarian and Pancreatic - Version 1.2025*. https://www.nccn.org/professionals/physician_gls/pdf/genetics_bop.pdf

NCCN. (2024b). *NCCN Guidelines Treatment by Cancer Type*. https://www.nccn.org/guidelines/category_1

NCI. (2024). *Definition of germline mutation* https://www.cancer.gov/publications/dictionaries/cancer-terms/def/germline-mutation

Pascual, J., Attard, G., Bidard, F. C., Curigliano, G., De Mattos-Arruda, L., Diehn, M., Italiano, A., Lindberg, J., Merker, J. D., Montagut, C., Normanno, N., Pantel, K., Pentheroudakis, G., Popat, S., Reis-Filho, J. S., Tie, J., Seoane, J., Tarazona, N., Yoshino, T., & Turner, N. C. (2022). ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. *Ann Oncol*, *33*(8), 750-768. https://doi.org/10.1016/j.annonc.2022.05.520

Pogoda, M., Hilke, F. J., Lohmann, E., Sturm, M., Lenz, F., Matthes, J., Muyas, F., Ossowski, S., Hoischen, A., Faust, U., Sepahi, I., Casadei, N., Poths, S., Riess, O., Schroeder, C., & Grundmann, K. (2019). Single Molecule Molecular Inversion Probes for High Throughput Germline Screenings in Dystonia. *Front Neurol*, *10*, 1332. https://doi.org/10.3389/fneur.2019.01332

Raby, B. A., & Blank, R. D. (2024, June 25). *Genetics: Glossary of terms*. https://www.uptodate.com/contents/genetics-glossary-of-terms?topicRef=2895&source=see_link

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., & Rehm, H. L. (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*, *17*(5), 405-424. https://doi.org/10.1038/gim.2015.30

Robson, M. E., Bradbury, A. R., Arun, B., Domchek, S. M., Ford, J. M., Hampel, H. L., Lipkin, S. M., Syngal, S., Wollins, D. S., & Lindor, N. M. (2015). American Society of Clinical Oncology Policy Statement Update: Genetic and Genomic Testing for Cancer

Susceptibility. *J Clin Oncol*, *33*(31), 3660-3667. https://doi.org/10.1200/jco.2015.63.0996

Schrijver, I., & Zehnder, J. L. (2024, May 6). *Chromosomal translocations, deletions, and inversions*. https://www.uptodate.com/contents/chromosomal-translocations-deletions-and-inversions

Slaught, C., Berry, E. G., Bacik, L., Skalet, A. H., Anadiotis, G., Tuohy, T., & Leachman, S. A. (2021). Clinical challenges in interpreting multiple pathogenic mutations in single patients. *Hereditary Cancer in Clinical Practice*, *19*(1), 15. https://doi.org/10.1186/s13053-021-00172-3

Tung, N., Ricker, C., Messersmith, H., Balmaña, J., Domchek, S., Stoffel, E. M., Almhanna, K., Arun, B., Chavarri-Guerra, Y., Cohen, S. A., Cragun, D., Crew, K. D., Hall, M. J., Idos, G., Lopez, G., Pal, T., Pirzadeh-Miller, S., Pritchard, C., Rana, H. Q., . . . Vidal, G. A. (2024). Selection of Germline Genetic Testing Panels in Patients With Cancer: ASCO Guideline. *Journal of Clinical Oncology*, *42*(21), 2599-2615. https://doi.org/10.1200/jco.24.00662

Wilson, C. L., Wang, Z., Liu, Q., Ehrhardt, M. J., Mostafavi, R., Easton, J., Mulder, H., Hedges, D. J., Wang, S., Rusch, M., Edmonson, M., Levy, S., Lanctot, J. Q., Currie, K., Lear, M., Patel, A., Sapkota, Y., Brooke, R. J., Moon, W., . . . Yasui, Y. (2020). Estimated number of adult survivors of childhood cancer in United States with cancer-predisposing germline variants. *Pediatr Blood Cancer*, *67*(2), e28047. https://doi.org/10.1002/pbc.28047

General Genetic Testing, Somatic Disorders

Policy Number: AHS – M2146 – General Genetic Testing, Somatic Disorders	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Genetic testing refers to the use of technologies that identify genetic variation, which include genomic, transcriptional, proteomic, and epigenetic alterations, for the prevention, diagnosis, and treatment of disease (Kohlmann & Slavotinek, 2024; Li et al., 2017).

Somatic variations or mutations are defined as a genetic alteration that occurs after conception in any of the cells of the body, except the germ cells, and therefore are not passed on to offspring (Li et al., 2017).

For guidance concerning Tumor Mutational Burden Testing (TMB) and/or Microsatellite instability analysis please refer to the AHS-M2178-Microsatellite Instability and Tumor Mutational Burden Testing policy.

II. Related Policies

Policy Number	Policy Title
AHS-M2145	General Genetic Testing, Germline Disorders
AHS-M2032	Whole Genome and Whole Exome Sequencing
AHS-M2178	Microsatellite Instability and Tumor Mutational Burden Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

This policy addresses the general use of somatic (tumor) genetic testing and applies to all tests for which a policy addressing a specific clinical condition is not available.

1) For diagnosis, selection of therapy, or prognostication (when there is a documented benefit based on the presence of such mutations in the tumor or neoplastic cells), genetic testing for a specific genetic mutation or mutations that have documented clinical utility **MEETS COVERAGE CRITERIA**.

2) Repeat testing **MEETS COVERAGE CRITERIA** in **either** of the following situations:

   a) For recurrence monitoring.

   b) When there is the possibility of further genetic alterations in the hematologic malignancy, primary tumor, or metastasis **and** knowledge of these changes would result in the addition, elimination, or alteration of non-investigational therapies.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

3) For all situations not described above, genetic testing (single gene or multi-gene panel testing) for somatic disorders **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note: For two or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
ACMG	American College of Medical Genetics and Genomics
ACTC1	*Actin alpha cardiac muscle 1*
AMP	Association For Molecular Pathology
APC	*Adenomatous polyposis coli*
APOB	*Apolipoprotein B*
ASCO	American Society of Clinical Oncology
ATP7B	*ATPase copper transporting beta*
BAG3	*BAG cochaperone 3*
BMPR1A	*Bone morphogenetic protein receptor type 1A*
BRCA	*Breast cancer gene*
BRCA1	*Breast cancer gene 1*
BRCA2	*Breast cancer gene 2*

BSG	British Sarcoma Group
CACNA1S	*Calcium voltage-gated channel subunit alpha1 S*
CALM1	*Calmodulin I*
CALM2	*Calmodulin 2*
CALM3	*Calmodulin 3*
CAP	College Of American Pathologists
CD	*Cluster of differentiation*
CD34	*Cluster of differentiation 34*
CGP	Comprehensive genomic profiling
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare and Medicaid Services
CNAs	Copy number alterations
CNV	Copy number variant
COL3A1	*Collagen type III alpha 1*
CTLA-4	*Cytotoxic T-lymphocyte-associated protein 4*
D842V	*Platelet-derived growth factor receptor alpha*
DES	*Desmin*
dMMR	Mismatch repair deficiency
DNA	Deoxyribonucleic acid
DOG1	Delay of germination
DSC2	*Desmocollin-2*
DSG2	*Desmoglein 2*
DSP	*Desmoplakin*
EGISTs	Extragastrointestinal stromal tumors
EP	Expected pathogenic
ESCAT	European Society for Medical Oncology Scale for Clinical Actionability of Molecular Targets
ESMO	European Society for Medical Oncology
EZH2	*Enhancer of zeste 2 polycomb repressive complex 2 subunit*
FBN1	*Fibrillin-1*
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin embedded
FISH	Fluorescence in situ hybridization
GIS	Genomic instability score
GISTs	Gastrointestinal stromal tumors
GLA	*Alpha-galactosidase A*
GRASP	Genome-Wide Repository of Associations Between SNPs And Phenotypes
HGSC	High-grade serous ovarian, fallopian tube, and peritoneal carcinoma

HOXC6	*Homeobox C6*
HRD	Homologous recombination deficiency
HRR	Homologous recombination repair
Indel	Insertion/deletion
KCNH2	*Potassium voltage-gated channel subfamily H member 2*
KCNQ1	*Potassium voltage-gated channel subfamily Q member 1*
KIT	*KIT proto-oncogene receptor tyrosine kinase*
LP	Likely pathogenic
LDLR	Low density lipoprotein receptor
LDTs	Laboratory-developed tests
LMNA	Lamin A/C
LOF	Loss of function
LOH	Loss of heterozygosity
LST	Large scale state transitions
MBR	Major breakpoint region
MCR	Minor cluster region
MLH1	*MutL homolog 1*
MSH2	*MutS homolog 2*
MSH6	*MutS homolog 6*
MSI	Microsatellite instability
MSK-IMPACT	Memorial Sloan Kettering- integrated mutation profiling of actionable cancer targets
MUTYH	*MutY DNA glycosylase*
MYBPC3	*Myosin binding protein C*
MYH7	*Myosin heavy chain 7*
MYH11	*Myosin heavy chain 11*
MYL2	*Myosin light chain 2*
MYL3	*Myosin light chain 3*
NCCN	National Comprehensive Cancer Network
NF2	*Neurofibromin 2*
NGS	Next generation sequencing
NSCLC	Non-small cell lung cancer
OCEANs	Oncogene Concatenated Enriched Amplicon Nanopore Sequencing
ORR	Overall response rate
OTC	*Ornithine transcarbamylase*
PARPi	Poly-ADP ribose inhibitors
PCR	Polymerase chain reaction
PCSK9	*Proprotein convertase subtilisin/kexin type 9*
PD-1/PD-L1	*Programmed death-1/ programmed death ligand-1*

PDGFRA	*Platelet-derived growth factor receptor alpha*
PKP2	*Plakophilin 2*
PMS2	*PMS1 homolog 2, mismatch repair system component*
poly-ADP	Polymeric adenosine diphosphate
PRKAG2	*Protein kinase AMP-activated non-catalytic subunit gamma 2*
PTEN	*Phosphatase and tensin homolog*
RB1	*RB transcriptional corepressor 1*
RBM20	*RNA binding motif 20*
RNA	Ribonucleic acid
RYR1	*Ryanodine receptor 1*
RYR2	*Ryanodine receptor 2*
SCL	Small cell lung cancer
SCN5A	*Sodium voltage-gated channel alpha subunit 5*
SCNEC	Small cell neuroendocrine carcinoma
SDHAF2	*Succinate dehydrogenase complex assembly factor 2*
SDHB	*Succinate dehydrogenase complex subunit B*
SDHC	*Succinate dehydrogenase complex subunit C*
SDHD	*Succinate dehydrogenase cytochrome b*
SMAD4	*Mothers against decapentaplegic homolog 4*
SNP	Single nucleotide polymorphism
SNV	single nucleotide variant
STK11	*Serine/threonine kinase 11*
STR	Short tandem repeat
TAI	Telomeric allelic imbalance
TF	Tumor fraction
TGFBR1	*Transforming growth factor, beta receptor I*
TGFBR2	*Transforming growth factor, beta receptor II*
TMB	Tumor mutational burden
TMEM43	*Transmembrane protein 43*
TNNC1	*Troponin C1, slow skeletal and cardiac type*
TP53	*Tumor protein P53*
TSC1	*Tuberous sclerosis complex 1*
TSC2	*Tuberous sclerosis complex 2*
UPD	Uniparental disomy
VUS	Variants of unknown significance
WES	Whole-exome sequencing
WT1	*Wilms' tumor 1*

V. Scientific Background

Gene mutations are referred to as "somatic" if they are not within the germline (i.e., within gametes); therefore, these mutations are not passed on from parent to offspring. Somatic mutations may arise *de novo* or later in life and are very common in neoplasms (Raby & Blank, 2024). There are many different types of somatic mutations, including single nucleotide polymorphisms (SNPs); structural variations such as deletions, inversions, or translocations, and smaller chromosomal abnormalities such as short tandem repeats or gene fusions. Most mutations do not result in disease (Kohlmann & Slavotinek, 2024).

Single nucleotide polymorphisms are the most common type of genetic mutation, including missense mutations. These mutations are single base-pair changes where one nucleotide replaces a different nucleotide. More than 65% of the diseases caused by genetic mutations are due to SNPs (Kohlmann & Slavotinek, 2024). Estimates based on whole genome sequencing have placed the average amount of SNPs in any given individual at 2.8 to 3.9 million (Kohlmann & Slavotinek, 2024). Insertion/deletion (Garrett et al.) polymorphisms are often a single nucleotide but may be up to four nucleotides. SNPs often lead to frameshift mutations that can cause premature stop codons and the failure of the allele (Kohlmann & Slavotinek, 2024).

Structural variations are usually classified as larger than 1000 base pairs. These include deletions, duplications, inversions, translocations, or ring chromosome formations. Due to the large number of genes affected, these variations commonly lead to severe genetic abnormalities; for example, a major cause of chronic myeloid leukemia is due to the translocation between chromosomes nine and 22, resulting in a fused gene. The most common structural variation is the copy number variant (CNV), referring to a differing number of DNA segment copies in different individuals. For example, one person may have three copies of a particular segment whereas another may only have two. These variations may lead to dysregulation, gain-of-function, or loss of function of the affected genes (Kohlmann & Slavotinek, 2024). The sensitive genes that require or produce precise quantities of a protein product tend to suffer more from these variations (Bacino, 2023).

Any size mutation may be pathogenic and must be categorized as to how likely the mutation is to cause disease. The American College of Medical Genetics and Genomics (ACMG) has classified mutations in five categories, which are as follows: pathogenic, likely pathogenic, uncertain significance, likely benign, and benign. The "likely pathogenic" and "likely benign" refer to weaker evidence than their respective pathogenic and benign categories, and "uncertain significance" refers to evidence that

does not meet criteria for benignity or pathogenicity or has conflicting evidence from both sides (Kohlmann & Slavotinek, 2024). Prediction algorithms have been used to interpret variants and to predict whether a variant will affect the gene function or splicing of the gene. These algorithms are publicly available but have a tendency of predicting the harmful impact of a variant. The specificity of these databases has been estimated at 60-80% (Li et al., 2017).

Due to the enormous number of variants, as well as the rate that variants are discovered, comprehensive databases of genetic variants have been published and are easily available. For example, the Genome-Wide Repository of Associations Between SNPs and Phenotypes (GRASP) database includes information from over 2000 studies and over one million variant-related results (Kohlmann & Slavotinek, 2024). Databases focusing on cancer-specific variants, reference sequences, and the general population are all available publicly (Li et al., 2017).

Spontaneous mutations accumulate in somatic cells over a lifetime. Early somatic mutations can cause developmental disorders while the accumulation of mutations throughout life can lead to cancer and contribute to aging (Martincorena & Campbell, 2015). Molecular profiles of tumors have clinical utility in guiding the clinical management of cancer patients, providing diagnostic or prognostic information, or identifying a potential treatment regimen (Li et al., 2017). Increasingly, somatic mutations are being identified in diseases other than cancer, such as neurodevelopmental diseases (Poduri et al., 2013).

A malignant neoplasm is another term for cancer, which may encompass many types including breast, prostate, skin, lung, rectum, colon, and brain. Gastrointestinal stromal tumors (GISTs) are considered rare neoplasms with approximately 95% of these cancers non-hereditary; GISTs are mainly identified by KIT protein expression with typical mutations in the *KIT* or platelet-derived growth factor receptor alpha (*PDGFRA*) genes (Morgan et al., 2023). These GISTs are the most common mesenchymal tumor of the gastrointestinal tract that originate from the cell of Cajal (Comandini et al., 2017). Primary prostate and lung tumors have been associated with different types of GISTs such as gastric and small bowel; genetic analysis of one patient found "that the gastric GIST and abdominal tumors were characterized by two different c-KIT mutations" (Comandini et al., 2017). Extragastrointestinal stromal tumors (EGISTs) are another type of rare neoplasm which also arise in the gastrointestinal tract. Liu et al. (2014) report that an EGIST was identified in the prostate of a patient. "The results of immunohistochemical staining showed positive immunoreactivity for cluster of

differentiation (CD)117 (c-kit), CD34 and DOG1 in the tumor. On mutation analysis, loss of heterozygosity of the c-kit gene was observed in the prostatic EGIST; however, the platelet-derived growth factor receptor-α (*PDGFRA*) gene was normal" (Liu et al., 2014). Due to the rarity of EGIST of the prostate, immunohistochemistry analysis is important to confirm a diagnosis.

Mutations of the *KIT* and *PDGFRA* genes in small cell neuroendocrine carcinoma (SCNEC) of the prostate have been researched by Terada (2012). A total of 706 malignant prostate tumors were identified, and four of these tumors were classified as SCNEC. Of these four tumors, three tumors were positive for *KIT*, and *PDGFRA,* among other genes. Molecular genotyping via PCR showed no *KIT* or *PDGFRA* mutations (Terada, 2012). Another study completed by McCabe et al. (2008) noted that homeobox C6 (HOXC6) is overexpressed in prostate cancers and completed an analysis of prostate cancer cells to identify which promoters are bound by HOXC6. "We show that HOXC6 directly regulates expression of bone morphogenic protein 7, fibroblast growth factor receptor 2, insulin-like growth factor binding protein 3, and platelet-derived growth factor receptor alpha (*PDGFRA*) in prostate cells" (McCabe et al., 2008). The researchers also note that *PDGFRA* is able to reduce the proliferation of prostate cancer cells, and that if HOXC6 is overexpressed, the effects of *PDGFRA* inhibition may be overcome. The fusion gene *FIP1L1-PDGFRA* has also been associated with chronic eosinophilic leukemia (Legrand et al., 2013).

Proprietary Testing

Clinical biomarkers are widely used for making personalized and actionable decisions for cancer treatment. TMB, the number of somatic mutations per mega base of the DNA in cancer cells, is an emerging biomarker associated with predicting the response to immunotherapy treatment (NCI, 2024). A high TMB value indicates better treatment outcomes, which is observed in patients with melanoma on CTLA-4 inhibitors and patients with melanoma, non-small cell lung carcinoma, bladder cancer, microsatellite instability cancers, and pan-tumors on PD-1/PD-L1 inhibitors. High TMB has also been associated with improved outcomes in patients on a combination of PD-1/PD-L1 and CTLA-4 inhibitors (Merino et al., 2020). TMB was originally measured with whole-exome sequencing (WES), but this method has limited clinical utility due to a six to eight week sequencing period and expensive costs. Alternatively, targeted NGS panels can reliably estimate TMB from a subset of the exome with reduced sequencing time and increased clinical application. Two FDA-approved products for calculating TMB include the FoundationOne CDx assay (Foundation Medicine Inc.) and MSK-IMPACT (Memorial

Sloan Kettering Cancer Center). Both of these tests, referred to as comprehensive genomic profiling (CGP), can identify all types of "molecular alterations (i.e., single nucleotide variants, small and large insertion-deletion alterations, copy number alterations, and structural variants) in cancer-related genes, as well as genomic signatures such as microsatellite instability (Bauml et al.), loss of heterozygosity, and TMB (Klempner et al., 2020). Studies show that TMB calculation from CGP has high concordance with TMB" measured from WES. On June 16, 2020, the FDA approved pembrolizumab for the treatment of adult and pediatric patients with a TMB value of greater than 10 mutations per mega base as determined by the FoundationOne CDx assay (FDA, 2020b).

Analysis of somatic mutations in solid tumors and hematologic malignancies using next generation sequencing has become common practice in oncology clinics as well as clinical trials. There are several known approved NGS tests for detection of somatic mutations. MyChoice HRD CDx, by Myriad Genetic Laboratories, was FDA-approved on October 23, 2019, and ONCO/Reveal Dx Lung & Colon Cancer Assay (O/RDx-LCCA) by Pillar Biosciences was FDA-approved on July 30, 2021. Myriad MyChoice® CDx is a next generation sequencing based in vitro diagnostic test that detects single nucleotide variants, insertions and deletions, and large rearrangement variants in protein coding regions and intron/exon boundaries of the *BRCA1* and *BRCA2* genes (Myriad Genetics, 2024). The ONCO/Reveal Dx Lung & Colon Cancer Assay (O/RDx-LCCA) by Pillar Biosciences, is a next generation sequencing test for detection of somatic mutations for non-small cell lung cancer (NSCLC) and colorectal cancer (CRC) tumor tissue. The test simultaneously detects clinically relevant mutations in *KRAS* for CRC and epidermal growth factor receptor (EGFR) for NSCLC in a single assay. In the accuracy study, positive percent agreement (Pappas et al.) and negative percent agreement (NPA) between O/RDx-LCCA and externally validated comparator method (CompO) was >99%. The authors conclude that O/RDx-LCCA "is a highly accurate assay for the detection of clinically relevant *KRAS* variants in CRC and EGFR variants in NSCLC" (Pillar Biosciences, 2020, 2021).

In 2020, the FDA approved Guardant360® CDx for tumor mutation profiling in patients with any solid malignant neoplasm. The Guardant360 CDx is also approved as a companion diagnostic to identify NSCLC patients with *EGFR* alterations who may benefit from treatment with Tagrisso® (osimertinib) (Guardant, 2020). In an analytical study, the positive and NPA for Guardant360 CDx relative to Therascreen® KRAS RGQ PCR was 0.71 and 1.00 respectively; overall percent agreement was 0.82 (Bauml et al.,

2021). In 2020, the FDA also approved Therascreen BRAF V600E RGQ PCR Kit by QIAGEN. This is a real-time PCR test for the qualitative detection of V600E mutations in the *BRAF* gene in human colorectal cancer (CRC) tumor tissue. Therascreen can help select patients with metastatic colorectal cancer (mCRC) whose tumors carry the *BRAF* V600E mutation for treatment with BRAFTOVI (encorafenib) in combination with cetuximab (QIAGEN, 2024).

Analytical Validity

Woodhouse et al. (2020) evaluated the analytical performance of FoundationOne Liquid CDx assay to detect genomic alterations in cancer patients. The assay was evaluated across more than 30 different cancer types in over 300 genes and greater than 30,000 gene variants. "Results demonstrated a 95% limit of detection of 0.40% variant allele fraction for select substitutions and insertions/deletions, 0.37% variant allele fraction for select rearrangements, 21.7% tumor fraction (USPSTF) for copy number amplifications, and 30.4% TF for copy number losses. The false positive variant rate was 0.013% (approximately one in 8,000). Reproducibility of variant calling was 99.59%" (Woodhouse et al., 2020). In comparison to in situ hybridization and immunohistochemistry, FoundationOne had an overall 96.3% positive percent agreement and > 99.9% negative percent agreement. "These study results demonstrate that FoundationOne Liquid CDx accurately and reproducibly detects the major types of genomic alterations in addition to complex biomarkers such as microsatellite instability, blood tumor mutational burden, and tumor fraction" (Woodhouse et al., 2020).

Thirunavukarasu et al. (2021) developed the Oncogene Concatenated Enriched Amplicon Nanopore Sequencing (OCEANS) method for rapid, accurate, and affordable somatic mutation detection. The OCEANS method involves amplified variants with low variant allele frequency and subsequently concatenating with Nanopore Sequencing. In this study, the 15-plex OCEANS melanoma panel was compared to NGS. OCEANS had a 100% sensitivity relative to NGS. Of the 9584 NGS-negative loci, OCEANS was able to detect an additional 97 variants; thus, relative to NGS, OCEANS had a 99.0% specificity and very low false positive rate. These 97 NGS-negative and OCEANS-positive results were believed to be true mutations, and droplet digital PCR (ddPCR) confirmation experiments supported this hypothesis. The authors conclude that "Integrating OCEANS with long-read and base modification detection capabilities of Nanopore Sequencing can enable development of comprehensive oncology panels" (Thirunavukarasu et al., 2021).

Clinical Utility and Validity

Advancements in technology and availability of sequencing, previously constrained by limitations of sequential single-gene testing on limited patient samples, have led to significant strides in our understanding of the genetic basis of inherited and somatic conditions. Variants detected by genetic testing include inherited germline variants and somatic mutations; next generation sequencing (Lamont et al.) has allowed for superior detection of these mutations (Konnick & Pritchard, 2016). The accuracy of NGS varies depending on how many genes are sequenced; fewer genes tend to result in higher accuracy since there will be more "probe-template overlap." Although Sanger sequencing remains the most accurate at >99.99% accuracy, it cannot sequence a large amount of genes in a timely fashion and is best used for sequencing of a specific gene (Hulick, 2024). NGS also can be used clinically to guide screening, preventive options, and cancer treatment such as the "analysis of tumor tissue or non-tumor tissue to identify genetic abnormalities that may be present in the germline and/or the tumor that could potentially match molecularly targeted therapies. As an example, a poly(ADP ribose) polymerase (PARP) inhibitor could be considered in a patient with an identified BRCA1 or BRCA2 mutation" (Hulick, 2024).

Next generation sequencing has been used to identify several types of somatic mutations associated with cancer and may help to single out therapeutic targets. Genetic mutations in *BRCA*1 & 2 are associated with breast and ovarian cancer. Kowalik et al. (2019) have identified somatic genetic mutations in *BRCA*1 & 2 for ovarian cancer prognostic purposes using NGS. Ovarian cancer tissue samples were used for the analysis. A total of three percent of mutations (6/201) were identified as somatic; with only 24% (49/201) of samples identified with a pathogenic mutation overall (Kowalik et al., 2019). The other 35 mutations were of germline origin. This corroborated the report by Nagahashi et al. (2019) which states that approximately 2.5% of *BRCA*1 & 2 mutations are somatic. Detection of a BRCA1/2 germline mutation is essential for planning individual therapy, where indicated, testing should be arranged as early as possible. It is the only way of identifying patients suitable for PARPi therapy and ensuring they receive the best possible treatment (Lux & Fasching, 2023).

The clinical validity of a genetic test depends primarily on the expressivity and penetrance of a given phenotype. Penetrance refers to the likelihood of developing a disease when the pathogenic mutation is present, and expressivity refers to the variations in the way the disease is expressed. For example, virtually any mutation in the *APC* gene will cause symptoms of familial adenomatous polyposis, thereby increasing the clinical validity of an *APC* assessment. Some conditions may not clinically manifest

at all despite a mutated genotype (Kohlmann & Slavotinek, 2024).

The clinical utility of a genetic test generally relies on available treatments for a condition. Conditions such as Huntington's Disease that do not have many options for treatment will have limited clinical utility compared to another condition even though the actual test is highly valid. Factors such as severity of the disease and management options affect the clinical utility of a genetic test (Kohlmann & Slavotinek, 2024).

Hayano et al. (2016); McCabe et al. (2008) noted that homeobox C6 (HOXC6) is overexpressed in prostate cancers and completed an analysis of prostate cancer cells to identify which promoters are bound by HOXC6.

In a multi-cohort, open-label, non-randomized study to establish the relationship between TMB and pembrolizumab treatment response, 790 patients were tested for TMB with the FoundationOne CDx assay. A total of 102/790 patients had high TMB (≥10 mutations per mega base) in solid tumors of anal, biliary, cervical, endometrial, mesothelioma, neuroendocrine, salivary, small cell lung, thyroid, and vulvar cancers. The overall response rate (ORR) in patients with a high TMB was 29%, with a 4% complete response rate and 25% partial response rate compared to an ORR of 6% in patients with a low TMB. The ORR was nearly 5-fold in patients with a high TMB. The authors conclude "TMB could be a novel and useful predictive biomarker for response to pembrolizumab monotherapy in patients with previously treated recurrent or metastatic advanced solid tumours" (Marabelle et al., 2020).

In a prospective study, Takeda evaluated the clinical application of the FoundationOne CDx Assay in decision-making for patients with advanced solid tumors. A total of 175 samples were analyzed using the FoundationOne assay and 153 of these patients were assessed for TMB. "The most common known or likely pathogenic variants were *TP53* mutations (n = 113), *PIK3CA* mutations (n = 33), *APC* mutations (n = 32), and *KRAS* mutations (n = 29). The median TMB was four mutations/Mb, and tumors with a high TMB (≥10 mutations/Mb) were more prevalent for lung cancer (11/32) than for other solid tumor types." From the 175 samples found to have known or likely pathogenic variants, 24 subjects (14%) received the optimal targeted therapy. The authors conclude that "such testing may inform the matching of patients with cancer with investigational or approved targeted drugs" (Takeda et al., 2021).

VI. Guidelines and Recommendations

Association for Molecular Pathology (AMP), American Society of Clinical

Oncology (ASCO), and College of American Pathologists (CAP)

The Joint Commission recommended that somatic variants be categorized by and reported based on their impact on clinical care. The Joint Commission notes that somatic variants include indels, single nucleotide variants (SNVs), fusion genes from genomic rearrangements, and CNVs and should focus on their impact on clinical care. Any variant may be considered a biomarker if it predicts response to therapy, influences prognosis, diagnosis, treatment decisions, or the gene function itself. The Joint Commission proposes four levels for these biomarkers which are as follows:

> "1. Level A, biomarkers that predict response or resistance to US FDA-approved therapies for a specific type of tumor or have been included in professional guidelines as therapeutic, diagnostic, and/or prognostic biomarkers for specific types of tumors;
> 2. Level B, biomarkers that predict response or resistance to a therapy based on well-powered studies with consensus from experts in the field, or have diagnostic and/or prognostic significance of certain diseases based on well-powered studies with expert consensus;
> 3. Level C, biomarkers that predict response or resistance to therapies approved by FDA or professional societies for a different tumor type (i.e., off-label use of a drug), serve as inclusion criteria for clinical trials, or have diagnostic and/or prognostic significance based on the results of multiple small studies;
> 4. Level D, biomarkers that show plausible therapeutic significance based on preclinical studies, or may assist disease diagnosis and/or prognosis themselves or along with other biomarkers based on small studies or multiple case reports with no consensus" (Li et al., 2017).

The Joint Commission also includes variants in different tiers based on the amount of evidence there is to support its significance. For example, tier 1 variants include significance of levels A and B, while tier 2 includes significance of levels C and D. Tier 3 is variants of unknown significance (VUS), such as variants in cancer genes that have not been reported in any other cancers. These variants are not typically seen in significant frequencies in the general population. When evaluating these variants, the type of mutation and gene function should be considered. Tier 4 is benign variants or likely benign variants. These alleles are often observed in significant amounts in general populations. Tier 3 variants should be reported while ensuring that the most important information is communicated to the patient (Li et al., 2017).

National Comprehensive Cancer Network (NCCN)

Multiple somatic mutations have been incorporated into the diagnostic workups recommended by the NCCN. Furthermore, the NCCN has several guidelines which recommend that gene expression profiling, or multiple gene testing, may be helpful, more efficient and/or cost-effective for selected patients (NCCN, 2024). Please see the individual policies.

American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP)

The ACMG and AMP released criteria on the types and severity of mutations, which are as follows:

- **Very strong evidence of pathogenicity**: Null variants (nonsense, frameshifts, canonical +/- 1-2 splice sites, initiation codon, exon deletions) in a gene where loss of function (LOF) is a known mechanism of disease. The guidelines note to use caution in genes where LOF is not a mechanism, if LOF variants are at the 3' end, if exon skipping occurs, and if multiple transcripts are present.
- **Strong:** Amino acid change to a pathogenic version, de novo mutations, established studies supporting a damaging gene or gene product, or if the prevalence of the variant is increased in affected individuals compared to healthy controls. The guidelines note to be careful of changes impacting splicing and if only the paternity has been confirmed.
- **Moderate:** Located in a mutational hot spot or well-established functional domain (e.g., active site of an enzyme) without a benign variation, absent from controls in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium, detected in *trans* with pathogenic variants for a recessive disorder, protein length changes, novel missense changes where a different missense change has been pathogenic before, and a possible de novo mutation.
- **Supporting:** Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease, missense variant in a gene with low rate of benign missense variation, if the mutation has evidence that it is deleterious, if the patient's phenotype is highly specific for disease with a single genetic cause.

The guidelines also list criteria for benign gene variants.

- **Stand-alone evidence of benignity:** Allele frequency is >5% in Exome

Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium.

- **Strong:** Allele frequency is greater than expected for disorder, observed in healthy adult with full penetrance at early age, lack of segregation in affected family members (although pathogenic variants may masquerade as nonsegregated), or well-established studies that show no damaging effect on protein production.
- **Supporting:** Missense variant of a gene for which truncating mutations are pathogenic, indels in repetitive region of unknown function, silent variants, VUS, or a *trans* version of a *cis* mutation (Richards et al., 2015).

American College of Medical Genetics and Genomics (ACMG)

The ACMG has released a list of genes for which secondary findings should be disclosed. Secondary findings refer to incidental findings unrelated to why a genetic test was originally ordered but are of significant clinical value to the patient. The portion of the table containing the conditions, the associated genes, and which variants should be report is listed below (Kalia et al., 2016; Miller et al., 2023; Miller et al., 2021):

Condition	Gene(s)	Variants to Report
Breast/ovarian cancer	*BRCA1, BRCA2*	LP (likely pathogenic), P (pathogenic)
Li-Fraumeni syndrome	*TP53*	LP, P
Peutz-Jeghers syndrome	*STK11*	LP, P
Juvenile polyposis syndrome	*BMPR1A, SMAD4*	LP, P
PTEN hamartoma syndrome	*PTEN*	LP, P
Lynch syndrome	*MLH1, MSH2, MSH6, PMS2*	LP, P
Familial adenomatous polyposis	*APC*	LP, P
MYH-associated polyposis	*MUTYH*	LP, P
Von Hippel Lindau syndrome	*VHL*	LP, P

Retinoblastoma	*RB1*	LP, P
Tuberous sclerosis complex	*TSC1, TSC2*	LP, P
Wilms tumor	*WT1*	LP, P
Multiple endocrine neoplasia 1 or 2	*MEN1 (1), RET (2)*	LP, P
Familial medullary thyroid cancer	*RET*	LP, P
Hereditary paraganglionoma-pheochromocytoma syndrome	*SDHD, SDHAF2, SDHC, SDHB*	LP, P
Neurofibromatosis type 2	*NF2*	LP, P
Marfan syndrome, Loeys-Dietz syndrome, familial thoracic aortic aneurysms and dissections	*FBN1, TGFBR1, TGFBR2, SMAD3, ACTA2, MYH11*	LP, P
Malignant hyperthermia	*RYR1, CACNA1S*	LP, P
Wilson disease (copper metabolism)	*ATP7B*	LP, P
Ornithine transcarbamylase deficiency (urea cycle)	*OTC*	All hemi, het, homozygous P and LP
Hereditary hemochromatosis	*HFE*	HFE p.Cys282Tyr homozygotes only
Hereditary hemorrhagic telangiectasia	*ACVRL1, ENG*	LP, P
Maturity-onset diabetes of the young	*HNF1A*	LP, P
RPE65-related retinopathy	*RPE65*	LP, P
Hereditary TTR amyloidosis	*TTR*	LP, P

Cardiac and/or blood vessel related		
Condition	Gene(s)	Variants to Report

Aortopathies	*FBN1, TGFBR1, TGFBR2, SMAD3, ACTA2, MYH11*	LP, P
Arrhythmogenic right ventricular cardiomyopathy	*PKP2, DSP, DSC2, TMEM43, DSG2*	LP, P
Catecholaminergic polymorphic ventricular tachycardia	*RYR2, CASQ2, TRDN*	LP, P
Dilated cardiomyopathy	*TNNT2, LMNA, FLNC, TTN, BAG3, DES, RBM20, TNNC1*	LP, P
Ehlers–Danlos syndrome, vascular type	*COL3A1*	LP, P
Familial hypercholesterolemia	*LDLR, APOB, PCSK9*	LP, P
Hypertrophic cardiomyopathy	*MYH7, MYBPC3, TNNI3, TPM1, MYL3, ACTC1, PRKAG2, MYL2*	LP, P
Long QT syndrome types 1 and 2	*KCNQ1, KCNH2*	LP, P
Long QT syndrome 3; Brugada syndrome	*SCN5A*	LP, P
Long QT syndrome types 14-16	*CALM1, CALM2, CALM3*	LP, P
Genes related to inborn errors of metabolism phenotypes		
Condition	Gene(s)	LP, P
Biotinidase deficiency	*BTD*	LP, P (2 variants)
Fabry disease	*GLA*	All hemi, het, homozygous P and LP
Ornithine transcarbamylase deficiency	*OTC*	All hemi, het, homozygous P and LP

Pompe disease	*GAA*	P and LP (2 variants)

American Society of Clinical Oncology (ASCO)

The ASCO published guidelines regarding genetic and genomic testing for cancer susceptibility. These guidelines state that the "ASCO recognizes that concurrent multigene testing (i.e., panel testing) may be efficient in circumstances that require evaluation of multiple high-penetrance genes of established clinical utility as possible explanations for a patient's personal or family history of cancer. Depending on the specific genes included on the panel employed, panel testing may also identify mutations in genes associated with moderate or low cancer risks and mutations in high-penetrance genes that would not have been evaluated on the basis of the presenting personal or family history… ASCO affirms that it is sufficient for cancer risk assessment to evaluate genes of established clinical utility that are suggested by the patient's personal and/or family history" (Robson et al., 2015).

ASCO released guidelines regarding somatic tumor testing for ovarian cancer. ASCO recommends that, forindividuals with ovaries "diagnosed with clear cell, endometrioid, or mucinous ovarian cancer should be offered somatic tumor testing for mismatch repair deficiency (dMMR). Somatic tumor testing for *BRCA1* and *BRCA2* pathogenic or likely pathogenic variants may be reserved for time of recurrence for [those] who have completed upfront therapy and are currently in observation, as presence of these mutations qualifies the patient for FDA-approved treatments" (Konstantinopoulos et al., 2020). In a 2021 update of these guidelines, ASCO adds "Implementation of techniques and pipelines enabling both SNV and CNV detection should be preferred, optimally by next-generation sequencing" (Pujol et al., 2021).

In 2022, a provisional opinion was released by ASCO. It addressed the appropriate use of tumor genomic testing in patients with metastatic or advanced solid tumors. A lack of clarity in clinical practice "as to when tumor genomic sequencing should be ordered, what type of assays should be performed, and how to interpret the results for treatment selection," was the impetus. In the opinion of ASCO, patients with metastatic or advanced cancer "should undergo genomic sequencing in a certified laboratory if the presence of one or more specific genomic alterations has regulatory approval as biomarkers to guide the use of or exclusion from certain treatments for their disease."

Additionally:

- "Multigene panel-based assays should be used if more than one biomarker-linked therapy is approved for the patient's disease."
- "Site-agnostic approvals for any cancer with a high tumor mutation burden, mismatch repair deficiency, or neurotrophic tyrosine receptor kinase (*NTRK*) provide a rationale for genomic testing for all solid tumors."
- "Multigene testing may also assist in treatment selection by identifying additional targets when there are few or no genotype-based therapy approvals for the patient's disease. For treatment planning, the clinician should consider the functional impact of the targeted alteration and expected efficacy of genomic biomarker-linked options relative to other approved or investigational treatments" (Chakravarty et al., 2022).

European Society for Medical Oncology (ESMO)

The ESMO recommends that "Mutational analysis inclusion in the diagnostic work-up of all GISTs should be considered standard practice [II, A] (with the possible exclusion of < 2 cm non-rectal GISTs)" (Casali et al., 2018). The article also states that "Mutational analysis for known mutations involving *KIT* and *PDGFRA* can confirm the diagnosis of GIST, if doubtful (particularly in rare CD117/DOG1-negative suspect GIST). Mutational analysis has a predictive value for sensitivity to molecular-targeted therapy and to prognostic value. Its inclusion in the diagnostic work-up of all GISTs should be considered standard practice" (Casali et al., 2018; Casali et al., 2022).

The ESMO Translational Research and Precision Medicine Working Group released clinical practice guidelines to define best practice for homologous recombination deficiency (HRD) testing in high-grade serous ovarian, fallopian tube and peritoneal carcinoma (HGSC). ESMO recommends that "pathological evaluation of the tumour tissue specimens used for assessment of somatic molecular alterations is essential" (Miller et al., 2020). Regarding homologous recombination repair (HRR) tests, *BRCA* germline and somatic mutation tests are recommended as they consistently identify the subgroup of ovarian cancer patients who benefit the most from poly-ADP ribose inhibitors (PARPi) therapy. There is insufficient evidence to determine the clinical validity of a panel of non-*BRCA* HRR genes and *BRCA1* or *RAD51C* promoter methylation to predict PARPi benefit. "In the first-line maintenance setting, germline and somatic *BRCA* mutation testing is routinely recommended to identify HGSC patients who should receive a PARPi" (Miller et al., 2020).

British Sarcoma Group (BSG)

The BSG has published guidelines on the management of GIST and state that most GIST cases are associated with a *KIT* or *PDGFRA* mutation. The guidelines recommend the following:

- "The diagnosis should be made by a pathologist experienced in the disease and include the use of immunohistochemistry and mutational analysis, which should be performed by an accredited laboratory.
- If neoadjuvant treatment with imatinib is planned, it is vital to confirm the diagnosis, since there is a wide differential. It may be necessary to perform a percutaneous core needle biopsy if the tumour is inaccessible to endoscopic ultrasound-guided biopsy. Mutational analysis is obligatory, since some GISTs are insensitive to imatinib (e.g. those with *D842V* mutation in exon 18 of *PDGFRA*)" (Judson et al., 2017).

These guidelines were updated in 2024 and recommended with the following minor changes:

- "The diagnosis of GIST should be established by a pathologist experienced in the disease and should include the use of IHC and, if necessary, molecular analysis (which should be performed by an accredited laboratory).
- If initial treatment with imatinib is planned, it is essential to confirm the diagnosis of GIST, since there is a wide differential. It may be necessary to perform a percutaneous core needle biopsy if the tumour is inaccessible to EUS biopsy. Molecular analysis is particularly critical, since some GISTs are insensitive to tyrosine kinase inhibitors (e.g. the PDGFRA exon 18 D842V mutation)" (Judson et al., 2024).

European Association of Urology (EAU)-European Association of Nuclear Medicine (EANM)-European Society for Radiotherapy and Oncology (ESTRO)-European Society of Urogenital Radiology (ESUR)-International Society of Geriatric Oncology (SIOG)

The EAU/EANM/ESTRO/ESUR/SIOG released guidelines on prostate cancer in 2021. These guidelines strongly recommend offering patients with Metastatic Castration-Resistant Prostate Cancer (mCRPC) "somatic molecular testing to identify patients

suitable for treatment with PARP inhibitors" (Mottet et al., 2021).

The American Urological Association / American Society for Radiation Oncology / Society of Urologic Oncology (AUA/ASTRO/SUO)

The AUA/ASTRO/SUO released guidelines on prostate cancer in 2021. These guidelines recommend that "clinicians should offer germline and somatic tumor genetic testing to identify DNA repair deficiency mutations and microsatellite instability status that may inform prognosis in patients with mCRPC and counseling regarding family risk as well as potential targeted therapies" (Lowrance et al., 2021).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

On July 30, 2021, the FDA approved ONCO/Reveal Dx Lung & Colon Cancer Assay (O/RDx-LCCA) by Pillar Biosciences. "The device is a qualitative next generation sequencing based in vitro diagnostic test that uses amplicon-based target enrichment technology for detection of single nucleotide variants (SNVs) and deletions in 2 genes from DNA isolated from formalin-fixed paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) and colorectal cancer (CRC) tumor tissue specimens" (FDA, 2021).

On July 18, 2020, the FDA approved Cobas® EZH2 Mutation Test, a somatic gene mutation detection system by Roche Molecular System, Inc. "The device is a real-time allele-specific PCR test for qualitative detection of single nucleotide mutations for *Y646N, Y646F or Y646X (Y646H, Y646S,* or *Y646C), A682G*, and *A692V* of the *EZH2* gene in DNA extracted from formalin fixed paraffin embedded (FFPE) human follicular lymphoma tumor tissue specimens" (FDA, 2020a).

On August 7, 2020, the FDA approved Guardant360 CDx, by Guardant Health, Inc. This device is a next generation sequencing based in vitro diagnostic device that uses targeted high throughput hybridization-based capture technology to detect SNVs, insertions, and deletions in 55 genes, copy number amplifications in two genes, and

fusions in four genes. Guardant360 CDx also utilizes circulating cell-free DNA collected in Streck Cell-Free DNA Blood Collection Tubes to identify NSCLC patients who may benefit from treatment with the targeted therapy (FDA, 2020c).

On April 15, 2020, the FDA approved Therascreen *BRAF* V600E RGQ PCR Kit by QIAGEN. The Therascreen *BRAF* V600E RGQ PCR Kit is a real-time PCR test for the qualitative detection of V600E mutations in the *BRAF* gene using genomic DNA extracted from FFPE human colorectal cancer (CRC) tumor tissue. The Therascreen *BRAF* V600E RGQ PCR Kit is an in vitro diagnostic device intended to be used as an aid in selecting patients with metastatic colorectal cancer (mCRC) whose tumors carry the *BRAF* V600E mutation for treatment with BRAFTOVI (encorafenib) in combination with cetuximab" (FDA, 2020d).

On October 23, 2019, the FDA approved MyChoice HRD CDx, by Myriad Genetic Laboratories, Inc. This device is a next generation sequencing based in vitro diagnostic device for detection of SNVs, insertions, deletions, and large rearrangement variants of the BRCA1 and BRCA2 genes. This test also determines the Genomic Instability Score (GIS), a measurement of Loss of Heterozygosity (LOH), Telomeric Allelic Imbalance (TAI), and Large Scale State Transitions (LST), which is used to identify ovarian cancer patients with positive HRD status (FDA, 2019).

On November 30, 2017, the FDA approved FoundationOne CDx, by Foundation Medicine, Inc. This device is a next generation sequencing based in vitro diagnostic device for detection of substitutions, insertion and deletion alterations (indels), and copy number alterations (CNAs) in 324 genes and select gene rearrangements, as well as genomic signatures including microsatellite instability (Bauml et al.) and TMB using DNA isolated from FFPE tumor tissue specimens (FDA, 2017).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81168	CCND1/IGH (t(11;14)) (eg, mantle cell lymphoma) translocation analysis, major breakpoint, qualitative and quantitative, if performed
81191	NTRK1 (neurotrophic receptor tyrosine kinase 1) (eg, solid tumors) translocation analysis

CPT	Code Description
81192	NTRK2 (neurotrophic receptor tyrosine kinase 2) (eg, solid tumors) translocation analysis
81193	NTRK3 (neurotrophic receptor tyrosine kinase 3) (eg, solid tumors) translocation analysis
81194	NTRK (neurotrophic-tropomyosin receptor tyrosine kinase 1, 2, and 3) (eg, solid tumors) translocation analysis
81233	BTK (Bruton's tyrosine kinase) (eg, chronic lymphocytic leukemia) gene analysis, common variants (eg, C481S, C481R, C481F)
81261	IGH@ (Immunoglobulin heavy chain locus) (eg, leukemias and lymphomas, B-cell), gene rearrangement analysis to detect abnormal clonal population(s); amplified methodology (eg, polymerase chain reaction)
81262	IGH@ (Immunoglobulin heavy chain locus) (eg, leukemias and lymphomas, B-cell), gene rearrangement analysis to detect abnormal clonal population(s); direct probe methodology (eg, Southern blot)
81263	IGH@ (Immunoglobulin heavy chain locus) (eg, leukemia and lymphoma, B-cell), variable region somatic mutation analysis
81264	IGK@ (Immunoglobulin kappa light chain locus) (eg, leukemia and lymphoma, B-cell), gene rearrangement analysis, evaluation to detect abnormal clonal population(s)
81265	Comparative analysis using Short Tandem Repeat (STR) markers; patient and comparative specimen (eg, pre-transplant recipient and donor germline testing, post-transplant non-hematopoietic recipient germline [eg, buccal swab or other germline tissue sample] and donor testing, twin zygosity testing, or maternal cell contamination of fetal cells)
81266	Comparative analysis using Short Tandem Repeat (STR) markers; each additional specimen (eg, additional cord blood donor, additional fetal samples from different cultures, or additional zygosity in multiple birth pregnancies) (List separately in addition to code for primary procedure)
81267	Chimerism (engraftment) analysis, post transplantation specimen (eg, hematopoietic stem cell), includes comparison to previously performed baseline analyses; without cell selection
81268	Chimerism (engraftment) analysis, post transplantation specimen (eg, hematopoietic stem cell), includes comparison to previously performed baseline analyses; with cell selection (eg, CD3, CD33), each cell type
81277	Cytogenomic neoplasia (genome-wide) microarray analysis, interrogation of genomic regions for copy number and loss-of-heterozygosity variants for chromosomal abnormalities

CPT	Code Description
81278	IGH@/BCL2 (t(14;18)) (eg, follicular lymphoma) translocation analysis, major breakpoint region (MBR) and minor cluster region (mcr) breakpoints, qualitative or quantitative
81305	MYD88 (myeloid differentiation primary response 88) (eg, Waldenstrom's macroglobulinemia, lymphoplasmacytic leukemia) gene analysis, p.Leu265Pro (L265P) variant
81314	PDGFRA (platelet-derived growth factor receptor, alpha polypeptide) (eg, gastrointestinal stromal tumor [GIST]), gene analysis, targeted sequence analysis (eg, exons 12, 18)
81315	PML/RARalpha, (t(15;17)), (promyelocytic leukemia/retinoic acid receptor alpha) (eg, promyelocytic leukemia) translocation analysis; common breakpoints (eg, intron 3 and intron 6), qualitative or quantitative
81316	PML/RARalpha, (t(15;17)), (promyelocytic leukemia/retinoic acid receptor alpha) (eg, promyelocytic leukemia) translocation analysis; single breakpoint (eg, intron 3, intron 6 or exon 6), qualitative or quantitative
81340	TRB@ (T cell antigen receptor, beta) (eg, leukemia and lymphoma), gene rearrangement analysis to detect abnormal clonal population(s); using amplification methodology (eg, polymerase chain reaction)
81341	TRB@ (T cell antigen receptor, beta) (eg, leukemia and lymphoma), gene rearrangement analysis to detect abnormal clonal population(s); using direct probe methodology (eg, Southern blot)
81342	TRG@ (T cell antigen receptor, gamma) (eg, leukemia and lymphoma), gene rearrangement analysis, evaluation to detect abnormal clonal population(s)
81347	SF3B1 (splicing factor [3b] subunit B1) (eg, myelodysplastic syndrome/acute myeloid leukemia) gene analysis, common variants (eg, A672T, E622D, L833F, R625C, R625L)
81348	SRSF2 (serine and arginine-rich splicing factor 2) (eg, myelodysplastic syndrome, acute myeloid leukemia) gene analysis, common variants (eg, P95H, P95L)
81357	U2AF1 (U2 small nuclear RNA auxiliary factor 1) (eg, myelodysplastic syndrome, acute myeloid leukemia) gene analysis, common variants (eg, S34F, S34Y, Q157R, Q157P)
81360	ZRSR2 (zinc finger CCCH-type, RNA binding motif and serine/arginine-rich 2) (eg, myelodysplastic syndrome, acute myeloid leukemia) gene analysis, common variant(s) (eg, E65fs, E122fs, R448fs)
81370	HLA Class I and II typing, low resolution (eg, antigen equivalents); HLA-A, -B, -C, -DRB1/3/4/5, and -DQB1
81371	HLA Class I and II typing, low resolution (eg, antigen equivalents); HLA-A, -B, and -DRB1 (eg, verification typing)

CPT	Code Description
81372	HLA Class I typing, low resolution (eg, antigen equivalents); complete (ie, HLA-A, -B, and -C)
81373	HLA Class I typing, low resolution (eg, antigen equivalents); one locus (eg, HLA-A, -B, or -C), each
81374	HLA Class I typing, low resolution (eg, antigen equivalents); one antigen equivalent (eg, B*27), each
81375	HLA Class II typing, low resolution (eg, antigen equivalents); HLA-DRB1/3/4/5 and -DQB1
81376	HLA Class II typing, low resolution (eg, antigen equivalents); one locus (eg, HLA-DRB1, -DRB3/4/5, -DQB1, -DQA1, -DPB1, or -DPA1), each
81377	HLA Class II typing, low resolution (eg, antigen equivalents); one antigen equivalent, each
81378	HLA Class I and II typing, high resolution (ie, alleles or allele groups), HLA-A, -B, -C, and -DRB1
81379	HLA Class I typing, high resolution (ie, alleles or allele groups); complete (ie, HLA-A, -B, and -C)
81380	HLA Class I typing, high resolution (ie, alleles or allele groups); one locus (eg, HLA-A, -B, or -C), each
81381	HLA Class I typing, high resolution (ie, alleles or allele groups); one allele or allele group (eg, B*57:01P), each
81382	HLA Class II typing, high resolution (ie, alleles or allele groups); one locus (eg, HLA-DRB1, -DRB3/4/5, -DQB1, -DQA1, -DPB1, or -DPA1), each
81383	HLA Class II typing, high resolution (ie, alleles or allele groups); one allele or allele group (eg, HLA-DQB1*06:02P), each
81400	Molecular pathology procedure, Level 1 (eg, identification of single germline variant [eg, SNP] by techniques such as restriction enzyme digestion or melt curve analysis)
81401	Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)
81402	Molecular pathology procedure, Level 3 (eg, >10 SNPs, 2-10 methylated variants, or 2-10 somatic variants [typically using non-sequencing target variant analysis], immunoglobulin and T-cell receptor gene rearrangements, duplication/deletion variants of 1 exon, loss of heterozygosity [LOH], uniparental disomy [UPD])
81403	Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)

CPT	Code Description
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81449	Solid organ neoplasm, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants and copy number variants or rearrangements, if performed; RNA analysis
81456	Solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes, genomic sequence analysis panel, interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; RNA analysis
81479	Unlisted molecular pathology procedure
81599	Unlisted multianalyte assay with algorithmic analysis
88237	Tissue culture for neoplastic disorders; bone marrow, blood cells
88239	Tissue culture for neoplastic disorders; solid tumor
88240	Cryopreservation, freezing and storage of cells, each cell line
88241	Thawing and expansion of frozen cells, each aliquot
88269	Chromosome analysis, in situ for amniotic fluid cells, count cells from 6-12 colonies, 1 karyotype, with banding
88271	Molecular cytogenetics; DNA probe, each (eg, FISH)
88272	Molecular cytogenetics; chromosomal in situ hybridization, analyze 3-5 cells (eg, for derivatives and markers)
88273	Molecular cytogenetics; chromosomal in situ hybridization, analyze 10-30 cells (eg, for microdeletions)
88274	Molecular cytogenetics; interphase in situ hybridization, analyze 25-99 cells
88275	Molecular cytogenetics; interphase in situ hybridization, analyze 100-300 cells
88280	Chromosome analysis; additional karyotypes, each study
88283	Chromosome analysis; additional specialized banding technique (eg, NOR, C-banding)
88285	Chromosome analysis; additional cells counted, each study
88289	Chromosome analysis; additional high resolution study
88291	Cytogenetics and molecular cytogenetics, interpretation and report
88299	Unlisted cytogenetic study
0268U	Hematology (atypical hemolytic uremic syndrome [aHUS]), genomic sequence analysis of 15 genes, blood, buccal swab, or amniotic fluid Proprietary test: Versiti™ aHUS Genetic Evaluation Lab/Manufacturer: Versiti™ Diagnostic Laboratories

CPT	Code Description
0444U	Oncology (solid organ neoplasia), targeted genomic sequence analysis panel of 361 genes, interrogation for gene fusions, translocations, or other rearrangements, using DNA from formalin-fixed paraffin-embedded (FFPE) tumor tissue, report of clinically significant variant(s) Proprietary test: Aventa FusionPlus ™ Lab/Manufacturer: Aventa Genomics, LLC
0523U	Oncology (solid tumor), DNA, qualitative, next-generation sequencing (NGS) of singlenucleotide variants (SNV) and insertion/deletions in 22 genes utilizing formalin-fixed paraffinembedded tissue, reported as presence or absence of mutation(s), location of mutation(s), nucleotide change, and amino acid change Proprietary test: oncoRevealTMCDx Lab/Manufacturer: Pillar Biosciences Inc, Pillar Biosciences Inc

Current Procedural Terminology© American Medical Association. All Rights reserved.
Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Bacino, C. (2023, Dec 14). *Genomic disorders: An overview*. https://www.uptodate.com/contents/genomic-disorders-an-overview

Bauml, J. M., Li, B. T., Velcheti, V., Govindan, R., Curioni-Fontecedro, A., Dooms, C., Takahashi, T., Duda, A. W., Odegaard, J. I., Cruz-Guilloty, F., Jin, L., Zhang, Y., Anderson, A., & Skoulidis, F. (2021). Clinical validation of Guardant360 CDx as a blood-based companion diagnostic for sotorasib. *Lung Cancer*. https://doi.org/10.1016/j.lungcan.2021.10.007

Casali, P. G., Abecassis, N., Aro, H. T., Bauer, S., Biagini, R., Bielack, S., Bonvalot, S., Boukovinas, I., Bovee, J., Brodowicz, T., Broto, J. M., Buonadonna, A., De Alava, E., Dei Tos, A. P., Del Muro, X. G., Dileo, P., Eriksson, M., Fedenko, A., Ferraresi, V., . . . Blay, J. Y. (2018). Gastrointestinal stromal tumours: ESMO-EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, *29*(Suppl 4), iv267. https://pubmed.ncbi.nlm.nih.gov/34560242/

Casali, P. G., Blay, J. Y., Abecassis, N., Bajpai, J., Bauer, S., Biagini, R., Bielack, S., Bonvalot, S., Boukovinas, I., Bovee, J. V. M. G., Boye, K., Brodowicz, T., Buonadonna, A., De Álava, E., Dei Tos, A. P., Del Muro, X. G., Dufresne, A., Eriksson, M., Fedenko, A., . . . Stacchiotti, S. (2022). Gastrointestinal stromal tumours: ESMO-EURACAN-GENTURIS Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*, *33*(1), 20-33. https://pubmed.ncbi.nlm.nih.gov/34560242/

Chakravarty, D., Johnson, A., Sklar, J., Lindeman, N. I., Moore, K., Ganesan, S., Lovly, C. M., Perlmutter, J., Gray, S. W., Hwang, J., Lieu, C., André, F., Azad, N., Borad, M., Tafe, L., Messersmith, H., Robson, M., & Meric-Bernstam, F. (2022). Somatic Genomic Testing in Patients With Metastatic or Advanced Cancer: ASCO Provisional Clinical Opinion. *Journal of Clinical Oncology*, *40*(11), 1231-1258. https://doi.org/10.1200/JCO.21.02767

Comandini, D., Damiani, A., & Pastorino, A. (2017). Synchronous GISTs associated with multiple sporadic tumors: a case report. *Drugs Context*, *6*, 212307. https://doi.org/10.7573/dic.212307

FDA. (2017). *FoundationOne CDx*. https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm?db=pma&id=407172

FDA. (2019). MyChoice HRD CDx. https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm?db=pma&id=440605

FDA. (2020a). Cobas® EZH2 Mutation Test. https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm?db=pma&id=455336

FDA. (2020b). FDA approves pembrolizumab for adults and children with TMB-H solid tumors. https://www.fda.gov/drugs/drug-approvals-and-databases/fda-approves-pembrolizumab-adults-and-children-tmb-h-solid-tumors

FDA. (2020c). Guardant360 CDx. https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm?db=pma&id=454228

FDA. (2020d). Therascreen BRAF V600E RGQ PCR Kit. https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm?db=pma&id=448074

FDA. (2021). ONCO/Reveal Dx Lung & Colon Cancer Assay (O/RDx-LCCA). https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm?db=pma&id=454588

Garrett, N., Mitchev, N., Osman, F., Naidoo, J., Dorward, J., Singh, R., Ngobese, H., Rompalo, A., Mlisana, K., & Mindel, A. (2019). Diagnostic accuracy of the Xpert CT/NG and OSOM Trichomonas Rapid assays for point-of-care STI testing among young women in South Africa: a cross-sectional study. *BMJ Open*, *9*(2), e026888. https://doi.org/10.1136/bmjopen-2018-026888

Guardant. (2020). Guardant Health Guardant360® CDx First FDA-Approved Liquid Biopsy for Comprehensive Tumor Mutation Profiling Across All Solid Cancers. https://www.globenewswire.com/news-release/2020/08/07/2075187/0/en/Guardant-Health-Guardant360-CDx-First-FDA-Approved-Liquid-Biopsy-for-Comprehensive-Tumor-Mutation-Profiling-Across-All-Solid-Cancers.html

Hayano, T., Matsui, H., Nakaoka, H., Ohtake, N., Hosomichi, K., Suzuki, K., & Inoue, I. (2016). Germline Variants of Prostate Cancer in Japanese Families. *PLOS ONE*, *11*(10), e0164233. https://doi.org/10.1371/journal.pone.0164233

Hulick, P. (2024, Feb 7). *Next-generation DNA sequencing (NGS): Principles and clinical applications*. https://www.uptodate.com/contents/next-generation-dna-sequencing-ngs-principles-and-clinical-applications

Judson, I., Bulusu, R., Seddon, B., Dangoor, A., Wong, N., & Mudan, S. (2017). UK clinical practice guidelines for the management of gastrointestinal stromal tumours (GIST). *Clin Sarcoma Res*, *7*, 6. https://doi.org/10.1186/s13569-017-0072-8

Judson, I., Jones, R. L., Wong, N. A. C. S., Dileo, P., Bulusu, R., Smith, M., & Almond, M. (2024). Gastrointestinal stromal tumour (GIST): British Sarcoma Group clinical practice guidelines. *British Journal of Cancer*. https://doi.org/10.1038/s41416-024-02672-0

Kalia, S. S., Adelman, K., Bale, S. J., Chung, W. K., Eng, C., Evans, J. P., Herman, G. E., Hufnagel, S. B., Klein, T. E., Korf, B. R., McKelvey, K. D., Ormond, K. E., Richards, C. S., Vlangos, C. N., Watson, M., Martin, C. L., & Miller, D. T. (2016). Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics [ACMG Statement]. *Genetics In Medicine*, *19*, 249. https://doi.org/10.1038/gim.2016.190

Klempner, S. J., Fabrizio, D., Bane, S., Reinhart, M., Peoples, T., Ali, S. M., Sokol, E. S., Frampton, G., Schrock, A. B., Anhorn, R., & Reddy, P. (2020). Tumor Mutational Burden as a Predictive Biomarker for Response to Immune Checkpoint Inhibitors: A Review of Current Evidence. *Oncologist*, *25*(1), e147-e159. https://doi.org/10.1634/theoncologist.2019-0244

Kohlmann, W., & Slavotinek, A. (2024, July 22). *Genetic testing*. https://www.uptodate.com/contents/genetic-testing

Konnick, E. Q., & Pritchard, C. C. (2016). Germline, hematopoietic, mosaic, and somatic variation: interplay between inherited and acquired genetic alterations in disease assessment. *Genome Med*, *8*(1), 100. https://doi.org/10.1186/s13073-016-0350-8

Konstantinopoulos, P. A., Norquist, B., Lacchetti, C., Armstrong, D., Grisham, R. N., Goodfellow, P. J., Kohn, E. C., Levine, D. A., Liu, J. F., Lu, K. H., Sparacio, D., & Annunziata, C. M. (2020). Germline and Somatic Tumor Testing in Epithelial Ovarian Cancer: ASCO Guideline. *Journal of Clinical Oncology*, *38*(11), 1222-1245. https://doi.org/10.1200/jco.19.02960

Kowalik, A., Zalewski, K., Kopczynski, J., Siolek, M., Lech, M., Hincza, K., Kalisz, J., Chrapek, M., Zieba, S., Furmanczyk, J., Jedlinski, M., Chlopek, M., Misiek, M., & Gozdz, S. (2019). Somatic mutations in BRCA1&2 in 201 unselected ovarian carcinoma samples - single institution study. *Pol J Pathol*, *70*(2), 115-126. https://doi.org/10.5114/pjp.2019.82905

Lamont, R. F., Sobel, J. D., Akins, R. A., Hassan, S. S., Chaiworapongsa, T., Kusanovic, J. P., & Romero, R. (2011). The vaginal microbiome: new information about genital tract flora using molecular based techniques. *Bjog*, *118*(5), 533-549. https://doi.org/10.1111/j.1471-0528.2010.02840.x

Legrand, F., Renneville, A., MacIntyre, E., Mastrilli, S., Ackermann, F., Cayuela, J. M., Rousselot, P., Schmidt-Tanguy, A., Fain, O., Michel, M., de Jaureguiberry, J. P., Hatron, P. Y., Cony-Makhoul, P., Lefranc, D., Sene, D., Cottin, V., Hamidou, M., Lidove, O., Baruchel, A., . . . Kahn, J. E. (2013). The Spectrum of FIP1L1-PDGFRA-Associated Chronic Eosinophilic Leukemia: New Insights Based on a Survey of 44 Cases. *Medicine (Baltimore)*, *92*(5), e1-e9. https://doi.org/10.1097/MD.0b013e3182a71eba

Li, M. M., Datto, M., Duncavage, E. J., Kulkarni, S., Lindeman, N. I., Roy, S., Tsimberidou, A. M., Vnencak-Jones, C. L., Wolff, D. J., Younes, A., & Nikiforova, M. N. (2017). Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn*, *19*(1), 4-23. https://doi.org/10.1016/j.jmoldx.2016.10.002

Liu, S., Yu, Q., Han, W., Qi, L., Zu, X., Zeng, F., Xie, Y., & Liu, J. (2014). Primary gastrointestinal stromal tumor of the prostate: A case report and literature review. *Oncol Lett*, *7*(6), 1925-1929. https://doi.org/10.3892/ol.2014.1968

Lowrance, W. T., Breau, R. H., Chou, R., Chapin, B. F., Crispino, T., Dreicer, R., Jarrard, D. F., Kibel, A. S., Morgan, T. M., Morgans, A. K., Oh, W. K., Resnick, M. J., Zietman, A. L., & Cookson, M. S. (2021). Advanced Prostate Cancer: AUA/ASTRO/SUO Guideline PART II. *J Urol*, *205*(1), 22-29. https://doi.org/10.1097/ju.0000000000001376

Lux, M. P., & Fasching, P. A. (2023). Breast Cancer and Genetic BRCA1/2 Testing in Routine Clinical Practice: Why, When and For Whom? *Geburtshilfe Frauenheilkd*, *83*(3), 310-320. https://doi.org/10.1055/a-1929-2629

Marabelle, A., Fakih, M., Lopez, J., Shah, M., Shapira-Frommer, R., Nakagawa, K., Chung, H. C., Kindler, H. L., Lopez-Martin, J. A., Miller, W. H., Jr., Italiano, A., Kao, S., Piha-Paul, S. A., Delord, J.-P., McWilliams, R. R., Fabrizio, D. A., Aurora-Garg, D., Xu, L., Jin, F., . . . Bang, Y.-J. (2020). Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. *The Lancet Oncology*, *21*(10), 1353-1365. https://doi.org/10.1016/S1470-2045(20)30445-9

Martincorena, I., & Campbell, P. J. (2015). Somatic mutation in cancer and normal cells. *Science*, *349*(6255), 1483-1489. https://doi.org/10.1126/science.aab4082

McCabe, C. D., Spyropoulos, D. D., Martin, D., & Moreno, C. S. (2008). Genome-wide analysis of the homeobox C6 transcriptional network in prostate cancer. *Cancer Res*, *68*(6), 1988-1996. https://doi.org/10.1158/0008-5472.Can-07-5843

Merino, D. M., McShane, L. M., Fabrizio, D., Funari, V., Chen, S. J., White, J. R., Wenz, P., Baden, J., Barrett, J. C., Chaudhary, R., Chen, L., Chen, W. S., Cheng, J. H., Cyanam, D.,

Dickey, J. S., Gupta, V., Hellmann, M., Helman, E., Li, Y., . . . Allen, J. (2020). Establishing guidelines to harmonize tumor mutational burden (TMB): in silico assessment of variation in TMB quantification across diagnostic platforms: phase I of the Friends of Cancer Research TMB Harmonization Project. *J Immunother Cancer*, *8*(1). https://doi.org/10.1136/jitc-2019-000147

Miller, D. T., Lee, K., Abul-Husn, N. S., Amendola, L. M., Brothers, K., Chung, W. K., Gollob, M. H., Gordon, A. S., Harrison, S. M., Hershberger, R. E., Klein, T. E., Richards, C. S., Stewart, D. R., & Martin, C. L. (2023). ACMG SF v3.2 list for reporting of secondary findings in clinical exome and genome sequencing: A policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *25*(8), 100866. https://doi.org/10.1016/j.gim.2023.100866

Miller, D. T., Lee, K., Chung, W. K., Gordon, A. S., Herman, G. E., Klein, T. E., Stewart, D. R., Amendola, L. M., Adelman, K., Bale, S. J., Gollob, M. H., Harrison, S. M., Hershberger, R. E., McKelvey, K., Richards, C. S., Vlangos, C. N., Watson, M. S., & Martin, C. L. (2021). ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genetics In Medicine*, *23*(8), 1381-1390. https://doi.org/10.1038/s41436-021-01172-3

Miller, R. E., Leary, A., Scott, C. L., Serra, V., Lord, C. J., Bowtell, D., Chang, D. K., Garsed, D. W., Jonkers, J., Ledermann, J. A., Nik-Zainal, S., Ray-Coquard, I., Shah, S. P., Matias-Guiu, X., Swisher, E. M., & Yates, L. R. (2020). ESMO recommendations on predictive biomarker testing for homologous recombination deficiency and PARP inhibitor benefit in ovarian cancer. *Annals of Oncology*, *31*(12), 1606-1622. https://www.sciencedirect.com/science/article/pii/S0923753420421647

Morgan, J., Raut, C., Duensing, A., & Keedy, V. (2023, April 11). *Clinical presentation, diagnosis, and prognosis of gastrointestinal stromal tumors*. https://www.uptodate.com/contents/clinical-presentation-diagnosis-and-prognosis-of-gastrointestinal-stromal-tumors

Mottet, N., van den Bergh, R. C. N., Briers, E., Van den Broeck, T., Cumberbatch, M. G., De Santis, M., Fanti, S., Fossati, N., Gandaglia, G., Gillessen, S., Grivas, N., Grummet, J., Henry, A. M., van der Kwast, T. H., Lam, T. B., Lardas, M., Liew, M., Mason, M. D., Moris, L., . . . Cornford, P. (2021). EAU-EANM-ESTRO-ESUR-SIOG Guidelines on Prostate Cancer-2020 Update. Part 1: Screening, Diagnosis, and Local Treatment with Curative Intent. *Eur Urol*, *79*(2), 243-262. https://doi.org/10.1016/j.eururo.2020.09.042

Myriad Genetics. (2024). What is Myriad myChoice® CDx? https://myriad.com/products-services/precision-medicine/mychoice-cdx/

Nagahashi, M., Shimada, Y., Ichikawa, H., Kameyama, H., Takabe, K., Okuda, S., & Wakai, T. (2019). Next generation sequencing-based gene panel tests for the management of solid tumors. *Cancer Sci*, *110*(1), 6-15. https://doi.org/10.1111/cas.13837

NCCN. (2024). *NCCN Guidelines*. https://www.nccn.org/guidelines/category_1

NCI. (2024). tumor mutational burden. https://www.cancer.gov/publications/dictionaries/cancer-terms/def/tumor-mutational-burden

Pappas, P. G., Kauffman, C. A., Andes, D. R., Clancy, C. J., Marr, K. A., Ostrosky-Zeichner, L., Reboli, A. C., Schuster, M. G., Vazquez, J. A., Walsh, T. J., Zaoutis, T. E., & Sobel, J. D. (2016). Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. *Clin Infect Dis*, *62*(4), e1-50. https://doi.org/10.1093/cid/civ933

Pillar Biosciences. (2020). Clinical and Analytical Validation of the CE IVD ONCO/Reveal™ Dx Lung and Colon Cancer Assay https://www.pillarbiosci.com/wp-content/uploads/2021/04/AMP_2020_Pillar_Biosciences_16NOV20_v1__LC103v4_Nick.pdf

Pillar Biosciences. (2021). Assay User Manual ONCO/Reveal™ Dx Lung and Colon Cancer Assay. https://www.pillarbiosci.com/wp-content/uploads/2021/04/20210409-CE-IVD-assay-User-Guide-UM-0044-ONCO_Reveal-Dx-Lung-and-Colon-Cancer-Assay-User-Manual-CE-8435-1-8436-3.pdf

Poduri, A., Evrony, G. D., Cai, X., & Walsh, C. A. (2013). Somatic Mutation, Genomic Variation, and Neurological Disease. *Science*, *341*(6141), 1237758. https://doi.org/10.1126/science.1237758

Pujol, P., Barberis, M., Beer, P., Friedman, E., Piulats, J. M., Capoluongo, E. D., Garcia Foncillas, J., Ray-Coquard, I., Penault-Llorca, F., Foulkes, W. D., Turnbull, C., Hanson, H., Narod, S., Arun, B. K., Aapro, M. S., Mandel, J.-L., Normanno, N., Lambrechts, D., Vergote, I., . . . Alés Martínez, J. E. (2021). Clinical practice guidelines for BRCA1 and BRCA2 genetic testing. *European Journal of Cancer*, *146*, 30-47. https://doi.org/10.1016/j.ejca.2020.12.023

QIAGEN. (2024). therascreen BRAF V600E RGQ PCR Kit. https://www.qiagen.com/us/products/diagnostics-and-clinical-research/oncology/therascreen-solid-tumor/therascreen-braf-v600e-rgq-pcr-kit/

Raby, B. A., & Blank, R. D. (2024, Oct 18). *Genetics: Glossary of terms*. https://www.uptodate.com/contents/genetics-glossary-of-terms

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., & Rehm, H. L. (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*, *17*(5), 405-424. https://doi.org/10.1038/gim.2015.30

Robson, M. E., Bradbury, A. R., Arun, B., Domchek, S. M., Ford, J. M., Hampel, H. L., Lipkin, S. M., Syngal, S., Wollins, D. S., & Lindor, N. M. (2015). American Society of Clinical Oncology Policy Statement Update: Genetic and Genomic Testing for Cancer

Susceptibility. *J Clin Oncol*, *33*(31), 3660-3667. https://doi.org/10.1200/jco.2015.63.0996

Takeda, M., Takahama, T., Sakai, K., Shimizu, S., Watanabe, S., Kawakami, H., Tanaka, K., Sato, C., Hayashi, H., Nonagase, Y., Yonesaka, K., Takegawa, N., Okuno, T., Yoshida, T., Fumita, S., Suzuki, S., Haratani, K., Saigoh, K., Ito, A., . . . Nishio, K. (2021). Clinical Application of the FoundationOne CDx Assay to Therapeutic Decision-Making for Patients with Advanced Solid Tumors. *The Oncologist*, *26*(4), e588-e596. https://doi.org/10.1002/onco.13639

Terada, T. (2012). Small cell neuroendocrine carcinoma of the prostate: incidence and a report of four cases with an examination of KIT and PDGFRA. *Prostate*, *72*(10), 1150-1156. https://doi.org/10.1002/pros.22464

Thirunavukarasu, D., Cheng, L. Y., Song, P., Chen, S. X., Borad, M. J., Kwong, L., James, P., Turner, D. J., & Zhang, D. Y. (2021). Oncogene Concatenated Enriched Amplicon Nanopore Sequencing for rapid, accurate, and affordable somatic mutation detection. *Genome Biology*, *22*(1), 227. https://doi.org/10.1186/s13059-021-02449-1

USPSTF. (2008). Screening for bacterial vaginosis in pregnancy to prevent preterm delivery: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med*, *148*(3), 214-219. https://doi.org/10.7326/0003-4819-148-3-200802050-00007

Woodhouse, R., Li, M., Hughes, J., Delfosse, D., Skoletsky, J., Ma, P., Meng, W., Dewal, N., Milbury, C., Clark, T., Donahue, A., Stover, D., Kennedy, M., Dacpano-Komansky, J., Burns, C., Vietz, C., Alexander, B., Hegde, P., & Dennis, L. (2020). Clinical and analytical validation of FoundationOne Liquid CDx, a novel 324-Gene cfDNA-based comprehensive genomic profiling assay for cancers of solid tumor origin. *PLOS ONE*, *15*(9), e0237802. https://doi.org/10.1371/journal.pone.0237802

Genetic Markers for Assessing Risk of Cardiovascular Disease

Policy Number: AHS – M2180 – Genetic Markers for Assessing Risk of Cardiovascular Disease	Prior Policy Name and Number, as applicable:
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Cardiovascular risk assessment comprises the means and processes to predict the probability of developing a cardiovascular disease. These are a group of tests and health factors that have been proven to indicate a person's chance of having a cardiovascular event such as a heart attack or stroke. This policy addresses genetic markers that are associated with cardiovascular disease risk. Terms such as male and female are used when necessary to refer to sex assigned at birth.

II. Related Policies

Policy Number	Policy Title
AHS-G2050	Cardiovascular Disease Risk Assessment
AHS-M2082	Genetic Testing for Lipoprotein(a) Variant(s) as a Decision Aid for Aspirin Treatment and/or CVD Risk Assessment
AHS-M2137	Genetic Testing for Familial Hypercholesterolemia
AHS-M2141	Testing of Homocysteine Metabolism-Related Conditions

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

1) To predict the risk of developing cardiovascular disease and/or the effectiveness of statin therapy, *KIF6* genotyping **DOES NOT MEET COVERAGE CRITERIA**.

2) To assess an individual's risk of developing cardiovascular disease, the following tests **DO NOT MEET COVERAGE CRITERIA**:

   a) Gene expression profiling.

   b) Genotyping for 9p21 single nucleotide polymorphisms.

   c) Panels that incorporate genetic risk factors with nongenetic biomarkers.

3) All other genetic tests for assessing cardiovascular disease risk **DO NOT MEET COVERAGE CRITERIA**.

IV. Table of Terminology

Term	Definition
ABI	Ankle-brachial index
ACC	American College of Cardiology
AHA	American Heart Association
Apo B	Apolipoprotein B
ASCVD	Atherosclerotic cardiovascular disease
CAC	Coronary artery calcium
CAD	Coronary artery disease
CCS	Canadian Cardiovascular Society
CHD	Coronary heart disease
CVD	Cardiovascular disease
EAS	European Atherosclerosis Society
ES	Endocrine Society
ESC	European Society of Cardiology
FDA	Food and Drug Administration
GRS	Genomic risk score
GWAS	Genome-wide association study
HDL	High-density lipoprotein
hsCRP	High-sensitivity C reactive protein
IDEAL	Incremental decrease in end points through aggressive lipid-lowering
KIF6	*Kinesin family member 6*
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol
LDT	Laboratory developed test

Lp(a)	Lipoprotein A
LPA	Apolipoprotein(a) locus
Lp-PLA2	Lipoprotein-associated phospholipase A2
MI	Myocardial infarction
PRSs	Polygenic risk scores
RNA	Ribonucleic acid
SNPs	Single nucleotide polymorphisms
TNT	Treating to new targets study
VLDL	Very low-density lipoprotein

V. Scientific Background

Statistics show that cardiovascular disease (including coronary heart disease, stroke, and hypertension) is America's leading health problem and the leading cause of death. According to the 2024 update of the heart disease and stroke statistics report released by the American Heart Association (AHA):

- Approximately 127.9 million people in this country suffer from some form of cardiovascular disease (encompassing coronary heart disease, heart failure, hypertension, and stroke).
- The direct and indirect costs of cardiovascular disease and stroke are about $422.3 billion and increasing every year.
- Coronary heart disease (CHD) was the leading cause (40.3%) of deaths attributable to CVD in the United States, followed by stroke (17.5%), other CVD (17.1%), high blood pressure (13.4%), heart failure (9.1%), diseases of the arteries (2.6%).
- Heart disease and stroke claim more lives each year in the United States than all forms of cancer and Chronic Lower Respiratory disease combined.
- On average, someone dies of a stroke every three minutes fourteen seconds in the United States.
- 11.5% of US adults reported cigarette use every day or some days (13.1% of males and 10.1% of females).
- Worldwide, high body mass index was attributed to 3.69 million deaths (AHA, 2024; Seth S. Martin, 2024).

Cardiovascular Risk Assessment

Traditionally, the most important indicators for cardiac risk are those of a person's health history. These include factors such as family history, age, weight, exercise, and cigarette smoking status (Wilson, 2023). Research has begun examining genetic markers and their ability to predict an individual’s risk of developing cardiovascular

disease.

Genetic Markers

According to the AHA in 2013, genomics serves several roles in cardiovascular health and disease, including disease prediction, discovery of genetic loci influencing CVD, functional evaluation of these genetic loci to understand mechanisms, and identification of therapeutic targets (Ganesh et al., 2013). The AHA notes that several clinically useful diagnostic tests have been discovered for single-gene CVDs. However, it is laborious to develop genetic testing for complex CVD because individual common variants have a smaller impact on risk. Furthermore, the influence of factors such as environmental variables, phenotypic heterogeneity, and pathogenic complexity, makes understanding the genomics behind complex CVDs more difficult. Characterization of the clinical phenotype requires consideration of the clinical details of the diseases and traits under study (Ganesh et al., 2013). Many genetic loci and polymorphisms have been proposed to influence cardiovascular risk. Palotie et al. (2016) identified 49,310 single nucleotide polymorphisms (SNPs), created a genomic risk score (GRS) based on those SNPs, and evaluated the GRS with five population cohorts totaling over 15,000 samples. The GRS was found to result in a hazard ratio of 1.74 for the three Finnish population cohorts (n = 12676) and a hazard ratio of 1.28 for the two Framingham cohorts (n = 3406). The GRS was also found to capture different trajectories of absolute risk, with men in the top 20% attaining 10% cumulative coronary heart disease (CHD) risk 12–18 years earlier than those in the bottom 20% (Palotie et al., 2016).

Gene expression changes, particularly in peripheral blood cells, may provide some information on CVD risk. Gene expression is thought to reflect the pathological state of the current cell, tissue, or organ system, therefore providing molecular insight on the status of cardiac tissue before clinical symptoms start to appear (Elashoff et al., 2011). Genetic variation may lead to vastly differing concentrations of many genetic products. For example, Zhernakova et al. (2018) evaluated the plasma concentrations of 92 cardiovascular disease-related proteins, of which 73 had a genetic association. Cis- and trans- isoforms of the protein quantitative trait loci produced genetic variation; this, combined with microbial variation, is responsible for 17.5% of inter-individual variation in plasma proteins (Zhernakova et al., 2018).

9p21 SNP

Genetic determinants of the development of CHD have been investigated in multiple studies with 46 SNPs across the genome significantly associated with an increased risk of disease (Deloukas et al., 2013). The strongest association with CHD risk was linked to SNPs around locus 9p21 (Palomaki et al., 2010; Patel et al., 2014; Paynter et al., 2009;

Samani et al., 2007; Wilson, 2023). However, despite clear association between variants and incident CHD, locus 9p21 SNPs have not been definitively shown to significantly improve CHD risk prediction compared with traditional risk factors (Dutta et al., 2011; Palomaki et al., 2010; Paynter et al., 2009; Virani et al., 2012). Trenkwalder et al. (2019) identified an association between the LPA locus and an increased risk of aortic valve stenosis, particularly in patients without CAD; however, 9p21 was not associated with aortic valve stenosis. The pathophysiologic impact of these genetic variants likely varies depending on other environmental factors or comorbid conditions (Wilson, 2023). Proprietary labs such as Celera Corporation (now owned by Quest Diagnostics) and deCODE Genetics offer tests revolving around analysis of this locus mutation (Quest, 2024).

Palomaki et al. (2010) performed a targeted systematic review of published literature to evaluate the clinical utility of 9p21 single nucleotide polymorphism testing. Forty-seven distinct data sets from 22 articles were analyzed, including 35,872 cases and 95,837 controls. The authors found a statistically significant association between 9p21 SNPs and heart disease that varied by age at disease onset, but the magnitude of the association was small. These risk alleles were found to associate more strongly with cardiovascular events in younger persons than cardiovascular events overall. However, the authors noted that the clinical utility of 9p21-related biomarkers cannot be assumed due to numerous complicating factors such as patient adherence (Palomaki et al., 2010).

Gransbo et al. (2013) evaluated if testing for 9p21 SNP adds useful information to CVD prediction beyond assessment of traditional risk factors. The common SNP variant rs4977574 on chromosome 9p21 was genotyped in 24,777 subjects who were free from CVD prior to the baseline examination. The genotyping of the 9p21 gene was not found to significantly improve predictive accuracy of CVD, although 9p21 significantly predicted CVD in the whole population in additive models. The authors evaluated the attributable risk conferred by 9p21 to be 13%. The investigators concluded that a variation of chromosome 9p21 alone "does not add clinically meaningful information in terms of CVD prediction beyond traditional risk factors at any age" (Gransbo et al., 2013).

Dehghan et al. (2016) evaluated the genome-wide association study (GWAS) for incident myocardial infarction (MI) and CHD in prospective cohort studies, which analyzed a total of 64297 individuals, including 3898 MI cases and 5465 CHD cases. A modest association between the 9p21 locus SNP (rs1333049) and MI, as well as marginal association with CHD was found. Among an inception cohort of those who experienced MI during follow-up, this risk allele was associated with a decreased risk of subsequent mortality. The authors concluded that the role of 9p21 locus may be

complex and that the protective effect of the risk alleles at 9p21 is unclear; however, the authors note that several other studies have found that the 9p21 locus increases the CHD risk for the first event only and not for subsequent events. Overall, the authors suggest further investigation of genetic causes of complex disorders (Dehghan et al., 2016).

Kessler et al. (2016) reviewed the impact of GWAS on the pathophysiology and therapy of CVD. The researchers found a correlation between the number of individuals participating in the study and the number of genome-wide significant variants detected by the GWAS. Technological advancement has led to denser genotyping, resulting in increased number of loci with genome-wide significant association for CAD. Over 50 genetic variants associated with cardiovascular risk have so far been identified. Almost all risk variants identified by GWAS were found in European individuals with the average European carrying several risk alleles associated with CHD. Most of these risk alleles were found in non-coding parts of the genome, suggesting that these variants affect gene regulation rather than protein structure. For example, the 9p21 locus encodes different isoforms of antisense non-coding RNA in the INK4 locus (ANRIL) which is involved in the preferred synthesis of non-circular/circular forms of the long non-coding RNA, affecting expression of multiple genes (Kessler et al., 2016). The authors state, however, that variants identified represent no more than 10% of the heritable risk. Although important progress was made with GWAS, further understanding of inheritance patterns is needed. The results available so far provide valuable insights into the pathophysiology of CAD and MI, but they are only a starting point for individualized treatment strategies (Kessler et al., 2016).

In summary, no studies were found that outlined changes to preventive and monitoring strategies, patient management, or improvement in clinical outcomes due to genotyping for 9p21 SNPs. There is a lack of published evidence regarding the clinical utility of genetic testing. Based on current literature, the contribution of 9p21 genotyping to overall cardiovascular risk assessment, above that of traditional risk factors, is not likely to be clinically important.

Kinesin family member 6 (KIF6)

Kinesin family member 6 (KIF6) is a protein encoded by the *KIF6* gene. This protein is involved in transport along microtubules, and the primary mutation of Trp719Arg affects its ability to bind its cargo. KIF6 has been studied as a marker for coronary artery disease and effectiveness of statin therapy. Initial studies of two trials with statins found an association between the *KIF6* p.Trp719Arg (rs20455, c.2155T>C) polymorphism and clinical outcomes with statin therapy, showing carriers of the 719Arg allele having greater reduction in clinical events compared with noncarriers

(Iakoubova et al., 2008). However, subsequent studies dispute the reported associations.

Hopewell et al. (2011) studied the effects of the *KIF6* Trp719Arg polymorphism (rs20455) on vascular risk and response to statin therapy in 18,348 participants from the Heart Protection Study. Participants received 40 mg simvastatin daily for four to six weeks before being randomly allocated 40 mg simvastatin daily or placebo for five years. The *KIF6* genotype was not significantly associated, among placebo-allocated participants, with the risks of incident major vascular events, major coronary events, revascularizations, or strokes. The investigators concluded that statin therapy significantly reduces the incidence of coronary and other major vascular events to a similar extent, irrespective of *KIF6* genotype. The authors further stated that use of *KIF6* genotyping to guide statin therapy is not warranted (Hopewell et al., 2011).

Ridker et al. (2011) evaluated the effect of polymorphism at rs20455 encoding the *KIF6* 719Arg allele on outcomes of 8,781 Caucasian trial participants in the Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) study. Rosuvastatin was equally effective at reducing cardiovascular event rates among carriers and noncarriers of the *KIF6* 719Arg allele, and carriers of the 719Arg allele were not found to have more vascular events or a significant difference in hsCRP or lipid levels. The authors concluded that for rosuvastatin there appears to be no clinical utility to screening for the *KIF6* genotype as a method to determine vascular risk or to predict statin efficacy (Ridker et al., 2011).

Arsenault et al. (2012) investigated if carriers of the *KIF6* 719Arg variant benefit more from statin therapy, in terms of CVD risk reduction, than noncarriers through two large prospective, randomized, double-blinded, controlled trials. A total of 10,001 patients with stable CHD and LDL-C levels <130 mg/dL (3.4 mmol/L) of the Treating to New Targets (TNT) study were randomly assigned to receive either 10- or 80-mg of atorvastatin per day and followed up for a median of 4.9 years. A total of 8,888 patients with a history of myocardial infarction from the Incremental Decrease in End Points Through Aggressive Lipid-Lowering (IDEAL) study were randomly assigned to receive 20–40 mg of simvastatin or 80 mg of atorvastatin and followed up for a median of 4.8 years. The investigators concluded that carriers of the *KIF6* 719Arg allele were not at increased cardiovascular risk and did not obtain consistent cardiovascular benefit from high-dose statin therapy compared with noncarriers (Arsenault et al., 2012).

Charland et al. (2014) investigated the impact of providing *KIF6* test results and risk information directly to 647 tested patients on six month statin adherence and persistence compared with concurrent non-tested matched controls. The investigators

observed that significantly more tested patients were adherent and persisted on therapy compared to the control group, with tested patients rating out at a 63.4% adherence (defined as ingesting medication on 80% of days), compared to 45% for the control group over six months (Charland et al., 2014). The test group also persisted on therapy more often than the control group (69.1% for the testing group, 53.3% for the control). The investigators concluded that the study provided the first evidence that pharmacogenetic testing may modify patient adherence (Charland et al., 2014).

Ruiz-Ramos et al. (2015) performed a systematic review and meta-analysis of previously published association studies between Trp719Arg polymorphism of *KIF6* and the development of CHD. This electronic search included papers published between 1993 and 2014. Twenty-three studies consisting of 38,906 subjects were identified (17,812 cases, 21,094 controls). A significant association was not found between Caucasian populations and the polymorphism or the CAD subgroup and the polymorphism, though the authors concluded that allele 719Arg may have a protective association to present CHD in all populations and the Trp719Arg polymorphism of the *KIF6* gene is an important risk factor for developing MI (Ruiz-Ramos et al., 2015).

Proprietary Testing

Cardiovascular Risk Panels/Profiles

Cardiovascular risk panels refer to combinations of cardiac markers that are used to assess risk of developing cardiovascular disease, major adverse cardiovascular events, or ischemic cerebrovascular events. Commercially available risk panels use different combinations of lipids, inflammatory, genetic, and metabolic markers. Risk panel's report the results of multiple individual tests, whereas quantitative risk scores generally use proprietary algorithms to combine the results of multiple markers into one score. The clinical utility of risk panels is lacking as the impact of results on patient management is unknown.

Quest Diagnostics currently offers cardiogenetic testing for CVD risk. Tests include Cardio IQ® 9p21 and Cardio IQ® KIF6 (Quest, 2024).

VI. Guidelines and Recommendations

U.S. Preventative Services Task Force (USPSTF)

In a 2018 recommendation, the USPSTF provided a general statement regarding nontraditional risk factors in CVD risk assessment, but did not directly address the use of commercial CVD risk panels:

"The USPSTF concludes that there are insufficient adequately powered clinical trials

evaluating the incremental effect of the ankle-brachial index (ABI), high-sensitivity C-reactive protein (hs-CRP) level, or coronary artery calcium (CAC) score in risk assessment and initiation of preventive therapy.
Furthermore, the clinical meaning of improvements in measures of calibration, discrimination, and reclassification risk prediction studies is uncertain" (Lin et al., 2018).

American College of Cardiology (ACC) and the American Heart Association (AHA)

The 2019 ACC and AHA guidelines do not address arterial compliance, lipoprotein-associated phospholipase, long-chain omega-3 fatty acids, 9p21 polymorphisms, *KIF6* genotyping, or endothelial function assessment as methods to assess initial CVD risk (Arnett et al., 2019). The 2013 ACC and AHA guidelines on the assessment of cardiovascular risk did not address assessment of 9p21 polymorphisms or KIF6 genotyping. However, the 2010 guidelines recommended against genotype testing for "CHD risk assessment in asymptomatic adults" (Goff et al., 2014; Greenland et al., 2010).

American Heart Association (AHA)

A policy statement from the AHA on genetics and cardiovascular disease states "although robust GWAS [genome wide association studies] evidence exists linking common variants to complex CVD, studies are not yet available to inform the clinical benefit of providing such genetic information to patients" (Ashley et al., 2012).

A 2022 statement notes that the use of polygenic risk factors shows promise for the prediction of cardiovascular disease, as "the identification of monogenic risk variants predisposing to cardiovascular conditions has been used clinically to inform surveillance and management plans. Relatively recent advances in population genetics have uncovered the polygenic basis of these and other cardiovascular conditions in most patients. These observations point to the possibility of using genetic profiling to inform clinical practice… Such PRSs [Polygenic Risk Scores] may be appropriately considered in select scenarios, given the current evidence base."

The AHA statement points to coronary artery disease (CAD) as one of the main areas where polygenic risk factors show promise; the discovery of common genetic variants associated with coronary artery disease has enabled this development of polygenic risk profiling. They also note that "scores have been shown to be strong predictors of subclinical coronary atherosclerosis and independently prognostic of CAD risk." The statement does not specifically mention individual genes such as 9p21 single nucleotide polymorphisms or *KIF6* genotyping—focusing instead on genome wide association studies (O'Sullivan et al., 2022).

Endocrine Society (ES)

This guideline was published with the intent to assess and treat dyslipidemia in patients with endocrine disorders. The guideline remarks that certain "advanced" lipid testing (assessment of markers such as Apo B, lipid fractionation, and Lp(a)) may be helpful in "characterizing" lipid abnormalities, but "add little" to risk prediction beyond the standard lipid profile. The guideline also notes that genetic tests for risk stratification may "modestly" improve risk prediction but are not available in clinical practice. The guideline does remark that Apo B and non-HDL cholesterol should be considered "risk-enhancing" factors (Newman et al., 2020).

Canadian Cardiovascular Society (CCS)

In 2021, the CCS published updated recommendations on the management of dyslipidemia for the prevention of cardiovascular disease in adults. A summary of the society's recommendations that involve genetic associations relevant to CVD risk assessment is provided below:

- We suggest that CAC (coronary artery calcium) screening might be considered for a subset of low-risk individuals 40 years or older with a family history of premature ASCVD (men 55 years or younger; women 65 years or younger), in addition to identifying known genetic causes of ASCVD such as elevated Lp(a) or FH (Weak Recommendation; Low-Quality Evidence)" (Pearson et al., 2021).

The 2021 guidelines reaffirmed those from 2016, stating that "Screening should be repeated every five years for men and women aged 40-75 years using the modified FRS or Cardiovascular Life Expectancy Model (CLEM) to guide therapy to reduce major CV events" (Pearson et al., 2021).

2021 European Society of Cardiology (ESC) and Other Societies on Cardiovascular Disease Prevention in Clinical Practice

In 2021, the Joint Task Force of the ESC and Other Societies on Cardiovascular Disease Prevention in Clinical Practice published an update to guidelines on cardiovascular disease prevention in clinical practice. The Task Force recognized that genetic screening could be useful in some conditions such as familial hypercholesterolemia. The authors note that the etiology of atherosclerotic cardiovascular disease (ASCVD) has genetic associations; but, genetic information is not currently used to guide preventive practice.

However, the ESC does not recommend genetic screening in the general population or the use of genetics in "preventive approaches." According to the Task Force, there is no consensus regarding which genes and their corresponding single nucleotide

polymorphisms that should be included in a genetic risk score. In terms of polygenic risk scores, they acknowledge potential towards using genetics for prevention, but a lack of clarity towards whether to use "risk factor-specific" or "outcome-specific" polygenic risk scores. Further evaluation of clinical utility is required (Frank L. J. Visseren et al., 2021).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/Medicare-Coverage-Database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81402	Molecular pathology procedure, Level 3 (eg, >10 SNPs, 2-10 methylated variants, or 2-10 somatic variants [typically using non-sequencing target variant analysis], immunoglobulin and T-cell receptor gene rearrangements, duplication/deletion variants of 1 exon, loss of heterozygosity [LOH], uniparental disomy [UPD])
81403	Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)
81404	Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)

CPT	Code Description
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons)
81407	Molecular pathology procedure, Level 8 (eg, analysis of 26-50 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of >50 exons, sequence analysis of multiple genes on one platform)
81408	Molecular pathology procedure, Level 9 (eg, analysis of >50 exons in a single gene by DNA sequence analysis)
81479	Unlisted molecular pathology procedure
81493	Coronary artery disease, mRNA, gene expression profiling by real-time RT-PCR of 23 genes, utilizing whole peripheral blood, algorithm reported as a risk score Proprietary test: Corus®CAD Lab/Manufacturer: CardioDx, Inc.
0401U	Cardiology (coronary heart disease [CAD]), 9 genes (12 variants), targeted variant genotyping, blood, saliva, or buccal swab, algorithm reported as a genetic risk score for a coronary event Proprietary test: CARDIO inCode-Score (CIC-SCORE) Lab/Manufacturer: GENinCode U.S. Inc
0439U	Cardiology (coronary heart disease [CHD]), DNA, analysis of 5 single-nucleotide polymorphisms (SNPs) (rs11716050 [LOC105376934], rs6560711 [WDR37], rs3735222 [SCIN/LOC107986769], rs6820447 [intergenic], and rs9638144 [ESYT2]) and 3 DNA methylation markers (cg00300879 [transcription start site {TSS200} of CNKSR1], cg09552548 [intergenic], and cg14789911 [body of SPATC1L]), qPCR and digital PCR, whole blood, algorithm reported as a 4-tiered risk score for a 3-year risk of symptomatic CHD Proprietary test: Epi+Gen CHD ™ Lab Manufacturer: Cardio Diagnostics, Inc
0440U	Cardiology (coronary heart disease [CHD]), DNA, analysis of 10 single-nucleotide polymorphisms (SNPs) (rs710987 [LINC010019], rs1333048 [CDKN2B-AS1], rs12129789 [KCND3], rs942317 [KTN1-AS1], rs1441433 [PPP3CA], rs2869675 [PREX1], rs4639796 [ZBTB41], rs4376434 [LINC00972], rs12714414 [TMEM18], and rs7585056 [TMEM18]) and 6 DNA methylation markers (cg03725309 [SARS1], cg12586707 [CXCL1, cg04988978 [MPO], cg17901584 [DHCR24-DT], cg21161138 [AHRR], and cg12655112 [EHD4]),

CPT	Code Description
	qPCR and digital PCR, whole blood, algorithm reported as detected or not detected for CHD Proprietary test: PrecisionCHDTM Lab/Manufacturer: Cardio Diagnostics, Inc
0466U	Cardiology (coronary artery disease [CAD]), DNA, genomewide association studies (564856 single-nucleotide polymorphisms [SNPs], targeted variant genotyping), patient lifestyle and clinical data, buccal swab, algorithm reported as polygenic risk to acquired heart disease Proprietary test: CardioRisk+ Lab/Manufacturer: Gene by Gene, Ltd, Open DNS, Ltd

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

AHA. (2024). *2024 Heart Disease and Stroke Statistics Update Fact Sheet At-a-Glance*. https://www.heart.org/-/media/PHD-Files-2/Science-News/2/2024-Heart-and-Stroke-Stat-Update/2024-Statistics-At-A-Glance-final_2024.pdf

Arnett, D. K., Blumenthal, R. S., Albert, M. A., Buroker, A. B., Goldberger, Z. D., Hahn, E. J., Himmelfarb, C. D., Khera, A., Lloyd-Jones, D., McEvoy, J. W., Michos, E. D., Miedema, M. D., Muñoz, D., Smith, S. C., Virani, S. S., Williams, K. A., Yeboah, J., & Ziaeian, B. (2019). 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation*, *140*(11), e596-e646. https://doi.org/doi:10.1161/CIR.0000000000000678

Arsenault, B. J., Boekholdt, S. M., Hovingh, G. K., Hyde, C. L., DeMicco, D. A., Chatterjee, A., Barter, P., Deedwania, P., Waters, D. D., LaRosa, J. C., Pedersen, T. R., & Kastelein, J. J. (2012). The 719Arg variant of KIF6 and cardiovascular outcomes in statin-treated, stable coronary patients of the treating to new targets and incremental decrease in end points through aggressive lipid-lowering prospective studies. *Circ Cardiovasc Genet*, *5*(1), 51-57. https://doi.org/10.1161/circgenetics.111.960252

Ashley, E. A., Hershberger, R. E., Caleshu, C., Ellinor, P. T., Garcia, J. G., Herrington, D. M., Ho, C. Y., Johnson, J. A., Kittner, S. J., Macrae, C. A., Mudd-Martin, G., Rader, D. J., Roden, D. M., Scholes, D., Sellke, F. W., Towbin, J. A., Van Eyk, J., & Worrall, B. B. (2012). Genetics and cardiovascular disease: a policy statement from the American Heart Association. *Circulation*, *126*(1), 142-157. https://doi.org/10.1161/CIR.0b013e31825b07f8

Charland, S. L., Agatep, B. C., Herrera, V., Schrader, B., Frueh, F. W., Ryvkin, M., Shabbeer, J., Devlin, J. J., Superko, H. R., & Stanek, E. J. (2014). Providing patients with pharmacogenetic test results affects adherence to statin therapy: results of the Additional KIF6 Risk Offers Better Adherence to Statins (AKROBATS) trial. *Pharmacogenomics J*, *14*(3), 272-280. https://doi.org/10.1038/tpj.2013.27

Dehghan, A., Bis, J. C., White, C. C., Smith, A. V., Morrison, A. C., Cupples, L. A., Trompet, S., Chasman, D. I., Lumley, T., Völker, U., Buckley, B. M., Ding, J., Jensen, M. K., Folsom, A. R., Kritchevsky, S. B., Girman, C. J., Ford, I., Dörr, M., Salomaa, V., . . . O'Donnell, C. J. (2016). Genome-Wide Association Study for Incident Myocardial Infarction and Coronary Heart Disease in Prospective Cohort Studies: The CHARGE Consortium. *PLOS ONE*, *11*(3), e0144997. https://doi.org/10.1371/journal.pone.0144997

Deloukas, P., Kanoni, S., Willenborg, C., Farrall, M., Assimes, T. L., Thompson, J. R., Ingelsson, E., Saleheen, D., Erdmann, J., Goldstein, B. A., Stirrups, K., Konig, I. R., Cazier, J. B., Johansson, A., Hall, A. S., Lee, J. Y., Willer, C. J., Chambers, J. C., Esko, T., . . . Samani, N. J. (2013). Large-scale association analysis identifies new risk loci for coronary artery disease. *Nat Genet*, *45*(1), 25-33. https://doi.org/10.1038/ng.2480

Dutta, A., Henley, W., Lang, I. A., Murray, A., Guralnik, J., Wallace, R. B., & Melzer, D. (2011). The coronary artery disease-associated 9p21 variant and later life 20-year survival to cohort extinction. *Circ Cardiovasc Genet*, *4*(5), 542-548. https://doi.org/10.1161/circgenetics.111.960146

Elashoff, M. R., Wingrove, J. A., Beineke, P., Daniels, S. E., Tingley, W. G., Rosenberg, S., Voros, S., Kraus, W. E., Ginsburg, G. S., Schwartz, R. S., Ellis, S. G., Tahirkheli, N., Waksman, R., McPherson, J., Lansky, A. J., & Topol, E. J. (2011). Development of a blood-based gene expression algorithm for assessment of obstructive coronary artery disease in non-diabetic patients. *BMC Med Genomics*, *4*, 26. https://doi.org/10.1186/1755-8794-4-26

Ganesh, S. K., Arnett, D. K., Assimes, T. L., Basson, C. T., Chakravarti, A., Ellinor, P. T., Engler, M. B., Goldmuntz, E., Herrington, D. M., Hershberger, R. E., Hong, Y., Johnson, J. A., Kittner, S. J., McDermott, D. A., Meschia, J. F., Mestroni, L., O'Donnell, C. J., Psaty, B. M., Vasan, R. S., . . . Waldman, S. A. (2013). Genetics and genomics for the prevention and treatment of cardiovascular disease: update: a scientific statement from the American Heart Association. *Circulation*, *128*(25), 2813-2851. https://doi.org/10.1161/01.cir.0000437913.98912.1d

Goff, D. C., Jr., Lloyd-Jones, D. M., Bennett, G., Coady, S., D'Agostino, R. B., Sr., Gibbons, R., Greenland, P., Lackland, D. T., Levy, D., O'Donnell, C. J., Robinson, J. G., Schwartz, J. S., Shero, S. T., Smith, S. C., Jr., Sorlie, P., Stone, N. J., & Wilson, P. W. (2014). 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*, *63*(25 Pt B), 2935-2959. https://doi.org/10.1016/j.jacc.2013.11.005

Gransbo, K., Almgren, P., Sjogren, M., Smith, J. G., Engstrom, G., Hedblad, B., & Melander, O. (2013). Chromosome 9p21 genetic variation explains 13% of cardiovascular disease incidence but does not improve risk prediction. *J Intern Med*, *274*(3), 233-240. https://doi.org/10.1111/joim.12063

Greenland, P., Alpert, J. S., Beller, G. A., Benjamin, E. J., Budoff, M. J., Fayad, Z. A., Foster, E., Hlatky, M. A., Hodgson, J. M., Kushner, F. G., Lauer, M. S., Shaw, L. J., Smith, S. C., Jr., Taylor, A. J., Weintraub, W. S., Wenger, N. K., Jacobs, A. K., Anderson, J. L., Albert, N., . . . Yancy, C. W. (2010). 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*, *56*(25), e50-103. https://doi.org/10.1016/j.jacc.2010.09.001

Hopewell, J. C., Parish, S., Clarke, R., Armitage, J., Bowman, L., Hager, J., Lathrop, M., & Collins, R. (2011). No impact of KIF6 genotype on vascular risk and statin response among 18,348 randomized patients in the heart protection study. *J Am Coll Cardiol*, *57*(20), 2000-2007. https://doi.org/10.1016/j.jacc.2011.02.015

Iakoubova, O. A., Tong, C. H., Rowland, C. M., Kirchgessner, T. G., Young, B. A., Arellano, A. R., Shiffman, D., Sabatine, M. S., Campos, H., Packard, C. J., Pfeffer, M. A., White, T. J., Braunwald, E., Shepherd, J., Devlin, J. J., & Sacks, F. M. (2008). Association of the Trp719Arg polymorphism in kinesin-like protein 6 with myocardial infarction and coronary heart disease in 2 prospective trials: the CARE and WOSCOPS trials. *J Am Coll Cardiol*, *51*(4), 435-443. https://doi.org/10.1016/j.jacc.2007.05.057

Kessler, T., Vilne, B., & Schunkert, H. (2016). The impact of genome-wide association studies on the pathophysiology and therapy of cardiovascular disease. *EMBO Mol Med*, *8*(7), 688-701. https://doi.org/10.15252/emmm.201506174

Lin, J. S., Evans, C. V., Johnson, E., Redmond, N., Coppola, E. L., & Smith, N. (2018). Nontraditional Risk Factors in Cardiovascular Disease Risk Assessment: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. *Jama*, *320*(3), 281-297. https://doi.org/10.1001/jama.2018.4242

Newman, C. B., Blaha, M. J., Boord, J. B., Cariou, B., Chait, A., Fein, H. G., Ginsberg, H. N., Goldberg, I. J., Murad, M. H., Subramanian, S., & Tannock, L. R. (2020). Lipid Management in Patients with Endocrine Disorders: An Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*, *105*(12). https://doi.org/10.1210/clinem/dgaa674

O'Sullivan, J. W., Raghavan, S., Marquez-Luna, C., Luzum, J. A., Damrauer, S. M., Ashley, E. A., O'Donnell, C. J., Willer, C. J., & Natarajan, P. (2022). Polygenic Risk Scores for Cardiovascular Disease: A Scientific Statement From the American Heart Association. *Circulation*, *146*(8), e93-e118. https://doi.org/doi:10.1161/CIR.0000000000001077

Palomaki, G. E., Melillo, S., & Bradley, L. A. (2010). Association between 9p21 genomic markers and heart disease: a meta-analysis. *Jama*, *303*(7), 648-656. https://doi.org/10.1001/jama.2010.118

Palotie, A., Havulinna, A. S., De Livera, A. M., Tikkanen, E., Sijbrands, E. J., Abraham, G., Schunkert, H., Yetukuri, L., Perola, M., Bhalala, O. G., Byars, S. G., Inouye, M., Samani, N. J., Ripatti, S., & Salomaa, V. (2016). Genomic prediction of coronary heart disease. *European Heart Journal*, *37*(43), 3267-3278. https://doi.org/10.1093/eurheartj/ehw450

Patel, R. S., Asselbergs, F. W., Quyyumi, A. A., Palmer, T. M., Finan, C. I., Tragante, V., Deanfield, J., Hemingway, H., Hingorani, A. D., & Holmes, M. V. (2014). Genetic variants at chromosome 9p21 and risk of first versus subsequent coronary heart disease events: a systematic review and meta-analysis. *J Am Coll Cardiol*, *63*(21), 2234-2245. https://doi.org/10.1016/j.jacc.2014.01.065

Paynter, N. P., Chasman, D. I., Buring, J. E., Shiffman, D., Cook, N. R., & Ridker, P. M. (2009). Cardiovascular disease risk prediction with and without knowledge of genetic variation at chromosome 9p21.3. *Ann Intern Med*, *150*(2), 65-72.

Pearson, G. J., Thanassoulis, G., Anderson, T. J., Barry, A. R., Couture, P., Dayan, N., Francis, G. A., Genest, J., Grégoire, J., Grover, S. A., Gupta, M., Hegele, R. A., Lau, D., Leiter, L. A., Leung, A. A., Lonn, E., Mancini, G. B. J., Manjoo, P., McPherson, R., . . . Wray, W. (2021). 2021 Canadian Cardiovascular Society Guidelines for the Management of Dyslipidemia for the Prevention of Cardiovascular Disease in Adults. *Can J Cardiol*, *37*(8), 1129-1150. https://doi.org/10.1016/j.cjca.2021.03.016

Quest. (2024). *Cardio IQ® 9p21 Genotype* https://testdirectory.questdiagnostics.com/test/test-detail/90648/cardio-iq-9p21-genotype?p=r&q=cardio%20IA%209p21%20genotype&cc=MASTER

Ridker, P., MacFadyen, J., Glynn, R., & Chasman, D. (2011). Kinesin-Like Protein 6 (KIF6) Polymorphism and the Efficacy of Rosuvastatin in Primary Prevention. *Circulation: Cardiovascular Genetics*, *4*(3), 312-317. https://doi.org/10.1161/CIRCGENETICS.110.959353

Ruiz-Ramos, D., Hernández-Díaz, Y., Tovilla-Zárate, C. A., Juárez-Rojop, I., López-Narváez, M. L., González-Castro, T. B., Torres-Hernández, M. E., & Baños-González, M. A. (2015). The Trp719Arg polymorphism of the KIF6 gene and coronary heart disease risk: systematic review and meta-analysis. *Hereditas*, *152*, 3. https://doi.org/10.1186/s41065-015-0004-7

Samani, N. J., Erdmann, J., Hall, A. S., Hengstenberg, C., Mangino, M., Mayer, B., Dixon, R. J., Meitinger, T., Braund, P., Wichmann, H. E., Barrett, J. H., Konig, I. R., Stevens, S. E., Szymczak, S., Tregouet, D. A., Iles, M. M., Pahlke, F., Pollard, H., Lieb, W., . . . Schunkert, H. (2007). Genomewide association analysis of coronary artery disease. *N Engl J Med*, *357*(5), 443-453. https://doi.org/10.1056/NEJMoa072366

Seth S. Martin, A. W. A., Zaid I. Almarzooq, Cheryl A.M. Anderson, Pankaj Arora, Christy L. Avery, Carissa M. Baker-Smith, Bethany Barone Gibbs, Andrea Z. Beaton, Amelia K. Boehme, Yvonne Commodore-Mensah, Maria E. Currie, Mitchell S.V. Elkind, Kelly R. Evenson, Giuliano Generoso, Debra G. Heard, Swapnil Hiremath, Michelle C. Johansen, Rizwan Kalani, Dhruv S. Kazi, Darae Ko, Junxiu Liu, Jared W. Magnani, Erin D. Michos, Michael E. Mussolino, Sankar D. Navaneethan, Nisha I. Parikh, Sarah M. Perman, Remy Poudel, Mary Rezk-Hanna, Gregory A. Roth, Nilay S. Shah, Marie-Pierre St-Onge, Evan L. Thacker, Connie W. Tsao, Sarah M. Urbut, Harriette G.C. Van Spall, Jenifer H. Voeks, Nae-Yuh Wang, Nathan D. Wong, Sally S. Wong, Kristine Yaffe, Latha P. Palaniappan and on behalf of the American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. (2024). 2024 Heart Disease and Stroke Statistics: A Report of US and Global Data From the American Heart Association. *Circulation*, *149*. https://doi.org/10.1161/CIR.0000000000001209

Trenkwalder, T., Nelson, C. P., Musameh, M. D., Mordi, I. R., Kessler, T., Pellegrini, C., Debiec, R., Rheude, T., Lazovic, V., Zeng, L., Martinsson, A., Gustav Smith, J., Gadin, J. R., Franco-Cereceda, A., Eriksson, P., Nielsen, J. B., Graham, S. E., Willer, C. J., Hveem, K., . . . Samani, N. J. (2019). Effects of the coronary artery disease associated LPA and 9p21 loci on risk of aortic valve stenosis. *Int J Cardiol*, *276*, 212-217. https://doi.org/10.1016/j.ijcard.2018.11.094

Virani, S. S., Brautbar, A., Lee, V.-V., MacArthur, E., Morrison, A. C., Grove, M. L., Nambi, V., Frazier, L., Wilson, J. M., Willerson, J. T., Boerwinkle, E., & Ballantyne, C. M. (2012). Chromosome 9p21 single nucleotide polymorphisms are not associated with recurrent myocardial infarction in patients with established coronary artery disease. *Circulation journal : official journal of the Japanese Circulation Society*, *76*(4), 950-956. https://doi.org/10.1253/circj.cj-11-1166

Visseren, F. L. J., Mach, F., Smulders, Y. M., Carballo, D., Koskinas, K. C., Bäck, M., Benetos, A., Biffi, A., Boavida, J.-M., Capodanno, D., Cosyns, B., Crawford, C., Davos, C. H., Desormais, I., Di Angelantonio, E., Franco, O. H., Halvorsen, S., Hobbs, F. D. R., Hollander, M., . . . Group, E. S. C. S. D. (2021). 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice: Developed by the Task Force for cardiovascular disease prevention in clinical practice with representatives of the European Society of Cardiology and 12 medical societies With the special contribution of the European Association of Preventive Cardiology (EAPC). *European Heart Journal*, *42*(34), 3227-3337. https://doi.org/10.1093/eurheartj/ehab484

Visseren, F. L. J., Mach, F., Smulders, Y. M., Carballo, D., Koskinas, K. C., Back, M., Benetos, A., Biffi, A., Boavida, J. M., Capodanno, D., Cosyns, B., Crawford, C., Davos, C. H., Desormais, I., Di Angelantonio, E., Franco, O. H., Halvorsen, S., Hobbs, F. D. R., Hollander, M., . . . Group, E. S. C. S. D. (2021). 2021 ESC Guidelines on cardiovascular

disease prevention in clinical practice. *Eur Heart J*, *42*(34), 3227-3337. https://doi.org/10.1093/eurheartj/ehab484

Wilson, P. (2023). Overview of the possible risk factors for cardiovascular disease - UpToDate. In B. Downey (Ed.), *UpToDate*. https://www.uptodate.com/contents/overview-of-the-possible-risk-factors-for-cardiovascular-disease

Zhernakova, D. V., Le, T. H., Kurilshikov, A., Atanasovska, B., Bonder, M. J., Sanna, S., Claringbould, A., Võsa, U., Deelen, P., Franke, L., de Boer, R. A., Kuipers, F., Netea, M. G., Hofker, M. H., Wijmenga, C., Zhernakova, A., Fu, J., LifeLines cohort, s., & consortium, B. (2018). Individual variations in cardiovascular-disease-related protein levels are driven by genetics and gut microbiome. *Nature Genetics*, *50*(11), 1524-1532. https://doi.org/10.1038/s41588-018-0224-7

Genetic Testing and Genetic Expression Profiling in Patients with Uveal Melanoma

Policy Number: AHS – M2071 – Genetic Testing and Genetic Expression Profiling in Patients with Uveal Melanoma	Prior Policy Name and Number, as applicable: AHS-M2071-Gene Expression Profiling for Uveal Melanoma
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Uveal melanoma develops from melanocytes in any part of the uveal tract, including the iris, ciliary body, and choroid (Diener-West et al., 2005). UM is the most common primary cancer of the eye and has a strong propensity for metastasis (Harbour & Chen, 2013). These melanomas have significant differences from cutaneous melanomas so the management of these two classes differ considerably (Albert et al., 1996; Harbour, 2022).

Gene expression assays measure the concentration of specific mRNAs being transcribed to assess the genes that are active in a particular cell or tissue. Analyses of gene expression can be clinically useful for disease classification, diagnosis, prognosis, and tailoring treatment to underlying genetic determinants of pharmacologic response (Steiling, 2023). Gene expression profiling has been proposed as a method of risk stratification for UM.

II. Related Policies

Policy Number	Policy Title
AHS-M2029	Molecular Testing for Cutaneous Melanoma

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" of this policy document.

1) For patients with primary, localized uveal melanoma (UM), gene expression profiling for uveal melanoma (e.g., DecisionDx-UM) **MEETS COVERAGE CRITERIA**.

2) For patients with primary, localized UM, the following genetic markers for UM **MEET COVERAGE CRITERIA** based on NCCN guidelines:
   a) Copy number assessment for chromosomes 3, 6, and/or 8
   b) Sequence analysis of the following genes:
      i) *BAP1*
      ii) *EIF1AX*
      iii) *PRAME*
      iv) *SF3B1*

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

3) All other testing for uveal melanoma (e.g., Uveal Melanoma Prognostic Genetic Test, DecisionDx-PRAME, DecisionDx-UMSeq) **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
aCGH	Array comparative genomic hybridization
AJCC	American Joint Committee on Cancer
ATRIP	*ATR interacting irotein*
BAP1	*BRCA1-Associated Protein 1*
BRAF	*Proto-oncogene B-Raf and v-Raf murine sarcoma viral oncogene homolog B*

BRCA1	*Hereditary breast and ovarian cancer-causing gene product*
CDC45	*Cell division cycle 45*
CDH1	*Cadherin 1*
CHEK1	*Checkpoint kinase 1*
CIZ1	*Cip1-interacting zinc finger protein*
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers for Medicare and Medicaid Services
COMS	Collaborative Ocular Melanoma Study
CYSLTR2	*Cysteinyl leukotriene receptor 2*
DNA	deoxyribonucleic acid
ECM1	*Extracellular Matrix Protein 1*
EIF1AX	*Eukaryotic translation initiation factor 1A*
EIF1B	*Eukaryotic translation initiation factor 1A X-Linked*
FDA	Food and Drug Administration
FEN1	*Flap structure-specific endonuclease 1*
FISH	fluorescence in situ hybridization
FXR1	*Fragile X mental retardation syndrome-related protein 1*
GEP	Gene expression profiling
GNA11	*Guanine nucleotide-binding protein subunit alpha-11*
GNAQ	*Guanine nucleotide-binding protein G(q) subunit alpha*
HTR2B	*5-hydroxytryptamine receptor 2B*
HUS1	HUS1 Checkpoint clamp component
ID2	Inhibitor of DNA binding 2
JAMA	Journal of the American Medical Association
LBD	Largest basal diameter
LCAs	Local coverage articles
LCDs	Local coverage determinations
LDTs	Laboratory-developed Tests
LIG1	*DNA ligase 1*
LMCD1	*LIM and cysteine rich domains 1*
LTA4H	*Leukotriene A4 hydrolase*
MAPK	*Mitogen-Activated Protein Kinases*
MCM10	*Minichromosome maintenance 10 Replication Initiation Factor*
MCM2	*Minichromosome maintenance complex component 2*
MCM4	*Minichromosome maintenance complex component 4*
MCM5	*Minichromosome maintenance complex component 5*
MLH3	*MutL Homolog 3*
MLPA	Multiplex Ligation-Dependent Probe Amplification
mRNA	Messenger Ribonucleic Acid

MRPS21	*Mitochondrial Ribosomal Protein S21*
MSH6	*MutS homolog 6*
MTUS1	*Microtubule associated scaffold protein 1*
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NICE	National Institute for Health and Care Excellence
NIH	National Institute of Health
OOTF	Ophthalmic Oncology Task Force
PCNA	*Proliferating cell nuclear antigen*
PCR	polymerase chain reaction
PLCB4	*1-Phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta-4*
POLD1	*Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1*
POLE	*DNA polymerase epsilon catalytic subunit*
PRAME	*Preferentially Expressed Antigen in Melanoma*
RAB31	*Ras-related protein*
RBM23	*RNA binding motif protein 23*
ROBO1	*roundabout guidance receptor 1*
RRs	Relative Risks
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAP130	*Histone deacetylase complex subunit SAP130*
SATB1	*special AT-rich sequence-binding protein-1*
SF3B1	*Splicing Factor [3b] Subunit B1*
SNPs	Single nucleotide polymorphism
TNM	Tumor-Node-Metastasis
UK	United Kingdom
UM	Uveal melanoma

V. Scientific Background

Uveal melanoma (Diener-West et al.) is the most common primary cancer in the eye, with an incidence of more than 7,000 new cases each year (Scott & Gerstenblith, 2018). The mortality rate at 15 years of diagnosis of the primary tumor is approximately 50% (Kujala et al., 2003); despite enucleation or definitive radiotherapy of the primary lesion, approximately half will develop a metastasis, and the average survival after metastasis is only 9-12 months (Carvajal et al., 2014; COMS, 2001; Diener-West et al., 2005; Kath et al., 1993; Onken et al., 2012; Rietschel et al., 2005). Tebentafusp is a United States Food and Drug Administration approved treatment for adults with advanced unresectable or metastatic uveal melanoma who are HLA-1 positive. Tebentadusp is a bispecific T cell engager targeting glycoprotein 100. The drug improved one-year overall survival rates compared to immunotherapy or

chemotherapy. Currently there is no effective treatment in preventing deaths from metastatic UM (Carvajal, 2024). Novel and innovative therapeutic targets for uveal melanoma are currently being investigated. These include liver-directed therapies, immunotherapy, and targeted-therapy on single compounds or combinational therapies (Mallone et al., 2020).

Uveal melanoma typically presents with visual disturbance, but may be asymptomatic (Mahendraraj et al., 2016). The diagnosis of UM is based upon fundoscopic examination by an experienced clinician, which is followed by ultrasound and/or fluorescein angiography. Biopsy is generally not indicated as the clinical diagnosis of UM has an accuracy of 99 percent (Pereira et al., 2013); however, molecular characterization of the tumor can provide important information about the risk of recurrence.

The molecular pathogenesis of UM is not completely characterized. It is not associated with the frequent *BRAF* mutations of cutaneous melanoma. UM has been associated with activating mutations in *GNAQ* or *GNA11* in greater than 80 percent of primary UMs leading to activation of downstream signaling pathways, including the mitogen-activated protein kinases (*MAPK*) pathway (Onken et al., 2008; Shoushtari & Carvajal, 2014; Van Raamsdonk et al., 2009; Van Raamsdonk et al., 2010). Inactivating somatic mutations have been found in the *BRCA1*-associated protein 1 (*BAP1*) gene in 84 percent of metastasizing tumors, implicating loss of *BAP1* in the progression of UM (Harbour et al., 2010). Germline mutations of *BAP1* in approximately five percent of patients with UMs have been associated with larger tumors and involvement of the ciliary body (Gupta et al., 2015). Recurring mutations occurring at codon 625 of the *SF3B1* gene and eukaryotic translation initiation factor 1A (*EIF1AX*) were associated with good prognosis (Harbour et al., 2013; Harbour, 2022; Martin et al., 2013). Other mutations such as *PLCB4, CYSLTR2, SF3B1,* and more are often observed (Carvajal, 2024).

Metastasis is common in UM. Approximately 50% of cases will have distal recurrence with the liver and lungs as the most common sites of metastasis. As many as 30% of patients with UM will die of a systemic metastasis within five years of diagnosis. The National Comprehensive Cancer Network (NCCN) considers *BAP1, PRAME, SF3B1*, and *EIF1AX* mutations to be associated with varying amounts of metastasis risk (NCCN, 2023). Cytogenetic changes may also confer increased metastasis risk. The most common cytogenetic changes in UM are monosomy of chromosome 3 (possibly the single strongest factor in predicting UM metastasis) and amplification of chromosome 8q; both of which are associated with poor prognosis. Other common cytogenetic alterations include amplification of chromosome 6p and loss of 1p (Amaro et al., 2017). Caines et al. (2015) organized four cytogenetic classes of prognostic risk based on

multiplex ligation-dependent probe amplification (MLPA) results. From best to worst, those classes are: "(i) normal chromosomes 3 and 8q; (Larsen et al.) chromosome 3 deletion, normal chromosome 8q; (iii) normal chromosome 3, chromosome 8q gain; and (iv) chromosome 3 deletion, chromosome 8q gain" (Caines et al., 2015).

Genetic analysis of UM can provide prognostic information for the risk of developing metastatic disease (Spagnolo et al., 2012) and "currently represents the gold standard in molecular prognosis" for uveal melanoma as it has a technical failure rate of only three percent (Mallone et al., 2020). Genetic expression profiling (GEP) determines the expression of multiple genes in a tumor and has been proposed as an additional method to stratify patients into prognostic risk groups. Castle Biosciences offers a gene expression profile for UM, called "Decision-DX." This test evaluates the gene expression of 15 genes, 12 as indicator genes and 3 as controls. The three control genes are *MRPS21, RBM23*, and *SAP130*, and the 12 indicators are *HTR2B, ID2, MTUS1, ECM1, ROBO1, SATB1, LTA4H, EIF1B, FXR1, CDH1, LMCD1,* and *RAB31* (Onken et al., 2010). The gene expression is reported in three classes of risk; class 1A with two percent chance of the cancer metastasizing over the next five years, class 1B with a 21% chance of metastasis, and class 2 with a 72% chance. Although the test does not change the course of treatment, it may still provide prognostic value for the patient (DecisionDX, 2024c).

Additionally, Decision-DX offers multiple tests for prognostication of UM. DecisionDX-UM*Seq* is a seven gene panel intended to identify somatic mutations relevant to UM. The seven genes are as follows: *GNAQ, GNA11, CYSLTR2, PLCB4, SF3B1*, exons 1-2 of *EIF1AX*, and all coding exons of *BAP1*. *GNAQ, GNA11, CYSLTR2,* and *PLCB4* are involved in G-protein-coupled receptor signaling, *EIF1AX* is involved with translation, *SF3B1* regulates transcript usage, and *BAP1* is a tumor suppressor on chromosome 3. This test will report any somatic mutations found in these seven genes, as well as an overview of any mutation found (DecisionDX, 2024b). DecisionDX also offers a test focusing on the preferentially expressed antigen in melanoma (*PRAME)* gene (compared to three control genes). The test reports whether the user is positive or negative, along with an overview. However, DecisionDX notes that the "exact clinical implications of *PRAME* are still under investigation" (DecisionDX, 2024a).

Another prognostic test available for UM is Impact Genetics' multiplex ligation-dependent probe amplification (MLPA). This test performs a copy number assessment on chromosomes 1, 3, 6, and 8 to detect monosomy, disomy, and trisomy, a microsatellite analysis on chromosome 3 to detect chromosome copy loss and/or isodisomy, and sequence analysis of *GNAQ, GNA11, SF3B1,* and *EIF1AX* (Impact, 2019b). The test combines these results with clinical and histomorphological data and predicts survival percentage at 3, 5, and 10 years (Impact, 2019a).

Analytical Validity

Plasseraud et al. (2017) examined the "technical reliability and correlation of molecular class with pathologic characteristics" of DecisionDx. The authors identified samples from de-identified clinical reports over a six year period. They found the inter-assay concordance of 16 samples (run on three consecutive days) to be 100% with strongly correlated discriminant scores (r^2 = .9944), inter-assay concordance of 46 samples performed in a one-year period to be 100% with an r^2 of .9747 for discriminant scores, and the inter-assay concordance of 12 assays concurrently run in duplicates to be 100% with an r^2 of .9934. Concordance between two sites assessing the same tumor was 100% with r^2 of 0.9818. Finally, the "technical success" of 5516 samples was 96.3% (Plasseraud et al., 2017).

Cook et al. (2018) investigated the validity of the DecisionDx-Melanoma test using formalin-fixed paraffin-embedded tumor tissue to analyze 31 genes. The authors evaluated samples from de-identified data over a three year period. They found inter-assay concordance on 168 specimens was 99% with strongly correlated discriminant scores (r^2 = 0.96). Inter-instrument concordance was 95% with a strongly correlated r^2 of .99. Overall, in tests that met tumor sample requirements, the technical success rate of the test was 98% (Cook et al., 2018).

Clinical Utility and Validity

In 2010, Onken et al. (2010) developed and validated the PCR-based 15-gene GEP assay comprising 12 discriminating genes and three endogenous control genes, analyzed the technical performance of the assay. A total of 609 samples were taken, and the authors defined an "undetectable" gene as "if its transcript was undetectable (i.e., no Ct value) after 40 qPCR cycles." A sample was said to have failed "if one or more endogenous controls was undetectable." By this definition, only 32 samples (of the 609) were said to have failed (Onken et al., 2010).

Damato et al. (2010) performed a study using MLPA to assess the correlation of chromosome 1p, 3, 6p, 6q, 8p, and 8q abnormalities with other risk factors and/or death. The authors examined 452 patients, and the ten-year disease-specific mortality rates were as follows: "0% in 133 tumors with no chromosome 3 loss, 55% in tumors with chromosome 3 loss but no chromosome 8q gain, and 71% in 168 tumors showing combined chromosome 3 loss and 8q gain." Lack of chromosome 6p gain was also noted as a prognosticator of poor survival. The authors concluded that "these results support the use of MLPA for routine clinical prognostication" (Damato et al., 2010)

Onken et al. (2012) further evaluated the prognostic accuracy of their GEP. A total of 459 patients from 12 independent centers were examined, and tumors were as

classified as "class 1" or "class 2." The authors then compared this classification to the 7th Edition clinical Tumor-Node-Metastasis (TNM) classification and chromosome 3 status (chromosome 3 was analyzed in the first 260 samples). The GEP assay was found to have correctly classified 446 of 459 samples, with 276 in class 1 and 170 in class 2. The authors also identified metastasis in three class 1 patients and 44 class 2 patients. GEP class was also found to have a strong independent association with metastasis than any other prognostic factor. The authors concluded that "the GEP assay had a high technical success rate and was the most accurate prognostic marker among all of the factors analyzed. The GEP provided a highly significant improvement in prognostic accuracy over clinical TNM classification and chromosome 3 status. Chromosome 3 status did not provide prognostic information that was independent of GEP" (Onken et al., 2012).

Larsen et al. (2014) evaluated the prognostic factors of the MLPA test and their associations with metastasis and survival. MLPA was used to identify cytogenetic changes in 36 patients. After adjusting for factors such as gender and age, chromosome 3 loss and 8q gain were identified to be "significant prognosticators" for poor survival. Chromosome 1p loss was also associated with metastatic death. Chromosome 6p gain and chromosome 6q loss did not show any associations with survival or metastasis, but the authors speculated this to be because of low occurrence (four each) (Larsen et al., 2014).

Correa and Augsburger (2016) conducted a prospective case series study of 299 patients to evaluate if any conventional clinical prognostic factors for metastasis from UM have prognostic value. The researchers found that GEP class was the strongest prognostic factor for metastatic death in this series. Using a two-term model including GEP class and "largest basal diameter" (LBD) led to strong, independent significance of each factor studied. The authors concluded that "both GEP and LBD of the tumor are independent prognostic factors for metastasis and metastatic death in multivariate analysis" (Correa & Augsburger, 2016).

Plasseraud et al. (2016) conducted a prospective, multicenter study "to document patient management differences and clinical outcomes associated with low-risk Class 1 and high-risk Class 2 results indicated by DecisionDx-UM testing." The initial results of the study indicated a low risk of metastasis for Class 1 patients (n = 37) compared to Class 2 patients (n = 33) (5% versus 36%, respectively). The authors found that the Class 1 patients (as determined by DecisionDx) had a 100% three year metastasis-free survival compared to 63% for Class 2 patients and that Class 2 patients received "significantly higher-intensity monitoring and more oncology/clinical trial referrals compared to Class 1 patients" (Plasseraud et al., 2016).

Aaberg et al. (2014) conducted a medical record review and cross-sectional survey of ophthalmologists to assess current clinical practices for UM and the impact of molecular prognostic testing on treatment decisions. The medical records for 191 Medicare patients was evaluated, with 88 (46%) patients having documented medical treatment actions or institutional policies related to surveillance plans. Of these 88, all GEP Class 1 UM patients were treated with low-intensity surveillance, while GEP Class 2 UM patients were treated with high-intensity surveillance. Patients with high metastatic risk (monosomy 3 or GEP Class 2) underwent more frequent surveillance with hepatic imaging and liver function testing every three to six months. High-risk patients were considered more suitable for adjuvant treatment protocols. The authors concluded that "the majority of ophthalmologists treating UM have adopted molecular diagnostic tests for the purpose of designing risk-appropriate treatment strategies" (Aaberg et al., 2014).

Worley et al. (2007) compared the gene expression-based classifier to the standard genetic prognostic marker, monosomy 3, for predicting metastasis in 67 primary UMs. The sensitivity and specificity for the molecular classifier (84.6% and 92.9%, respectively) were superior to monosomy 3 detected by aCGH (58.3% and 85.7%, respectively) and FISH (50.0% and 72.7%, respectively). The researchers concluded that "molecular classification based on gene expression profiling of the primary tumor was superior to monosomy 3 and clinicopathologic prognostic factors for predicting metastasis in UM" (Worley et al., 2007).

Recent studies have shown that even after controlling for gene expression profile, tumor size (≥ 12 mm) is an independent predictor of metastasis at five years (Walter et al., 2017; Weis et al., 2016). Weis et al. (2016) also noted that no published studies indicate that patients at high risk for future metastasis (GEP class 2) benefit from adjuvant therapy in reducing metastasis rates (Nathan et al., 2015).

Cai et al. (2018) compared the prognostic accuracy of gene expression profiling (GEP, Class 1 or 2) with *PRAME* status and Tumor-Node-Metastasis (TNM) staging in patients with uveal melanoma. A total of 128 patients were labeled Class 1 by the GEP, and 112 patients were labeled Class 2. *PRAME* status was negative in 157 cases and positive in 83 cases. TNM was stage I in 26 cases, IIA in 67 cases, IIB in 50 cases, IIIA in 59 cases and IIIB in 38 cases. Metastatic disease was detected in 59 cases after median follow-up of 29 months. GEP class was found to be associated with metastasis (Cai et al., 2018).

Kucherlapati (2018) examined groups of genes to identify gene correlations in UM survival. Genes with significant alteration include *MCM2, MCM4, MCM5, CDC45, MCM10, CIZ1, PCNA, FEN1, LIG1, POLD1, POLE, HUS1, CHECK1, ATRIP, MLH3,* and

MSH6. Exon 4 skipping in *CIZ1* was previously identified as an early serum biomarker in lung cancer. *MLH3* was found to have splicing variations with deletions to both Exon 5 and Exon 7 (Kucherlapati, 2018).

Szalai et al. (2018) evaluated the deterministic properties of UM, including mutation rate and metastatic rate. The metastatic rate was based on patients with three mutations: *BAP1, SF3B1,* and *EIF1AX*. The authors found that tumors with smaller thicknesses had a higher mutation rate and that tumors with only an *EIF1AX* mutation did not metastasize. Further, the authors identified a small peak in metastatic rate at one year and a large peak at 3.5 years post-treatment for *BAP1* mutations, and peaks at two to three years and seven years post-treatment for *SF3B1* mutations (Szalai et al., 2018).

Decatur et al. (2016) evaluated the associations between GEP classification, driver mutations, and patient outcomes in UM. A total of 81 patients treated by enucleation were examined. The GEP classified 35 patients as class 1 and 42 as class 2 (four were unknown). The authors then performed a multiple regression analysis. *BAP1* mutations were associated with class 2 GEP and older patients, *EIF1AX* mutations were associated with class 1 GEP, and *GNA11* mutations were not associated with any analyzed features. Class 2 GEP was identified as the prognostic factor most related to metastasis and melanoma-specific mortality, with relative risks (RRs) of 9.4 and 15.7 respectively. *BAP1* mutations were also strongly related to metastasis, with RRs of 10.6 and 9.0 respectively (Decatur et al., 2016).

Schefler et al. (2019) examined the relationship between *PRAME* expression, GEP class, and clinical features in UM cases. This retrospective, multicenter chart review study included 148 patients with UM. All patients underwent GEP and *PRAME* mRNA expression testing. The Tumor, Node, Metastasis (TNM) staging system was used to separate patients; a total of 51 patients were stage I, 33 patients were stage IIA, 34 patients were stage IIB, 20 patients were stage IIIA, and 10 patients were stage IIIB. The authors note, "There was no association between higher TNM stage and positive *PRAME* status ($p = 0.129$). *PRAME* expression was found to be independent of gender, patient age, and tumor thickness. *PRAME* expression was statistically associated with LBD [largest basal diameter] and tumor volume. Higher GEP class was associated with higher TNM staging" (Schefler et al., 2019). Additional research is needed to clarify these results.

VI. Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

The NCCN notes that gene expression profiling of a biopsy specimen may provide prognostic information that can assist with eligibility of clinical trials or affect management. Specifically, the guidelines state, "Biopsy of the primary tumor may provide prognostic information that can help inform frequency of follow-up and may be needed for eligibility for clinical trials. If biopsy is performed, molecular/chromosomal is preferred over cytology alone" (NCCN, 2023).

The NCCN divides the "risk of distant metastasis" into three risk groups, low, medium, and high.

- The following markers are considered **low risk**: Class 1A, disomy of chromosome 3, gain of chromosome 6p, *EIF1AX* mutations, tumor stage T1 (AJCC).
- The following markers are considered **medium risk**: Class 1B, *SF3B1* mutations, tumor stage T2 and tumor stage T3 (AJCC).
- The following markers are considered **high risk**: Class 2, monosomy of chromosome 3, gain of chromosome 8q, *BAP1* mutations, *PRAME* mutations, tumor stage T4 (AJCC)(NCCN, 2023).

Regarding extraocular recurrence or metastasis, the NCCN states that results "should be confirmed histologically whenever possible or if clinically indicated. Biopsy techniques may include FNA or core. Obtain tissue for genetic analysis (screening for mutations that may be potential targets for treatment or determine eligibility for a clinical trial from either biopsy of the metastasis (preferred) or archival material if the patient is being considered for targeted therapy. Consider broader genomic profiling if the test results might guide future decisions or eligibility for participation in a clinical trial" (NCCN, 2023).

American Joint Committee on Cancer (AJCC)

The 7th edition of the American Joint Committee on Cancer (AJCC) classification system recommends using tumor size to predict survival and has been validated internationally. The guidelines from the AJCC Ophthalmic Oncology Task Force (OOTF) note that "the OOTF recognizes that future modifications of the AJCC staging system are inevitable. Future modifications are likely to involve incorporation of a patient's genetic and molecular UM characteristics" (AJCC, 2015).

The AJCC 8th edition updates and corrections document notes that "only minor adjustments are introduced in the AJCC Cancer Staging Manual, 8th Edition" regarding UM (AJCC, 2018). The document also states, "Prognostic biopsies of conservatively treated uveal melanomas that allow analysis of their cytogenic, gene expression, and molecular genetic features are increasingly common. However, evidence for a long-term association between these characteristics and survival according to the anatomic extent of the tumor is still incomplete" (AJCC, 2018).

United Kingdom (Van Raamsdonk et al.) Uveal Melanoma Guideline Development Group

United Kingdom (Van Raamsdonk et al.) uveal melanoma guideline development group published guidelines which were accredited by the National Institute for Health and Care Excellence (NICE). These guidelines state that: "Prognostic factors of UM are multi-factorial and include clinical, morphological, immunohistochemical and genetic features. There are several different cytogenetic and molecular techniques for evaluating genetic changes in UM but there is insufficient comparative data. No evidence was found that demonstrated one technique was superior to another" (Nathan et al., 2015).

Consensus-Based Provincial Clinical Practice Guideline

In 2016, a consensus-based guideline on the management of UM was published by a group of content experts from medical, radiation, and surgical oncology fields. These guidelines state, "Two genetic tests more precisely identify patients with worse prognosis: testing for monosomy 3 and gene-expression profiling (GEP)" (Weis et al., 2016).

National Institute of Health - National Cancer Institute Guideline (NIH-NCI)

The 2023 guidelines from the NIH specifies molecular features as key prognostic indicators. These are in addition to staging algorithms from the AJCC, which they acknowledge as the current classification system to define melanoma of the uveal tract. Key prognostic indicators from the NIH guideline specifically include:

"Molecular Features

1. Chromosomal alterations
   a. Chromosome 3 status (loss or no loss; complete or partial).
   b. Chromosome 6p status (gain or no gain).
   c. Chromosome 8q status (gain or no gain).

   Indicate:
   - Technique used for assessing chromosome status may include the following:
     - Karyotyping.
     - Fluorescence in situ hybridization.
     - Comparative genomic hybridization.
     - Loss of heterozygosity using DNA polymorphism analysis (e.g. single nucleotide polymorphism, microsatellite).
     - Other.
   - How specimen was obtained may include the following:
     - Enucleation.

- Local resection.
- Biopsy.
- Fine-needle aspiration biopsy.

- For needle biopsies, whether cytopathologic evaluation was performed to confirm the presence of tumor cells.

2. Gene-expression profile: class 1 or class 2
   Indicate:
   - Technique used for gene-expression profiling (e.g., microarray, pathologic complete response).
   - How specimen was obtained (e.g., enucleation, local resection, biopsy, fine-needle aspiration biopsy).
   - For needle biopsies, whether cytopathologic evaluation was performed to confirm the presence of tumor cells" (NIH, 2023).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81347	SF3B1 (splicing factor [3b] subunit B1) (eg, myelodysplastic syndrome/acute myeloid leukemia) gene analysis, common variants (eg, A672T, E622D, L833F, R625C, R625L)

CPT	Code Description
81401	Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat) **Gene:** *PRAME (preferentially expressed antigen in melanoma) (eg, melanoma), expression analysis*
81479	Unlisted molecular pathology procedure
81552	Oncology (uveal melanoma), mRNA, gene expression profiling by real-time RT-PCR of 15 genes (12 content and 3 housekeeping), utilizing fine needle aspirate or formalin-fixed paraffin-embedded tissue, algorithm reported as risk of metastasis
81599	Unlisted multianalyte assay with algorithmic analysis

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Aaberg, T. M., Jr., Cook, R. W., Oelschlager, K., Maetzold, D., Rao, P. K., & Mason, J. O., 3rd. (2014). Current clinical practice: differential management of uveal melanoma in the era of molecular tumor analyses. *Clin Ophthalmol*, *8*, 2449-2460. https://doi.org/10.2147/opth.s70839

AJCC. (2015). International Validation of the American Joint Committee on Cancer's 7th Edition Classification of Uveal Melanoma. *JAMA Ophthalmol*, *133*(4), 376-383. https://doi.org/10.1001/jamaophthalmol.2014.5395

AJCC. (2018). *AJCC 8th Edition Updates and Corrections*. https://www.facs.org/quality-programs/cancer-programs/american-joint-committee-on-cancer/updates-and-corrections/

Albert, D. M., Ryan, L. M., & Borden, E. C. (1996). Metastatic ocular and cutaneous melanoma: a comparison of patient characteristics and prognosis. *Arch Ophthalmol*, *114*(1), 107-108. https://doi.org/10.1001/archopht.1996.01100130103030

Amaro, A., Gangemi, R., Piaggio, F., Angelini, G., Barisione, G., Ferrini, S., & Pfeffer, U. (2017). The biology of uveal melanoma. *Cancer Metastasis Rev*, *36*(1), 109-140. https://doi.org/10.1007/s10555-017-9663-3

Cai, L., Paez-Escamilla, M., Walter, S. D., Tarlan, B., Decatur, C. L., Perez, B. M., & Harbour, J. W. (2018). Gene Expression Profiling and PRAME Status Versus Tumor-Node-Metastasis Staging for Prognostication in Uveal Melanoma. *Am J Ophthalmol*. https://doi.org/10.1016/j.ajo.2018.07.045

Caines, R., Eleuteri, A., Kalirai, H., Fisher, A. C., Heimann, H., Damato, B. E., Coupland, S. E., & Taktak, A. F. (2015). Cluster analysis of multiplex ligation-dependent probe amplification data in choroidal melanoma. *Mol Vis*, *21*, 1-11. https://pubmed.ncbi.nlm.nih.gov/25684974/

Carvajal, R. D. (2024, April 3). *Metastatic uveal melanoma*. https://www.uptodate.com/contents/management-of-metastatic-uveal-melanoma

Carvajal, R. D., Sosman, J. A., Quevedo, J. F., Milhem, M. M., Joshua, A. M., Kudchadkar, R. R., Linette, G. P., Gajewski, T. F., Lutzky, J., Lawson, D. H., Lao, C. D., Flynn, P. J., Albertini, M. R., Sato, T., Lewis, K., Doyle, A., Ancell, K., Panageas, K. S., Bluth, M., . . . Schwartz, G. K. (2014). Effect of Selumetinib versus Chemotherapy on Progression-Free Survival in Uveal Melanoma: A Randomized Clinical Trial. *JAMA*, *311*(23), 2397-2405. https://doi.org/10.1001/jama.2014.6096

COMS. (2001). Assessment of metastatic disease status at death in 435 patients with large choroidal melanoma in the Collaborative Ocular Melanoma Study (COMS): COMS report no. 15. *Arch Ophthalmol*, *119*(5), 670-676. https://doi.org/10.1001/archopht.119.5.670

Cook, R. W., Middlebrook, B., Wilkinson, J., Covington, K. R., Oelschlager, K., Monzon, F. A., & Stone, J. F. (2018). Analytic validity of DecisionDx-Melanoma, a gene expression profile test for determining metastatic risk in melanoma patients. *Diagn Pathol*, *13*(1), 13. https://doi.org/10.1186/s13000-018-0690-3

Correa, Z. M., & Augsburger, J. J. (2016). Independent Prognostic Significance of Gene Expression Profile Class and Largest Basal Diameter of Posterior Uveal Melanomas. *Am J Ophthalmol*, *162*, 20-27.e21. https://doi.org/10.1016/j.ajo.2015.11.019

Damato, B., Dopierala, J. A., & Coupland, S. E. (2010). Genotypic Profiling of 452 Choroidal Melanomas with Multiplex Ligation-Dependent Probe Amplification. *Clinical Cancer Research*, *16*(24), 6083. https://doi.org/10.1158/1078-0432.CCR-10-2076

Decatur, C. L., Ong, E., Garg, N., Anbunathan, H., Bowcock, A. M., Field, M. G., & Harbour, J. W. (2016). Driver Mutations in Uveal Melanoma: Associations With Gene Expression Profile and Patient Outcomes. *JAMA Ophthalmol*, *134*(7), 728-733. https://doi.org/10.1001/jamaophthalmol.2016.0903

DecisionDX. (2024a). *DecisionDx-PRAME Summary*. https://castlebiosciences.com/tests/prognostic/decisiondx-um/prame

DecisionDX. (2024b). *DecisionDx-UMSeq Summary*. https://castlebiosciences.com/tests/prognostic/decisiondx-um/umseq

DecisionDX. (2024c). *Overview of DecisionDx®-UM*. https://castlebiosciences.com/tests/prognostic/decisiondx-um/how-it-works

Diener-West, M., Reynolds, S. M., Agugliaro, D. J., Caldwell, R., Cumming, K., Earle, J. D., Hawkins, B. S., Hayman, J. A., Jaiyesimi, I., Jampol, L. M., Kirkwood, J. M., Koh, W. J., Robertson, D. M., Shaw, J. M., Straatsma, B. R., & Thoma, J. (2005). Development of

metastatic disease after enrollment in the COMS trials for treatment of choroidal melanoma: Collaborative Ocular Melanoma Study Group Report No. 26. *Arch Ophthalmol*, *123*(12), 1639-1643. https://doi.org/10.1001/archopht.123.12.1639

Gupta, M. P., Lane, A. M., DeAngelis, M. M., Mayne, K., Crabtree, M., Gragoudas, E. S., & Kim, I. K. (2015). Clinical Characteristics of Uveal Melanoma in Patients With Germline BAP1 Mutations. *JAMA Ophthalmol*, *133*(8), 881-887. https://doi.org/10.1001/jamaophthalmol.2015.1119

Harbour, J. W., & Chen, R. (2013). The DecisionDx-UM Gene Expression Profile Test Provides Risk Stratification and Individualized Patient Care in Uveal Melanoma. *PLOS Currents Evidence on Genomic Tests*. https://pubmed.ncbi.nlm.nih.gov/23591547/

Harbour, J. W., Onken, M. D., Roberson, E. D., Duan, S., Cao, L., Worley, L. A., Council, M. L., Matatall, K. A., Helms, C., & Bowcock, A. M. (2010). Frequent mutation of BAP1 in metastasizing uveal melanomas. *Science*, *330*(6009), 1410-1413. https://doi.org/10.1126/science.1194472

Harbour, J. W., Roberson, E. D., Anbunathan, H., Onken, M. D., Worley, L. A., & Bowcock, A. M. (2013). Recurrent mutations at codon 625 of the splicing factor SF3B1 in uveal melanoma. *Nat Genet*, *45*(2), 133-135. https://doi.org/10.1038/ng.2523

Harbour, J. W., Shih, Helen. (2022, August 31). *Initial management of uveal and conjunctival melanomas*. https://www.uptodate.com/contents/initial-management-of-uveal-and-conjunctival-melanomas

Impact. (2019a). Benefits of our test. https://impactgenetics.com/wp-content/uploads/2019/03/UM-Patient-Brochure-11Mar2019.pdf

Impact. (2019b). Uveal Melanoma Genetic Prognostic Test. https://impactgenetics.com/wp-content/uploads/2019/03/UM-Test-Description-11Mar2019.pdf

Kath, R., Hayungs, J., Bornfeld, N., Sauerwein, W., Hoffken, K., & Seeber, S. (1993). Prognosis and treatment of disseminated uveal melanoma. *Cancer*, *72*(7), 2219-2223. https://pubmed.ncbi.nlm.nih.gov/7848381/

Kucherlapati, M. (2018). Examining transcriptional changes to DNA replication and repair factors over uveal melanoma subtypes. *BMC Cancer*, *18*(1), 818. https://doi.org/10.1186/s12885-018-4705-y

Kujala, E., Makitie, T., & Kivela, T. (2003). Very long-term prognosis of patients with malignant uveal melanoma. *Invest Ophthalmol Vis Sci*, *44*(11), 4651-4659. https://doi.org/10.1167/iovs.03-0538

Larsen, A. C., Holst, L., Kaczkowski, B., Andersen, M. T., Manfe, V., Siersma, V. D., Kolko, M., Kiilgaard, J. F., Winther, O., Prause, J. U., Gniadecki, R., & Heegaard, S. (2014). MicroRNA expression analysis and Multiplex ligation-dependent probe amplification in metastatic and non-metastatic uveal melanoma. *Acta Ophthalmol*, *92*(6), 541-549. https://doi.org/10.1111/aos.12322

Mahendraraj, K., Lau, C. S., Lee, I., & Chamberlain, R. S. (2016). Trends in incidence, survival, and management of uveal melanoma: a population-based study of 7,516 patients from the Surveillance, Epidemiology, and End Results database (1973–2012). In *Clin Ophthalmol* (Vol. 10, pp. 2113-2119). https://doi.org/10.2147/opth.s113623

Mallone, F., Sacchetti, M., Lambiase, A., & Moramarco, A. (2020). Molecular Insights and Emerging Strategies for Treatment of Metastatic Uveal Melanoma. *Cancers*, *12*(10), 2761. https://doi.org/10.3390/cancers12102761

Martin, M., Masshofer, L., Temming, P., Rahmann, S., Metz, C., Bornfeld, N., van de Nes, J., Klein-Hitpass, L., Hinnebusch, A. G., Horsthemke, B., Lohmann, D. R., & Zeschnigk, M. (2013). Exome sequencing identifies recurrent somatic mutations in EIF1AX and SF3B1 in uveal melanoma with disomy 3. *Nat Genet*, *45*(8), 933-936. https://doi.org/10.1038/ng.2674

Nathan, P., Cohen, V., Coupland, S., Curtis, K., Damato, B., Evans, J., Fenwick, S., Kirkpatrick, L., Li, O., Marshall, E., McGuirk, K., Ottensmeier, C., Pearce, N., Salvi, S., Stedman, B., Szlosarek, P., & Turnbull, N. (2015). Uveal Melanoma UK National Guidelines. *Eur J Cancer*, *51*(16), 2404-2412. https://doi.org/10.1016/j.ejca.2015.07.013

NCCN. (2023). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) Melanoma: Uveal Version 1.2023 — May 4, 2023* https://www.nccn.org/professionals/physician_gls/pdf/uveal.pdf

NIH. (2023, May 12, 2023). *Intraocular (Uveal) Melanoma Treatment (PDQ®)–Health Professional Version*. https://www.cancer.gov/types/eye/hp/intraocular-melanoma-treatment-pdq

Onken, M. D., Worley, L. A., Char, D. H., Augsburger, J. J., Correa, Z. M., Nudleman, E., Aaberg, T. M., Jr., Altaweel, M. M., Bardenstein, D. S., Finger, P. T., Gallie, B. L., Harocopos, G. J., Hovland, P. G., McGowan, H. D., Milman, T., Mruthyunjaya, P., Simpson, E. R., Smith, M. E., Wilson, D. J., . . . Harbour, J. W. (2012). Collaborative Ocular Oncology Group report number 1: prospective validation of a multi-gene prognostic assay in uveal melanoma. *Ophthalmology*, *119*(8), 1596-1603. https://doi.org/10.1016/j.ophtha.2012.02.017

Onken, M. D., Worley, L. A., Long, M. D., Duan, S., Council, M. L., Bowcock, A. M., & Harbour, J. W. (2008). Oncogenic mutations in GNAQ occur early in uveal melanoma. *Invest Ophthalmol Vis Sci*, *49*(12), 5230-5234. https://doi.org/10.1167/iovs.08-2145

Onken, M. D., Worley, L. A., Tuscan, M. D., & Harbour, J. W. (2010). An accurate, clinically feasible multi-gene expression assay for predicting metastasis in uveal melanoma. *J Mol Diagn*, *12*(4), 461-468. https://doi.org/10.2353/jmoldx.2010.090220

Pereira, P. R., Odashiro, A. N., Lim, L. A., Miyamoto, C., Blanco, P. L., Odashiro, M., Maloney, S., De Souza, D. F., & Burnier, M. N. (2013). Current and emerging treatment options for uveal melanoma. *Clin Ophthalmol*, *7*, 1669-1682. https://doi.org/10.2147/opth.s28863

Plasseraud, K. M., Cook, R. W., Tsai, T., Shildkrot, Y., Middlebrook, B., Maetzold, D., Wilkinson, J., Stone, J., Johnson, C., Oelschlager, K., & Aaberg, T. M. (2016). Clinical Performance and Management Outcomes with the DecisionDx-UM Gene Expression Profile Test in a Prospective Multicenter Study. *J Oncol*, *2016*, 5325762. https://doi.org/10.1155/2016/5325762

Plasseraud, K. M., Wilkinson, J. K., Oelschlager, K. M., Poteet, T. M., Cook, R. W., Stone, J. F., & Monzon, F. A. (2017). Gene expression profiling in uveal melanoma: technical reliability and correlation of molecular class with pathologic characteristics. *Diagn Pathol*, *12*(1), 59. https://doi.org/10.1186/s13000-017-0650-3

Rietschel, P., Panageas, K. S., Hanlon, C., Patel, A., Abramson, D. H., & Chapman, P. B. (2005). Variates of survival in metastatic uveal melanoma. *J Clin Oncol*, *23*(31), 8076-8080. https://doi.org/10.1200/jco.2005.02.6534

Schefler, A. C., Koca, E., Bernicker, E. H., & Correa, Z. M. (2019). Relationship between clinical features, GEP class, and PRAME expression in uveal melanoma. *Graefes Arch Clin Exp Ophthalmol*, *257*(7), 1541-1545. https://doi.org/10.1007/s00417-019-04335-w

Scott, J. F., & Gerstenblith, M. R. (2018). Noncutaneous Melanoma. In J. F. Scott & M. R. Gerstenblith (Eds.), *Noncutaneous Melanoma*. Codon Publications Copyright © 2018 Codon Publications. https://doi.org/10.15586/codon.noncutaneousmelanoma.2018

Shoushtari, A. N., & Carvajal, R. D. (2014). GNAQ and GNA11 mutations in uveal melanoma. *Melanoma Res*, *24*(6), 525-534. https://doi.org/10.1097/cmr.0000000000000121

Spagnolo, F., Caltabiano, G., & Queirolo, P. (2012). Uveal melanoma. *Cancer Treat Rev*, *38*(5), 549-553. https://doi.org/10.1016/j.ctrv.2012.01.002

Steiling, K., Christenson, Stephanie. (2023, October 03). *Tools for genetics and genomics: Gene expression profiling*. https://www.uptodate.com/contents/tools-for-genetics-and-genomics-gene-expression-profiling

Szalai, E., Jiang, Y., van Poppelen, N. M., Jager, M. J., de Klein, A., Kilic, E., & Grossniklaus, H. E. (2018). Association of Uveal Melanoma Metastatic Rate With Stochastic Mutation Rate and Type of Mutation. *JAMA Ophthalmol*. https://doi.org/10.1001/jamaophthalmol.2018.2986

Van Raamsdonk, C. D., Bezrookove, V., Green, G., Bauer, J., Gaugler, L., O'Brien, J. M., Simpson, E. M., Barsh, G. S., & Bastian, B. C. (2009). Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. *Nature*, *457*(7229), 599-602. https://doi.org/10.1038/nature07586

Van Raamsdonk, C. D., Griewank, K. G., Crosby, M. B., Garrido, M. C., Vemula, S., Wiesner, T., Obenauf, A. C., Wackernagel, W., Green, G., Bouvier, N., Sozen, M. M., Baimukanova, G., Roy, R., Heguy, A., Dolgalev, I., Khanin, R., Busam, K., Speicher, M. R., O'Brien, J., & Bastian, B. C. (2010). Mutations in GNA11 in uveal melanoma. *N Engl J Med*, *363*(23), 2191-2199. https://doi.org/10.1056/NEJMoa1000584

Walter, S., Department of Ophthalmology, B. P. E. I., University of Miami, Miami, FL, Chao, D. L., Department of Ophthalmology, U. S. o. M., San Francisco, CA, Schiffman, J. C., Department of Ophthalmology, B. P. E. I., University of Miami, Miami, FL, Feuer, W. J., Department of Ophthalmology, B. P. E. I., University of Miami, Miami, FL, Harbour, J. W., Department of Ophthalmology, B. P. E. I., University of Miami, Miami, FL, & Sylvester Comprehensive Cancer Center, U. o. M. M. S. o. M., Miami, FL. (2017). Tumor diameter contributes prognostic information that enhances the accuracy of gene expression profile molecular classification in uveal melanoma. *Invest Ophthalmol Vis Sci*, *56*(7), 4334-4334. http://iovs.arvojournals.org/article.aspx?articleid=2334273

Weis, E., Salopek, T., McKinnon, J., Larocque, M., Temple-Oberle, C., Cheng, T., McWhae, J., Sloboda, R., & Shea-Budgell, M. (2016). Management of uveal melanoma: a consensus-based provincial clinical practice guideline. *Curr Oncol*, *23*(1), e57-64. https://doi.org/10.3747/co.23.2859

Worley, L. A., Onken, M. D., Person, E., Robirds, D., Branson, J., Char, D. H., Perry, A., & Harbour, J. W. (2007). Transcriptomic versus chromosomal prognostic markers and clinical outcome in uveal melanoma. *Clin Cancer Res*, *13*(5), 1466-1471. https://doi.org/10.1158/1078-0432.ccr-06-2401

Genetic Testing for Alpha- and Beta- Thalassemia

Policy Number: AHS – M2131 – Genetic Testing for Alpha- and Beta-Thalassemia	Prior Policy Name and Number, as applicable: • AHS-M2131 Genetic Testing for Alpha Thalassemia
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Alpha-thalassemia is characterized by an impaired production of the alpha globin chains of hemoglobin, leading to a relative excess of gamma globin chains (fetus and newborn), or excess beta globin chains (children and adults) mainly due to deletion or mutation of the alpha globin genes. There are four alpha thalassemia syndromes, reflecting the loss of function of one, two, three, or all four of these alpha chain genes varying in severity from non-symptomatic to incompatibility with extrauterine life (Benz Jr & Angelucci, 2024; Martin & Thompson, 2013).

Beta-thalassemia is similarly characterized by impaired production of hemoglobin components but affects the beta chains instead of the alpha chains. This creates excess alpha globin chains, leading to hemolytic anemia, impaired iron handling, and other clinical symptoms (Benz Jr, 2023).

When pursuing genetic testing for alpha- or beta-thalassemia, genetic counseling is strongly recommended.

For guidance on prenatal screening and preconception screening for alpha- or beta-thalassemia, please see AHS-M2179-Prenatal Screening (Genetic).

II. Related Policies

Policy Number	Policy Title

AHS-M2170	Red Blood Cell Molecular Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Genetic testing to confirm an alpha- or beta-thalassemia diagnosis **MEETS COVERAGE CRITERIA** in **any** of the following situations:
   a) For individuals for whom one parent is a known carrier of alpha- or beta-thalassemia.
   b) For individuals for whom other testing to diagnose the cause of microcytic anemia has been inconclusive.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

2) For all other situations not described above, genetic testing for alpha- or beta-thalassemia **DOES NOT MEET COVERAGE CRITERIA**.

IV. Table of Terminology

Term	Definition
ACMG	American College of Medical Genetics and Genomics
ACOG	American College of Obstetrics and Gynecology
APHL	The Association of Public Health Laboratories
BCL11A	*B-cell CLL/lymphoma 11a*
BSH	British Society for Haematology
CCMG	Canadian College of Medical Geneticists
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers for Medicare and Medicaid
CRISPR/Cas9	Clustered Regularly Interspaced Short Palindromic Repeated/CRISPR-associated protein 9
DNA	Deoxyribonucleic acid
FDA	Food And Drug Administration
Hb	Hemoglobin
HBA1	*Alpha Globin 1*
HBA2	*Alpha Globin 2*

HBB	Hemoglobin, subunit beta
HbF	Hemoglobin F
HE	Electrophoresis
HHPLC	Hb high performance liquid chromatography
HLA	Human leukocyte antigens
LDTs	Laboratory-developed tests
MCH	Mean cell hemoglobin
MCV	Mean corpuscular volume
MDA	Multiple displacement amplification
NGS	Next-generation sequencing
NHS	National Health Service
PCR	Polymerase chain reaction
PGT	Preimplantation genetic testing
PHE	Public Health England
SCT	Sickle cell and thalassemia
SNP	Single nucleotide polymorphism
SOGC	Society of Obstetricians and Gynaecologists of Canada
β+	Reduced expression
β0	Absent expression

V. Scientific Background

Thalassemias result from deficiencies in hemoglobin biosynthesis due to mutations in or near the two globin gene clusters which encode the globin polypeptide subunits of hemoglobin (Benz Jr & Angelucci, 2024). Normal hemoglobin is a heterotetramer of two alpha globin chains and two beta globin chains (hemoglobin A) or two gamma globin chains (hemoglobin F). Well over 100 mutations have been documented to affect the biosynthesis or post-translational stability of the globin subunits needed for successful production of the large amounts of Hb needed for normal red cell homeostasis. Globin chain synthesis is very tightly controlled, such that the ratio of production of alpha to non-alpha chains is almost exactly 1:1 (Benz Jr, 2024b).

Alpha thalassemia refers to thalassemias that result from impaired or absent production of alpha globin, leading to a relative excess of gamma globin (fetus and newborn), or excess beta globin (children and adults). Excess beta globin chains can form soluble homotetramers, but they are nonfunctional and unstable. This may lead to increased hemolysis and a variety of clinical manifestations, such as anemia, thrombosis, and skeletal changes. A diagnosis of alpha thalassemia is often confirmed by genetic testing, as assessment of the hemoglobin gene is inexpensive and convenient (Benz Jr, 2024b).

The clinical severity is directly attributable to the net deficit of alpha globin synthesis but is complicated by the number of alpha globin genes affected, which of the two alpha globin loci is affected, and the degree to which the mutation blocks gene expression. In addition, combinations of defects in both alpha and beta globulins can balance each other out. Thus, understanding the broad spectrum of clinical severity in alpha thalassemia requires a detailed knowledge of the underlying genetic defect and the impact of these defects on the overall levels and balance of globin chain synthesis (Benz Jr, 2023).

The majority of cases of alpha thalassemia are attributable to deletion of alpha globin alleles, especially in Asia and Africa (Steinberg, 1999). However, more detailed analysis of globin gene sequences suggests that some fairly common forms of alpha thalassemia that appear to arise from a deletion of one copy of an alpha globin gene are actually due to unequal crossover and recombination events that fuse the two alpha globin genes together into one (Benz Jr, 2024b). Additionally, non-deletion alleles are also common, especially in the Mediterranean area, which contain mutations producing highly unstable alpha globin variants unable to produce intact hemoglobin (Benz Jr & Angelucci, 2024). Current research continues to identify novel mutations and improve thalassemia screening (He et al., 2018).

Beta-thalassemia is similar to alpha-thalassemia, with the beta chains of hemoglobin affected instead of the alpha chains. However, excess alpha globin chains do not form soluble homotetramers, causing them to aggregate when they accumulate in erythroid precursors. This causes clinical symptoms to be more severe, although the symptoms themselves are similar to alpha-thalassemia (anemia, iron overload, and so on) (Benz Jr, 2023; Benz Jr & Angelucci, 2024). There are two beta globin genes compared to four for the alpha chain. As with alpha-thalassemia, the severity of clinical presentation depends on the genotype of the beta globin genes (i.e. the ratio of beta to alpha globin chains). Mutations may result in a reduced expression (β^{+}) or absent expression (β^{0}). β^{0} phenotypes are generally transfusion-dependent as they produce very little (if any) adult hemoglobin (Benz Jr, 2023).

Due to the frequency of thalassemias worldwide, carrier screening may be useful, particularly in areas such as Southeast Asia, Africa, and the Indian subcontinent. Both primary thalassemias are autosomal recessive genetic disorders so parents who are heterozygous carriers would have a 25% chance to have an affected child despite being asymptomatic themselves. Identification of an affected fetus could alter decisions during the pregnancy (Yates, 2023).

Below is a table summarizing the clinical genotypes and phenotypes of both thalassemia syndromes (Benz Jr, 2024a; Benz Jr & Angelucci, 2024; Steinberg, 2024) (figure from Benz Jr).

Severity	Genotype	Anemia	Hemoglobin Analysis
Alpha Thalassemias			
Silent carrier	α α / α -	None	Normal, <3% Hb Barts (gamma globin tetramer) at birth
Minor	α α / - - or α - / α -	Mild Microcytic	Normal, 3 to 8% Hb Barts at birth
Hb H disease	α - / - -	Moderate Microcytic	up to 30% HbH (beta globin tetramer), present in adults, up to 4% HbA_2 (alpha and delta globin)
Major (fetal hydrops)	- - / - -	Severe Microcytic, usually fatal	Hb Barts, Hb Portland (zeta and gamma globin), and HbH present, HbA, HbF, and HbA_2 absent
Beta Thalassemias			
Minor (trait or carrier)	β / $β^0$ or β / β+	Mild Microcytic	HbA_2 (4% or more); HbF (up to 5%)
Intermedia (non-transfusion-dependent)	β+ / β+	Moderate Microcytic	HbA_2 (4% or more); HbF (up to 50%)
Major (transfusion-dependent)	$β^0$ / $β^0$ or $β^0$ / β+	Severe microcytic with target cells (typical Hb 3 to 4 g/dL)	HbA_2 (5% or more); HbF (up to 95%); no HbA

Analytical Validity

He et al. (2017) examined a next-generation sequencing (NGS) panel's utility for thalassemia screening in Southwestern China. A total of 951 individuals were tested, and the NGS screen found 471 carriers (49.5%) of thalassemia. In comparison, traditional methods (defined as "red cell indexes and hemoglobin electrophoresis, then

DNA sequencing") identified only 209 carriers (22%) of thalassemia, missing 217 alpha-thalassemia carriers and 47 beta-thalassemia carriers (He et al., 2017). In a separate study by Zhang et al. (2019), because of studying 3,973 subjects that underwent hematological examinations and additional NGS and Gap-PCR due to being suspected thalassemia carriers, the researchers found that "approximately 2.88% thalassemia carriers would be missed by traditional genetic analysis. In addition, four novel thalassemia mutations and one novel abnormal hemoglobin mutation were identified." This research further corroborated the increased effectiveness of using NGS in screening thalassemia in an area of high disease prevalence (Zhang et al., 2019).

Shook et al. (2020) evaluated the accuracy of a specific pattern in hemoglobin separation tests. The authors desired to find if an "FSA" pattern corresponded to a final diagnosis of the sickle cell trait (HbAS), or a final diagnosis of sickle beta-thalassemia ($HbS\beta^+$). Traditionally, the FSA pattern has indicated a diagnosis of $HbS\beta^+$; however, the authors hypothesized that the FSA pattern truly indicates a diagnosis of HbAS instead. 31 newborns with an initial screening result of the FSA pattern (a suspected diagnosis of $HbS\beta^+$) were included. 30 of these newborns underwent protein-base confirmatory testing and 17 underwent confirmatory genetic testing. Of the newborns undergoing protein confirmatory testing, 23 had an "FSA" pattern, establishing a diagnosis of HbAS. Of the 8 remaining newborns with an FSA pattern, 7 underwent genetic testing which identified HbAS as well. Genetic testing also confirmed positive HbAS results in 10 newborns that tested initially positive by protein testing. The authors concluded that genetic testing had utility in newborn screening for hemoglobinopathies (Shook et al., 2020).

Chen et al. (2021) established an effective NGS protocol for four-factor preimplantation genetic testing (PGT) to diagnose α- and β-thalassemia. Three couples, in whom both partners were α- and β-double thalassemia carriers, underwent PGT and a total of 35 biopsied trophectoderm samples underwent multiple displacement amplification (MDA). Using NGS-based single-nucleotide polymorphism (SNP) haplotyping, these samples were analyzed. "51.5% (17/33) of the embryos were diagnosed as unaffected non-carriers or carriers. Of the 17 unaffected embryos, nine (52.9%) were tested further and identified as euploid via NGS-based aneuploid screening, in which five had HLA types matching affected children." The authors conclude that NGS-SNP was effective in performing PGT for multipurpose detection (Chen et al., 2021).

Clinical Utility and Validity

Nosheen et al. (2015) evaluated a preliminary screening program for beta-thalassemia. The screening program focused on families of beta-thalassemia major children. 98

samples were taken, and 57 were found to have a beta-thalassemia trait with elevated hemoglobin alpha 2. The mean hemoglobin alpha 2 level of the carriers was 5.2±0.56% compared to 2.34±0.57% in normal subjects. The authors suggested that screening programs and counseling for carriers could decrease incidence of beta-thalassemia major (Nosheen et al., 2015).

Satirapod et al. (2019) evaluated the clinical outcomes of using preimplantation genetic testing (PGT) in couples at risk of passing on beta thalassemia. Two components of PGT were used, PGT for monogenic disease (used for diagnosis) and PGT for aneuploidy (intended to identify chromosomal aberrations) A total of 15 couples were included and a total of 106 embryos were tested. After preimplantation testing, 12 of 15 individuals were able to obtain satisfactory genetic testing results (defined as non-disease affected embryos without chromosomal aberration and transfer within first two cycles). Of these, nine individuals had successful implantations and eight individuals had successful pregnancies with live births (deemed a 53.33% success rate). PGT assessment of genetic status was confirmed by pre- and post-natal genetic testing. Overall, the authors concluded that combined PGT-A and PGT-M was a useful technology to prevent beta-thalassemia in the offspring of recessive carriers. To increase the diagnostic efficiency of PGT-M, multiple displacement amplification (MDA) may be utilized as the first step. This conclusion was drawn by Fu et al. (2019), who found in a retrospective cohort study, that from 2,315 embryos tested, MDA yielded a 96.99% diagnostic efficiency, versus a PCR group, which only yielded 88.15%. MDA also enabled statistically significantly more embryos to be available for transfer as well when compared to the PCR group (74.28% vs 64.28%, respectively, $P < 0.001$) (Fu et al., 2019).

Chen et al. (2020) also conducted a similar study that evaluated "the efficacy of preimplantation genetic testing (PGT) for α- and β-double thalassemia combined with aneuploidy screening using next-generation sequencing (NGS)." From 12 couples that each carried both α- and β- thalassemia mutations, the researchers were able to facilitate 11 healthy live births from examining 112 embryos. This NGS-based SNP (single nucleotide polymorphism) haplotyping was demonstrated to "reduce misdiagnosis by linkage analyses with multiple SNP loci" and increase the number of diagnosis results, including those from detecting aneuploidy and identified mutation sites, in a single PGT cycle. It was also found that NGS-based SNP haplotyping could be performed "through directly detecting mutation sites with NGS and using affected embryos or gametes as probands." This procedure benefits in eliminating multiple biopsies as well (Chen et al., 2020).

Dan et al. (2023) conducted a literature mini-review of the current state of beta-thalessemia management. Currently, beta-thalessemia requires lifelong management

strategies that include regular blood transfusions and iron chelation therapy for severe cases. The review discussed the limitations of current therapies and advocates for continued research into hematopoietic stem cell transplantation and gene therapy as new treatment approaches. Several novel therapeutic methods are being explored for clinical utility. For example, Luspatercept is a recently FDA-approved therapy that works by inhibiting the Smad2/3 signaling pathway, which is involved in erythropoiesis (the process of producing red blood cells). By modifying this pathway, Luspatercept helps to reduce the ineffective erythropoiesis that is a hallmark of beta-thalessemia. It is particularly useful for patients who are transfusion-dependent and has purported promise in improving iron balance and reducing the frequency of blood transfusions; however, the cost of Luspatercept is $170,000 annually per patient. Hydroxyurea is a drug that is well-known in the treatment of blood disorders, such as sickle cell disease, but could also have potential benefits in treating beta-thalessemia. Gene therapy focuses on strategies to increase the production of gamma globin chains, thereby increasing HbF levels. This approach could potentially correct the underlying genetic defects causing thalassemia. Gene therapy techniques include: (1) CRISPR/Cas9 gene editing (involves editing the BCL11A gene, a key regulator of hemoglobin production, to enhance HbF synthesis) and (2) Lentiviral Gene Transfer (therapies that involve a lentiviral vector to insert a functional copy of the beta-globin gene into the patients' hematopoietic stem cells). One example of lentiviral gene transfer is Zynteglo gene therapy, the first FDA-approved genetic treatment for beta-thalessemia (Dan et al., 2023).

VI. Guidelines and Recommendations

American College of Medical Genetics and Genomics (ACMG)

In 2021, ACMG released an updated guideline for screening for autosomal recessive and X-linked conditions during pregnancy and preconception. Their practice resource aims to recommend "a consistent and equitable approach for offering carrier screening to all individuals during pregnancy and preconception" and replaces any earlier ACMG position statements on prenatal/preconception expanded carrier screening.

The ACMG provides carrier screening recommendations during pregnancy that are split into three tiers. Tier 1 includes recommended screenings for all couples considering pregnancy or pregnant women. The tier 1 recommendations include disorders that have significant health impacts, for which prenatal diagnosis and potential interventions might be available; this tier includes screenings relevant to this policy, such as sickle cell disease, alpha-thalassemia, and beta-thalassemia. Tier 2 includes additional screenings that may be offered based on the family history or ancestry that suggest higher risk of specific genetic conditions. In some cases,

expanded carrier screening can be considered, which may test for less common hemoglobinopathies. Tier 3 screenings are "optional" and can be considered based on individual or family history factors. These screenings may include rare genetic disorders or less common variants of hemoglobinopathies. The ACMG provides the following specific recommendations:

- "Carrier screening enables those screened to consider their reproductive risks, reproductive options, and to make informed decisions."
- "The phrase "expanded carrier screening" be replaced by "carrier screening."
- "Adopting a more precise tiered system based on carrier frequency:
    - Tier 4: <1/200 carrier frequency (includes Tier 3) genes/condition will vary by lab
    - Tier 3: ≥ 1/200 carrier frequency (includes Tier 2) includes X-linked conditions
    - Tier 2: ≥1/100 carrier frequency (includes Tier 1)
    - Tier 1: *CF* [Cystic Fibrosis] + *SMA* [spinal muscular atrophy] + Risk Based Screening
        - "Tier 1 screening conveys the recommendations previously adopted by ACMG and ACOG" and "adopts an ethnic and population neutral approach when screening for cystic fibrosis and spinal muscular atrophy. Beyond these two conditions, additional carrier screening is determined after risk assessment, which incorporates personal medical and family history as well as laboratory and imaging information where appropriate"
        - "Tier 2 carrier screening stems from an ACOG recommendation for conditions that have a severe or moderate phenotype and a carrier frequency of at least 1/100." However, "data demonstrate that carrier screening for two common conditions using a carrier frequency threshold of 1/100 may not be equitable across diverse populations. Others have shown that limiting the carrier frequency to ≥1/100 creates missed opportunities to identify couples at risk for serious conditions."
        - "We define Tier 3 screening as carrier screening for conditions with a carrier frequency ≥1/200 . . . Tier 2 and Tier 3 screening prioritize carrier frequency as a way to think about conditions most appropriate for screening in the general population. However, when ACOG proposed this level, they did not specify whether it was thinking about carrier frequency in terms of the global population or subpopulations. We use "carrier frequency" to mean in any ethnic group with reasonable representation in the United States."
        - "Tier 4 includes genes less common than those in Tier 3 and can identify additional at-risk couples. Tier 4 has no lower limit carrier screening frequency and can greatly extend the number of conditions screened . . .

the clinical validity at this level of carrier screening may be less compelling, therefore we suggest reserving this level of screening for consanguineous pregnancies (second cousins or closer) and in couples where family or medical history suggests Tier 4 screening might be beneficial . . . Importantly, patients should understand that their chance of being a carrier for one or more conditions increases as the number of conditions screened is increased."

- "All pregnant patients and those planning a pregnancy should be offered Tier 3 carrier screening.
- Tier 4 screening should be considered:
  - When a pregnancy stems from a known or possible consanguineous relationship (second cousins or closer);
  - When a family or personal medical history warrants.
- ACMG does NOT recommend:
  - Offering Tier 1 and/or Tier 2 screening, because these do not provide equitable evaluation of all racial/ethnic groups.
  - Routine offering of Tier 4 panels.
- "Carrier screening paradigms should be ethnic and population neutral and more inclusive of diverse populations to promote equity and inclusion."
- "All pregnant patients and those planning a pregnancy should be offered Tier 3 carrier screening for autosomal recessive [Table 1 & 5] . . . conditions."
- "Reproductive partners of pregnant patients and those planning a pregnancy may be offered Tier 3 carrier screening for autosomal recessive conditions [Table 1 & 5] when carrier screening is performed simultaneously with their partner."
- "When Tier 1 or Tier 2 carrier screening was performed in a prior pregnancy, Tier 3 screening should be offered" (Gregg et al., 2021).

Table 1. Autosomal recessive genes for screening with carrier frequency ≥1/50.

OMIM gene	OMIM gene name	Maximum carrier frequency[a]	OMIM phenotype	Conditions
141900	*HBB*	0.119837	603903	Sickle cell anemia β-thalassemia
			613985	
613208	*XPC*	0.050885	278720	Xeroderma pigmentosum
606933	*TYR*	0.049337	203100	Oculocutaneous albinism type 1A and 1B
			606952	
613815	*CYP21A2*	0.048459	201910	Congenital adrenal hyperplasia due to 21-hydroxylase deficiency
612349	*PAH*	0.046068	261600	Phenylketonuria
602421	*CFTR*	0.040972	219700	Cystic fibrosis
600985	*TNXB*	0.035134	606408	Ehlers–Danlos-like syndrome due to tenascin-X deficiency
606869	*HEXA*	0.033146	272800	Tay–Sachs disease
121011	*GJB2*	0.026200	220290	Nonsyndromic hearing loss recessive 1A
			601544	Nonsyndromic hearing loss dominant 3A
602858	*DHCR7*	0.023709	270400	Smith–Lemli–Opitz syndrome
277900	*ATP7B*	0.021983	606882	Wilson disease
608034	*ASPA*	0.019856	271900	Canavan disease
607008	*ACADM*	0.016583	201450	Medium-chain acyl-coenzyme A dehydrogenase deficiency
602716	*NPHS1*	0.015994	256300	Finnish congenital nephrotic syndrome
601785	*PMM2*	0.015877	212065	Carbohydrate-deficient glycoprotein syndrome type Ia
607440	*FKTN*	0.015660	611615	Cardiomyopathy, dilated, 1X
			253800	Walker–Warburg congenital muscular dystrophy
605646	*SLC26A4*	0.015422	600791	Deafness autosomal recessive 4
			274600	Pendred syndrome
126340	*ERCC2*	0.015255	610756	Cerebrooculofacioskeletal syndrome 2
			601675	Trichothiodystrophy 1, photosensitive
603297	*DYNC2H1*	0.014817	613091	Short-rib thoracic dysplasia 3 with or without polydactyly

OMIM Online Mendelian Inheritance in Man.[55]
[a]Values round to ≥ 0.02 (two decimal places).

Table 5. Genes that were ascertained for screening outside of the gnomAD criteria[a].

OMIM gene	OMIM gene name	Published carrier frequency[b]	Rationale for inclusion	Ethnic group	OMIM phenotype	Conditions
141800	*HBA1*	U[c]	Carrier frequency	SEA and others	604131	α-Thalassemia
141850	*HBA2*	U[c]	Carrier frequency	SEA and others	604131	α-Thalassemia
600354	*SMN1*	1/60[18]	ACOG/ACMG and carrier frequency	US panethnic	253300	
					253550	Spinal muscular
					253400	atrophy types: I, II, III, IV
					271150	
604982	*HPS1*	1/59[56–58]	Carrier frequency	PR	203300	Hermansky Pudlak S. 1
606118	*HPS3*	1/59[56]	Carrier frequency	PR	614072	Hermansky Pudlak S. 3
603722	*ELP1*	1/32[59]	ACOG/ACMG and carrier frequency	AJ	223900	Familial dysautonomia
606829	*FXN*	1/60–1/100[60]	Carrier frequency	Caucasians[d]	229300	Friedreich ataxia
238331	*DLD*	~1/100[59,61]	Carrier frequency	AJ	246900	Dihydrolipoamide dehydrogenase deficiency
161650	*NEB*	1/168[59]	Carrier frequency	AJ	256030	Nemaline myopathy 2
606397	*CLRN1*	1/120[59]	Carrier frequency	AJ	276902	Usher syndrome 3a
604610	*BLM*	1/100[59]	ACMG and carrier frequency	AJ	210900	Bloom syndrome

ACMG American College of Medical Genetics and Genomics, *ACOG* American College of Obstetricians and Gynecologists, *AJ* Ashkenazi Jewish (≥2% of the US population), *OMIM* Online Mendelian Inheritance in Man,[55] *PR* Puerto Rican, *SEA* South East Asian.
[a]Carrier frequency of a sequence variant is <1/200, if reported in gnomAD.[50]
[b]Diagnostic laboratory data was not used for carrier frequency data.
[c]Specific data for general US population not available; however, recognized as common among many US immigrant populations.[62]
[d]This term is no longer used by the journal but is used in the original article to which these studies refer. We have therefore not changed the term but recognize it does not accurately describe the ancestry of the populations originally studied.[46]

(Gregg et al., 2021)

Canadian College of Medical Geneticists (CCMG) and Society of Obstetricians and Gynaecologists of Canada (SOGC)

The CCMG and SOGC published a joint guideline titled "Carrier Screening for Thalassemia and Hemoglobinopathies in Canada" in 2008. Their recommendations addressing thalassemia's/hemoglobinopathies are listed below:

1. "Carrier screening for thalassemia and hemoglobinopathies should be offered to a woman if she and/or her partner are identified as belonging to an ethnic population whose members are at higher risk of being carriers. Ideally, this screening should be done pre-conceptionally or as early as possible in the pregnancy. (II-2A)
2. Screening should consist of a complete blood count, as well as hemoglobin electrophoresis or hemoglobin high performance liquid chromatography. This investigation should include quantitation of HbA2 and HbF. In addition, if there is microcytosis (mean cellular volume < 80 fL) and/or hypochromia (mean cellular hemoglobin < 27 pg) in the presence of a normal hemoglobin electrophoresis or high-performance liquid chromatography the patient should be investigated with a brilliant cresyl blue stained blood smear to identify H bodies. A serum ferritin (to exclude iron deficiency anemia) should be performed simultaneously. (III-A)
3. If a woman's initial screening is abnormal (e.g., showing microcytosis or hypochromia with or without an elevated HbA2, or a variant Hb on electrophoresis or high-performance liquid chromatography) then screening of the partner should be performed. This would include a complete blood count as well as hemoglobin electrophoresis or HPLC, HbA2 and HbF quantitation, and H body staining. (III-A)
4. If both partners are found to be carriers of thalassemia or an Hb variant, or of a combination of thalassemia and a hemoglobin variant, they should be referred for genetic counselling. Ideally, this should be prior to conception, or as early as possible in the pregnancy. Additional molecular studies may be required to clarify the carrier status of the parents and thus the risk to the fetus. (II-3A)
5. Prenatal diagnosis should be offered to the pregnant woman/couple at risk for having a fetus affected with a clinically significant thalassemia or hemoglobinopathy. Prenatal diagnosis should be performed with the patient's informed consent. If prenatal diagnosis is declined, testing of the child should be done to allow early diagnosis and referral to a pediatric hematology centre, if indicated. (II-3A)
6. Prenatal diagnosis by DNA analysis can be performed using cells obtained by chorionic villus sampling or amniocentesis. Alternatively, for those who decline

invasive testing and are at risk of hemoglobin Bart's hydrops fetalis (four-gene deletion α-thalassemia), serial detailed fetal ultrasound for assessment of the fetal cardiothoracic ratio (normal < 0.5) should be done in a centre that has experience conducting these assessments for early identification of an affected fetus. If an abnormality is detected, a referral to a tertiary care centre is recommended for further assessment and counselling. Confirmatory studies by DNA analysis of amniocytes should be done if a termination of pregnancy is being considered. (II-3A)

7. The finding of hydrops fetalis on ultrasound in the second or third trimester in [individuals] with an ethnic background that has an increased risk of α-thalassemia should prompt immediate investigation of the pregnant patient and her partner to determine their carrier status for α-thalassemia. (III-A)" (Langlois et al., 2008).

The Thalassemia Longitudinal Cohort

The report on the Thalassemia Longitudinal Cohort recommends: "Obtaining genotyping to confirm the diagnosis and HLA typing for transplant evaluation for all patients who require chronic transfusion is strongly recommended. For pediatric patients, annual comprehensive follow up should include assessment of the availability of a related donor as well as a recommendation to bank cord blood and obtain HLA typing on all subsequently born full siblings" (Tubman et al., 2015).

American College of Obstetrics and Gynecology (ACOG)

The ACOG Committee Opinion #691 ("Carrier Screening for Genetic Conditions") states that: "Couples at risk of having a child with a hemoglobinopathy may benefit from genetic counseling to review their risk, the natural history of these disorders, prospects for treatment and cure, availability of prenatal genetic testing, and reproductive options. Prenatal diagnostic testing for the mutation responsible for sickle cell disease is widely available. Testing for α-thalassemia and β-thalassemia is possible if the mutations and deletions have been previously identified in both parents. These DNA-based tests can be performed using chorionic villi obtained by chorionic villus sampling or using cultured amniotic fluid cells obtained by amniocentesis. For some couples, preimplantation genetic diagnosis in combination with in vitro fertilization may be a desirable alternative to avoid termination of an affected pregnancy. Preimplantation genetic diagnosis has been successfully performed for sickle cell disease and most types of β-thalassemia" (ACOG, 2017). This was reaffirmed in 2020.

In 2022, ACOG put out a new practice bulletin regarding hemoglobinopathies in pregnancy (originally published in 2007) and reaffirmed this in 2023 with a few additions (ACOG, 2022).

- ACOG "recommends offering universal hemoglobinopathy testing to persons planning pregnancy or at the initial prenatal visit if no prior testing results are available for interpretation."
- "Hemoglobinopathy testing may be performed using hemoglobin electrophoresis or molecular genetic testing (eg, expanded carrier screening that includes sickle cell disease [SCD] and other hemoglobinopathies)."
- "The use of noninvasive prenatal diagnosis for SCD with cell-free fetal DNA is still experimental and currently not recommended" (ACOG, 2022).
-

National Health Service (NHS)

The NHS released standards for antenatal laboratories working with the NHS sickle cell and thalassaemia (SCT) screening program (NHS, 2021). The referral guidelines for antenatal screening specimens are as follows:

Biological mother carrier state	Biological father carrier state	Further studies by DNA analysis
No abnormalities detected	Testing of baby's biological father not required	None required
Any abnormal Hb or thalassaemia	No abnormality detected	None required
HbS	HbS or HbC	None required until PND
HbS	HbO^{Arab}, D^{Punjab}, E, Lepore, β thalassaemia or δβ thalassaemia	If PND is being considered send bloods for mutation confirmation, provided it does not limit options available
HbS	HPFH	Send bloods for mutation confirmation. PND is not usually indicated when HPFH has been confirmed
HbS + α thalassaemia	Assess risk as per HbS alone, unless family origins indicate a high risk of α^0 thalassaemia	Assess risk as per HbS alone, unless family origins indicate a high risk of a^0 thalassaemia
HbC	HbS	None required until PND
HbD	HbS	If PND is being considered send bloods for mutation confirmation, provided it does not limit options available
HbO^{Arab}	HbS, β thalassaemia or δβ thalassaemia	If PND is being considered send bloods for mutation confirmation, provided it does not limit options available
Hb Lepore	HbS, E, O^{Arab}, Lepore, β thalassaemia or δβ thalassaemia	If PND is being considered send bloods for mutation confirmation, provided it does not limit options available.
HbE	β thalassaemia, Hb Lepore, δβ thalassaemia, HbS	If PND is being considered send bloods for mutation confirmation, provided it does not limit options available
HbE	α thalassaemia (MCH < 25pg)	Send bloods for mutation confirmation if high risk family origins for α^0 thalassaemia, consider impact on options available
β or δβ thalassaemia	HbS, E, O^{Arab}, Lepore, β thalassaemia or δβ thalassaemia	If PND is being considered send bloods for mutation confirmation, provided it does not limit options available
β or δβ thalassaemia	α thalassaemia (high risk family origins)	Send bloods for mutation confirmation if high risk family origins for α^0 thalassaemia, consider impact on options available
HPFH	HbS, E, O^{Arab}, Lepore, β or δβ thalassaemia	Send bloods for mutation confirmation. PND is not usually indicated when HPFH has been confirmed
α thalassaemia (MCH < 27pg but ≥ 25pg)	Testing of baby's biological father not required	None required
α thalassaemia (MCH < 25pg)		
1. Low risk α^0 thal family origins in either biological parent	Testing of baby's biological father not required	None required
2. High risk α^0 thal family origins in both biological parents or unknown	Test baby's biological father and if MCH < 25pg irrespective of any other phenotype detected	Send maternal and paternal bloods for mutation confirmation, consider impact on options available.

The Association of Public Health Laboratories (APHL)

The Association of Public Health Laboratories (APHL) states that "Molecular testing can be added to resolve cases when the newborn has been transfused with packed red blood cells. Since the newborn's phenotype is masked by the donor, DNA testing can be used to identify any abnormal hemoglobins" (APHL, 2015).

Public Health England (PHE)

The PHE highlights the importance of antenatal screening. If the baby's mother is identified as a carrier, the biological father should also be tested. Both prenatal diagnosis and genetic counselling are recommended by the PHE (PHE, 2022).

British Society for Haematology (BSH)

The BSH provides the following recommendations:

- "Antenatal screening/testing of pregnant [individuals] should be carried out according to the guidelines of the NHS Sickle Cell and Thalassaemia Screening programme.
- Laboratories performing antenatal screening should utilize methods capable of detecting significant variants and be capable of quantitating haemoglobins A_2 and F at the cut-off points required by the national antenatal screening program" (Ryan et al., 2010).

Genetic counseling is also permitted for prospective parents.

The Thalassemia International Foundation (TIF)

The TIF provided recommendations for the management of transfusion dependent Thalassemia. The following recommendations were made (TIF, 2021):

- "Molecular genetic testing is available in clinical laboratories and may be useful for predicting the clinical phenotype in some cases as well as enabling presymptomatic diagnosis of at-risk family members and prenatal diagnosis.
- Molecular analysis is not required to confirm the diagnosis of a β carrier, but it is necessary to confirm the α thalassemia carrier status (grade A)
- Since the prevalent pathogenic variants of the β globin gene are limited in each at-risk population, a PCR method designed to detect the common specific mutation simultaneously should be used initially (grade B)

- β globin gene sequence analysis may be considered first if the affected individual is not of an ancestry at high risk or if targeted analysis reveals only one or no pathogenic variant (grade B)
- α thalassemias are mainly due to deletions of different length and they can be detected preferentially by reverse dot blot and Gap-PCR (grade B)
- Methods that may be used to detect rare or unknown deletions include: Southern blotting (now fallen into abeyance), quantitative PCR, long-range PCR and, above all, MLPA (grade B)" (TIF, 2021).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81257	HBA1/HBA2 (alpha globin 1 and alpha globin 2) (eg, alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis; common deletions or variant (eg, Southeast Asian, Thai, Filipino, Mediterranean, alpha3.7, alpha4.2, alpha20.5, Constant Spring)
81258	HBA1/HBA2 (alpha globin 1 and alpha globin 2) (eg, alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis; known familial variant

CPT	Code Description
81259	HBA1/HBA2 (alpha globin 1 and alpha globin 2) (eg, alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis; full gene sequence
81269	HBA1/HBA2 (alpha globin 1 and alpha globin 2) (eg, alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis; duplication/deletion variants
81361	HBB (hemoglobin, subunit beta) (eg, sickle cell anemia, beta thalassemia, hemoglobinopathy); common variant(s) (eg, HbS, HbC, HbE)
81362	HBB (hemoglobin, subunit beta) (eg, sickle cell anemia, beta thalassemia, hemoglobinopathy); known familial variant(s)
81363	HBB (hemoglobin, subunit beta) (eg, sickle cell anemia, beta thalassemia, hemoglobinopathy); duplication/deletion variant(s)
81364	HBB (hemoglobin, subunit beta) (eg, sickle cell anemia, beta thalassemia, hemoglobinopathy); full gene sequence
S3845	Genetic testing for alpha-thalassemia
S3846	Genetic testing for hemoglobin E beta-thalassemia
96040	Medical genetics and genetic counseling services, each 30 minutes face-to-face with patient/family
S0265	Genetic counseling, under physician supervision, each 15 minutes

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

ACOG. (2007). ACOG Practice Bulletin No. 78: hemoglobinopathies in pregnancy. *Obstet Gynecol*, *109*(1), 229-237. https://doi.org/10.1097/00006250-200701000-00055

ACOG. (2017). ACOG Commitee Opinion 691: Carrier Screening for Genetic Conditions. *Obstet Gynecol*, *129*, e41-e55. https://doi.org/10.1097/AOG.0000000000001952

ACOG. (2018). ACOG Publications. *Obstetrics & Gynecology*, *131*(1). https://journals.lww.com/greenjournal/Fulltext/2018/01000/ACOG_Publications.31.aspx

ACOG. (2022). Hemoglobinopathies in Pregnancy. https://www.acog.org/clinical/clinical-guidance/practice-advisory/articles/2022/08/hemoglobinopathies-in-pregnancy

APHL. (2015). *Hemoglobinopathies: Current Practices for Screening, Confirmation and Follow-up*. Association of Public Health Laboratories Retrieved from

https://www.cdc.gov/ncbddd/sicklecell/documents/nbs_hemoglobinopathy-testing_122015.pdf
Benz Jr, E. J. (2023). *Pathophysiology of thalassemia*. https://www.uptodate.com/contents/pathophysiology-of-thalassemia
Benz Jr, E. J. (2024a). Classical thalassemia syndromes (genotypes and laboratory findings). In *UpToDate*. Waltham. MA.
Benz Jr, E. J. (2024b). *Molecular pathology of the thalassemic syndromes*. https://www.uptodate.com/contents/molecular-genetics-of-the-thalassemia-syndromes
Benz Jr, E. J., & Angelucci, E. (2024, April 5). *Clinical manifestations and diagnosis of the thalassemias*. https://www.uptodate.com/contents/clinical-manifestations-and-diagnosis-of-the-thalassemias
Chen, D., Shen, X., Wu, C., Xu, Y., Ding, C., Zhang, G., Xu, Y., & Zhou, C. (2020). Eleven healthy live births: a result of simultaneous preimplantation genetic testing of α- and β-double thalassemia and aneuploidy screening. *Journal of assisted reproduction and genetics*, *37*(3), 549-557. https://doi.org/10.1007/s10815-020-01732-7
Chen, D., Shen, X., Xu, Y., Ding, C., Ye, Q., Zhong, Y., Xu, Y., & Zhou, C. (2021). Successful four-factor preimplantation genetic testing: α- and β-thalassemia, human leukocyte antigen typing, and aneuploidy screening. *Systems Biology in Reproductive Medicine*, *67*(2), 151-159. https://doi.org/10.1080/19396368.2020.1832158
Dan, M., Gutu, B.-I., Severin, E., & Tanase, V.-G. (2023). Innovative and Needs-led research on β-thalassemia treatment methods. *Frontiers in Hematology*, *1*. https://doi.org/10.3389/frhem.2022.1085952
Fu, Y., Shen, X., Chen, D., Wang, Z., & Zhou, C. (2019). Multiple displacement amplification as the first step can increase the diagnostic efficiency of preimplantation genetic testing for monogenic disease for β-thalassemia. *J Obstet Gynaecol Res*, *45*(8), 1515-1521. https://doi.org/10.1111/jog.14003
Gregg, A. R., Aarabi, M., Klugman, S., Leach, N. T., Bashford, M. T., Goldwaser, T., Chen, E., Sparks, T. N., Reddi, H. V., Rajkovic, A., & Dungan, J. S. (2021). Screening for autosomal recessive and X-linked conditions during pregnancy and preconception: a practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *23*(10), 1793-1806. https://doi.org/10.1038/s41436-021-01203-z
He, J., Song, W., Yang, J., Lu, S., Yuan, Y., Guo, J., Zhang, J., Ye, K., Yang, F., Long, F., Peng, Z., Yu, H., Cheng, L., & Zhu, B. (2017). Next-generation sequencing improves thalassemia carrier screening among premarital adults in a high prevalence population: the Dai nationality, China. *Genet Med*, *19*(9), 1022-1031. https://doi.org/10.1038/gim.2016.218
He, S., Li, J., Li, D. M., Yi, S., Lu, X., Luo, Y., Liang, Y., Feng, C., Chen, B., Zheng, C., & Qiu, X. (2018). Molecular characterization of alpha- and beta-thalassemia in the Yulin region of Southern China. *Gene*, *655*, 61-64. https://doi.org/10.1016/j.gene.2018.02.058

Langlois, S., Ford, J. C., Chitayat, D., Chitayat, D., Désilets, V. A., Farrell, S. A., Geraghty, M., Langlois, S., Nelson, T., Nikkel, S. M., Shugar, A., Skidmore, D., Allen, V. M., Audibert, F., Blight, C., Désilets, V. A., Gagnon, A., Johnson, J.-A., Langlois, S., . . . Wyatt, P. (2008). Carrier Screening for Thalassemia and Hemoglobinopathies in Canada. *Journal of Obstetrics and Gynaecology Canada*, *30*(10), 950-959. https://doi.org/10.1016/s1701-2163(16)32975-9

Martin, A., & Thompson, A. A. (2013). Thalassemias. *Pediatr Clin North Am*, *60*(6), 1383-1391. https://doi.org/10.1016/j.pcl.2013.08.008

NHS. (2021). *SCT Screening: handbook for antenatal laboratories*. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1039978/Referral_guidelines_for_antenatal_screening_specimens_final.pdf

Nosheen, A., Ahmad, H., Qayum, I., Siddiqui, N., Abbasi, F. M., & Iqbal, M. S. (2015). Premarital genetic screening for beta thalassemia carrier status of indexed families using HbA2 electrophoresis. *J Pak Med Assoc*, *65*(10), 1047-1049.

PHE. (2022). *Sickle cell and thalassaemia screening: commission and provide*. https://www.gov.uk/government/collections/sickle-cell-and-thalassaemia-screening-commission-and-provide

Ryan, K., Bain, B. J., Worthington, D., James, J., Plews, D., Mason, A., Roper, D., Rees, D. C., de la Salle, B., & Streetly, A. (2010). Significant haemoglobinopathies: guidelines for screening and diagnosis. *Br J Haematol*, *149*(1), 35-49. https://doi.org/10.1111/j.1365-2141.2009.08054.x

Satirapod, C., Sukprasert, M., Panthan, B., Charoenyingwattana, A., Chitayanan, P., Chantratita, W., Choktanasiri, W., Trachoo, O., & Hongeng, S. (2019). Clinical utility of combined preimplantation genetic testing methods in couples at risk of passing on beta thalassemia/hemoglobin E disease: A retrospective review from a single center. *PLoS One*, *14*(11), e0225457. https://doi.org/10.1371/journal.pone.0225457

Shook, L. M., Haygood, D., & Quinn, C. T. (2020). Clinical Utility of Confirmatory Genetic Testing to Differentiate Sickle Cell Trait from Sickle-beta(+)-Thalassemia by Newborn Screening. *Int J Neonatal Screen*, *6*(1). https://doi.org/10.3390/ijns6010007

Steinberg, M. H. (1999). Management of sickle cell disease. *N Engl J Med*, *340*(13), 1021-1030. https://www.nejm.org/doi/10.1056/NEJM199904013401307

Steinberg, M. H. (2024). *Structure and function of normal hemoglobins*. https://www.uptodate.com/contents/structure-and-function-of-normal-hemoglobins

TIF. (2021). 2021 GUIDELINES FOR THE MANAGEMENT OF TRANSFUSION DEPENDENT THALASSAEMIA (TDT). https://www.thalassemia.org/boduw/wp-content/uploads/2021/06/TIF-2021-Guidelines-for-Mgmt-of-TDT.pdf

Tubman, V. N., Fung, E. B., Vogiatzi, M., Thompson, A. A., Rogers, Z. R., Neufeld, E. J., & Kwiatkowski, J. L. (2015). Guidelines for the Standard Monitoring of Patients with

Thalassemia: Report of the Thalassemia Longitudinal Cohort. *J Pediatr Hematol Oncol*, *37*(3), e162-169. https://doi.org/10.1097/mph.0000000000000307

Yates, A. (2023). *Prenatal screening and testing for hemoglobinopathy*. https://www.uptodate.com/contents/prenatal-screening-and-testing-for-hemoglobinopathy

Zhang, H., Li, C., Li, J., Hou, S., Chen, D., Yan, H., Chen, S., Liu, S., Yin, Z., Yang, X., Tan, J., Huang, X., Zhang, L., Fang, J., Zhang, C., Li, W., Guo, J., & Lei, D. (2019). Next-generation sequencing improves molecular epidemiological characterization of thalassemia in Chenzhou Region, P.R. China. *Journal of clinical laboratory analysis*, *33*(4), e22845-e22845. https://doi.org/10.1002/jcla.22845

Genetic Testing for Breast, Ovarian, Pancreatic, and Prostate Cancers

Policy Number: AHS – M2003 – Genetic Testing for Breast, Ovarian, Pancreatic, and Prostate Cancers	Prior Policy Name and Number, as applicable: AHS – M2003 – *BRCA*
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

BRCA1 and *BRCA2* are two distinct tumor suppressor genes involved in a common DNA repair process (Roy et al., 2012). Germline mutations of *BRCA* genes are associated with an increased risk of breast and ovarian cancer, as well as other cancer types, including pancreatic and prostate cancer to a lesser extent (Paul & Paul, 2014).

"Prior to 2020, the NCCN Guidelines for Genetic/Familial High-Risk Assessment: Breast and Ovarian (Breast, Ovarian, and Pancreatic as of 2020) focused largely on testing criteria for BRCA1/2 and appropriate risk management for carriers of a BRCA1 or BRCA2 P/LP variant. Sections on LFS and Cowden syndrome/PTEN hamartoma tumor syndrome (PHTS) were also included. Based on strong evidence that genes beyond *BRCA1/2*, *TP53*, and *PTEN* confer markedly increased risk of breast and/or ovarian cancers, these Guidelines have been expanded" (NCCN, 2024b) to include *BRCA1, BRCA2, CDH1, PALB2, PTEN*, and *TP53* as high-penetrance breast cancer susceptibility genes, as well as some moderate-penetrance susceptibility genes. These susceptibility genes are often found on multi-gene panels, in addition to *BRCA1/2*.

II. Related Policies

Policy Number	Policy Title
AHS-M2004	Lynch Syndrome
AHS-M2020	Molecular Diagnostics for Breast Cancer Prognosis
AHS-M2081	Genetic Testing for Li-Fraumeni Syndrome
AHS-M2087	Genetic Testing for PTEN Hamartoma Tumor Syndrome

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

The following coverage criteria are designed from National Comprehensive Cancer Network (NCCN) guidelines. "NCCN recommendations have been developed to be inclusive of individuals of all sexual and gender identities to the greatest extent possible. In this [policy], the terms males and females refer to sex assigned at birth."

Consideration of both maternal and paternal family histories is necessary in the evaluation of an individual's for risk of carrying a mutation in the BRCA1 or BRCA2 gene; each lineage must be considered separately.

1) For individuals without a diagnosis of a *BRCA*-related cancer who are at least 18 years of age, who have received genetic counseling, and who are in a family with a pathogenic familial gene mutation associated with breast cancer, the following testing **MEETS COVERAGE CRITERIA**:
   a) Testing restricted to the known familial mutation.
   b) Comprehensive genetic testing, including muti-gene panel testing, when the specific familial mutation is unknown.

2) For individuals who have received genetic counseling, multi-gene panel testing (see Note 1, Note 2) for gene mutations associated with breast, ovarian, pancreatic, or prostate cancer **MEETS COVERAGE CRITERIA** when **one** of the following conditions are met:
   a) The individual has been diagnosed with breast cancer **and** meets at least one of the following conditions:
      i) Was diagnosed before 51 years of age.
      ii) Was diagnosed at any age with at least one of the following conditions:
         (a) High-risk (see Note 3), HER2-negative breast cancer.
         (b) Lobular breast cancer with a personal or family history of diffuse gastric cancer.
         (c) Metastatic breast cancer.
         (d) Triple negative breast cancer.
         (e) Two or more primary breast cancer diagnoses.
         (f) Male breast cancer.

(g) Has at least one close blood relative (see Note 4) with **any** of the following:

(i) Breast cancer before 51 years of age.

(ii) Breast cancer at any age in a male.

(iii) Ovarian cancer at any age.

(iv) Pancreatic cancer at any age.

(v) Prostate cancer that is metastatic or a high- or very-high risk group prostate cancer (see Note 5) at any age.

(h) Has a combined total of at least three diagnosed cancers (breast or prostate) in a single lineage of close blood relatives (see Note 4) (including the individual).

iii) Is of Ashkenazi Jewish ancestry

b) The individual has been diagnosed at any age with ovarian cancer (including fallopian tube cancer or peritoneal cancer).

c) The individual has been diagnosed at any age with pancreatic cancer.

d) The individual has been diagnosed at any age with prostate cancer with at least **one** of the following conditions:

i) Has metastatic cancer, the tumor has intraductal/cribriform histology, or a high- or very-high risk group (see Note 5).

ii) Has **any** of the following family history:

(a) Ashkenazi Jewish ancestry.

(b) At least one close blood relative (See Note 4) with **any** of the following:

(i) Breast cancer before 51 years of age.

(ii) Ovarian cancer at any age.

(iii) Pancreatic cancer at any age.

(iv) Prostate cancer that is metastatic, tumor with intraductal/cribriform histology, or a high- or very-high risk group (see Note 5) at any age.

(c) Two or more close blood relatives (See Note 4) with either breast or prostate cancer (any grade) at any age.

e) The individual has a gene mutation associated with breast, ovarian, pancreatic, or prostate cancer that was detected by tumor genomic profiling in the absence of germline mutation testing.

f) The individual meets testing criteria for Li-Fraumeni syndrome (LFS), Lynch syndrome, or Cowden syndrome/PTEN hamartoma tumor syndrome.

3) For individuals with a known family history of *BRCA*-related cancer who have received genetic counseling and are at least 18 years of age, multi-gene panel testing (see Note 1, Note 2) for gene mutations associated with breast, ovarian, pancreatic, or prostate cancer **MEETS COVERAGE CRITERIA** only if the family mutation is unknown (i.e., family member is unavailable for testing or testing results are unavailable) **and** at least one of the following conditions are met:

   a) The individual has at least one first- or second-degree blood relative (see Note 4) meeting any of the above criteria for an individual with cancer, **except as noted below**:

      i) Only first-degree relatives of an individual affected with pancreatic cancer should be offered testing.

   b) The individual has a family member with breast, ovarian, tubal, or peritoneal cancer with positive screening results (5-year probability of 5% or greater) from a tool (see Note 6) designed to identify a family history that may be associated with an increased risk for potentially harmful mutations in breast cancer susceptibility genes (*BRCA1* or *BRCA2*).

4) For individuals 18 years of age or older who are of Ashkenazi Jewish ancestry and who do not meet any of the above criteria, testing for the three known founder mutations (185delAG and 5385insC in *BRCA1*; 6174delT in *BRCA2*) **MEETS COVERAGE CRITERIA.**

5) Testing for gene mutations associated with breast, ovarian, pancreatic, or prostate cancer **DOES NOT MEET COVERAGE CRITERIA** for **any** of the following:

   a) General population screening.

   b) Women diagnosed with breast cancer who are over 65 years of age and who have no close blood relative (see Note 4) with breast, ovarian, pancreatic, or prostate cancer, as there is a low probability that testing will have findings of documented clinical utility.

   c) Individuals diagnosed with localized prostate cancer with Gleason Score <7 and who have no close blood relative (see Note 4) with breast, ovarian, pancreatic, or prostate cancer, as there is a low probability that testing will have findings of documented clinical utility.

   d) In all other situations not specified above.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

6) Testing family members for a variant of uncertain significance **DOES NOT MEET COVERAGE CRITERIA**.

7) For all other purposes, including, but not limited to, testing of the general population, genetic testing for susceptibility to breast, ovarian, pancreatic, or prostate cancer **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

Note 2: When germline multigene panel testing is performed in individuals with breast, ovarian, pancreatic, or prostate cancer, the panel should at minimum include the following high-penetrance breast, ovarian, pancreatic, and prostate cancer susceptibility genes: *ATM, BRCA1, BRCA2, BRIP1, CDH1, CDKN2A, CHEK2, EPCAM, HOXB13, MLH1, MSH2, MSH6, PALB2, PTEN, RAD51C, RAD51D, STK11,* and *TP53*.

Note 3: High-risk disease includes the following:

- The individual has four or more positive lymph nodes.
- The individual has triple negative receptor status cancer.
- The individual has cancer that had an incomplete pathologic response to chemotherapy.

Note 4: Close blood relatives include 1st-degree relatives (e.g., parents, siblings, and children), 2nd-degree relatives (e.g., grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings), and 3rd-degree relatives (great-grandparents, great-aunts, great-uncles, great-grandchildren, and first cousins), all of whom are on the same side of the family.

Note 5: Risk groups are defined in NCCN Guidelines for Prostate Cancer https://www.nccn.org/professionals/physician_gls/pdf/prostate.pdf

Note 6: According to the USPSTF recommendation in 2019, the risk tools evaluated by the USPSTF include the Ontario Family History Assessment Tool, Manchester Scoring System, Referral Screening Tool, Pedigree Assessment Tool, 7-Question Family History Screening Tool, International Breast Cancer Intervention Study instrument (Tyrer-Cuzick),

and brief versions of BRCAPRO. They do not specifically state the preference of one tool over any of the others listed. According to the USPSTF, "these tools should be used to guide referrals to genetic counseling" (USPSTF, 2019).

IV. Table of Terminology

Term	Definition
AA	African American
ACOG	The American College of Obstetricians and Gynecologists
AJ	Ashkenazi Jewish
ASBS	The American Society of Breast Surgeons
ASCO	American Society of Clinical Oncology
ATM	Ataxia telangiectasia mutated
BRCA	*Breast cancer gene*
BRCA1	*Breast cancer gene 1*
BRCA2	*Breast cancer gene 2*
CDH1	*cadherin 1*
CDRH	Center for Devices and Radiological Health
CHEK2	*Checkpoint kinase 2*
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CRPC	Castrate-resistant prostate cancer
dMMR	Mismatch despair deficiency
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetraacetic acid
ER	Estrogen receptor
FANCC	FA complementation group c
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
FGFR2	Fibroblast growth factor receptor 2
GIS	Genomic instability score
HBOC	Hereditary Breast and Ovarian Cancer
HER-2	*Human epidermal growth factor 2*
LDTs	Laboratory-developed tests
LFS	Li-Fraumeni syndrome
MSH2	*Muts homolog 2*
MSI	Microsatellite instability
NBN	*Nibrin*

NCCN	National Comprehensive Cancer Network
NGS	Next-generation sequencing
NICE	National Institute for Health and Care Excellence
OCCR	Ovarian cancer cluster region
PALB2	*Partner and localizer of brca2*
PARP	*Poly ADP-ribose polymerase*
PCR	Polymerase chain reaction
PMS2	Pms1 homolog 2, mismatch repair system component
PR	Progesterone receptor
PTEN	*Phosphatase and tensin homolog*
RAD51C	*Rad51 paralog c*
RAD51D	*RAD51 homolog C*
RECQL	*RecQ Like Helicase*
RET	*Ret proto-oncogene*
SMARCA4	*Swi/snf related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4*
TMB	Tumor mutational burden
TP53	*Tumor protein p53*
USPSTF	The U.S. Preventive Services Task Force

V. Scientific Background

BRCA1 and *BRCA2* are critical genes in the process of homologous recombination repair of double-strand DNA breaks (Walsh, 2015). Both genes are very large (occupying about 70 kb) and encode a combined total of 49 exons. They are considered tumor suppressor genes and a loss of function on either gene increases the cancer risk (Pan & Xie, 2017). *BRCA1* is thought to regulate c-Abl kinase activity (as loss of *BRCA1* results in a constitutively activated c-Abl kinase) whereas *BRCA2* is thought to regulate Rad51, which repairs DNA damage such as chromosomal breaks (Yoshida & Miki, 2004).

Different regions of mutation may confer different types of risk. For example, *BRCA2* has an area called the ovarian cancer cluster region (OCCR) in which mutations predispose the patient for ovarian cancer. Mutations outside the OCCR are more likely to result in breast cancer compared to mutations in the OCCR. On *BRCA1*, mutations closer to the 3' end of the gene may result in higher risk than mutations closer to the 5' end (Meric-Bernstam et al., 2013). Other gene defects that affect homologous recombination include hypermethylation of *RAD51C* or *ATR* mutation. However, these are considered to have a phenotype of "BRCAness" and behave like *BRCA*-deficient genes even if the *BRCA* gene itself is normal (Walsh, 2015).

Although the probability of cancer development in carriers is variable, estimates of penetrance in individuals with a pathogenic variant in *BRCA1* or *BRCA2* range from 46% to 87% lifetime risk for breast cancer, and 16.5% to 63% lifetime risk for ovarian cancer (Petrucelli et al., 2016). *BRCA1* and *BRCA2* mutations account for about 5 – 10% of breast cancers and 10 – 18% of ovarian cancers (Walsh, 2015). *BRCA* mutations are inherited in an autosomal dominant fashion and are highly penetrant (Isaacs & Peshkin, 2024).

It is clinically important to recognize these carriers to guide management of cancer and identify unaffected women with a *BRCA* mutation who will benefit from enhanced surveillance; in addition, recognizing carriers helps physicians tailor care to improve outcomes and more efficiently use health-care resources. Adherence to guidelines for managing cancer risk has the potential to have a significant individual and population health impact on morbidity and mortality (Buchanan et al., 2017). For example, *BRCA* deficient cancers are often targeted for a certain class of drugs called poly(ADP-ribose) polymerase (PARP) inhibitors. These inhibitors target enzymes responsible for the base excision repair pathway. A cell can survive with the loss of either the base excision repair pathway or the homologous recombination mechanism, but not both. Since *BRCA*-deficient cells already have a faulty homologous recombination mechanism, the *BRCA*-deficient cell dies when the PARP inhibitor shuts down the base excision repair pathway. *BRCA*-deficient cells have been shown to be affected 1000 times more by these PARP inhibitors than wild-type cells (Walsh, 2015).

Numerous proprietary tests exist for the assessment of *BRCA* or its related genes such as *RAD51*. For example, gene panels such as Ambry Genetics' panel include 25 genes such as *BRCA1*, *BRCA2*, *CHEK2*, *ATM*, *RAD51C*, and *BRIP1*. This test is performed by next generation sequencing or Sanger sequencing (except for *EPCAM*) with a turnaround time of 2-3 weeks. Ambry has several proprietary tests such BRCAplus and BreastNext (Ambry, 2023). Another gene panel that has been developed to identify genetic mutations associated with inherited breast and ovarian cancers is the AmpliSeq for Illumina *BRCA* Plus, Extended Hereditary Breast and Ovarian Research Panel. This panel assesses germline variants in 11 genes known to harbor mutations related to breast and ovarian cancer:
ATM, *BRCA1*, *BRCA2*, *CHEK2*, *PALB2*, *RAD51C*, *RAD51D*, *NBN*, *CDH1*, *SMARCA4*, and *TP53*. However, though these community panels boasts the convenience of being made-to-order, Illumina warns that they do not have associated performance metrics (Illumina, 2021). myChoice CDx by Myriad Genetics, Inc. is a tumor test that determines homologous recombination deficiency status by detecting *BRCA1* and *BRCA2* (sequencing and large rearrangement) variants. This next generation sequencing-based *in vitro* diagnostic assay focuses on assessing genomic instability by using loss of heterozygosity, telomeric allelic imbalance and large-scale state transitions from

tumor tissue specimens. The results can then be used to guide treatment and therapy for ovarian cancer patients with positive homologous recombination deficiency, which is defined by the presence of *BRCA1/2* mutations and/or positive Genomic Instability Score (Myriad Genetics, 2024).

Clinical Utility and Validity

A study performed by Kuchenbaecker et al. (2017) assessed the cumulative risk of breast and ovarian cancer based on mutation position. A sample of 9856 patients was analyzed, with 6036 patients carrying a *BRCA1* mutation and 3820 with a *BRCA2* mutation. 5046 patients were unaffected by either type of cancer and 4810 had breast cancer, ovarian cancer, or both at baseline. The breast cancer assessment was based on 3886 carriers, and the ovarian cancer assessment was based on 5066 women. The authors evaluated the cumulative risk of breast cancer to 80 years to be 72% for *BRCA1* mutation carriers and 69% for *BRCA2* carriers. Cumulative risk for ovarian cancer to 80 years was found to be 44% for *BRCA1* carriers and 17% for *BRCA2* carriers. *BRCA2* mutations outside the OCCR were found to have a higher risk of breast cancer than mutations inside it (hazard ratio: 1.93 for OCCR ranges 5′ to c.2830, c.2831 to c.6401, c.6402 to 3) but no difference in overall ovarian cancer risk. Mutations closer to the 3′ or 5′ ends of *BRCA1* were found to have a higher risk of breast cancer compared to the middle third of the gene and the third closest to the 3′ end had the highest hazard ratio of 1.51 compared to the third closest to the 5′ end (1.43) (Kuchenbaecker et al., 2017).

A meta-analysis of 44 articles was performed to assess the difference in risk factors between *BRCA1* and *BRCA2* carriers. Factors such as breastfeeding, coffee, infertility, and more were examined between both genotypes, and the only risk factor that revealed an association of any kind was age at first live birth for *BRCA1* carriers. Breast cancer risk was found to decrease for *BRCA1* women over 30 compared to women under 30, and the same was found for women from 25-29 compared to women under 25. However, the authors stressed that more research was required (Friebel et al., 2014).

However, the importance of *BRCA* testing has not only been explored for lifestyle choices or transient states; factors such as ethnicity can also play a role in the predisposition of patients to breast cancer. Palmer et al. (2020) delved into the risks of breast cancer in African American (AA) women associated with inherited mutations in breast cancer predisposition genes. Using germline DNA samples and drawing from 10 epidemiologic studies encompassing 5054 affected African American women and 4993 unaffected African American women, Palmer et al. (2020) sequenced mutations in 23 cancer predisposition genes using a QIAseq multiplex amplicon panel and found that

pathogenic mutations could be identified in 10.3% of women with estrogen receptor (ER)-negative breast cancer, 5.2% of women with ER-positive breast cancer, and 2.3% of unaffected women. Mutations in *BRCA1*, *BRCA2*, and *PALB2* were associated with an overall increased risk for breast cancer, while *RAD51D* mutations were observed specifically to be linked to higher risk of ER-negative disease. Other mutations the researchers found to be of interest were in *CHEK2*, *ATM*, *ERCC3*, *FANCC,* and *RECQL*. Thus, it was concluded that the study corroborated the use and "validity of current breast cancer testing panels for use in AA women" (Palmer et al., 2020).

A study using next generation sequencing (NGS) to identify *BRCA* mutations was performed by Lang et al. 4034 patients were screened (2991 breast cancer patients, 1043 healthy controls). *BRCA* mutations were found in 247 of the breast cancer patients or 8.3%. 13.9% (16/115) of the *BRCA1* mutations were of the "c.5470_5477del" variation, and several clinical characteristics such as high KI67 index and high tumor grade were related to *BRCA* mutations, *BRCA2* carriers were also found to have poorer disease-free survival among HER2 positive patients (Lang et al., 2017).

Tomao et al. (2019) investigated the ability of *BRCA* mutational status on predicting hematologic toxicity with platinum-based chemotherapy. 176 patients were included, with 58 *BRCA* mutation carriers (40 *BRCA1,* 18 *BRCA2*, 118 controls). The authors identified several differences in hematologic toxicity between the two groups; the *BRCA* positive group was observed to have significantly higher frequency in "thrombocytopenia (24% vs 5%), anemia (21% vs 7%; p = 0.006) and neutropenia (62% vs 27%)". The authors also noted that granulocyte-colony stimulating growth factors injection (12% versus 1%,) and dose delay (19% versus 27%) were more likely in the *BRCA* positive group (odds ratio = 2.567 for granulocyte-colony stimulating growth factors injection and 3.860 for dose delay). Overall, the authors concluded that "germline *BRCA* ½ mutations are associated with a higher hematologic toxicity in patients with ovarian cancer who underwent platinum-based chemotherapy" (Tomao et al., 2019).

Yoo et al. (2020) conducted *BRCA1/NGS* for 262 hereditary breast and ovarian cancer (HBOC) syndrome patients, and the results were confirmed by using multiplex ligation-dependent probe amplification and direct Sanger sequencing. A multigene panel test was also performed on 120 patients who did not possess *BRCA1/2* pathogenic variants but who met NCCN criteria for testing. The researchers reported that pathogenic variants in *BRCA1/2* were detected in 30 HBOC patients (11.5%), and four out of the 120 patients possessed pathogenic variants of *MSH2 PMS2, CHEK2* and *PALB2*, which were also detectable by multigene panel testing. The results suggested to the authors that "Multi-gene panel testing could be a significant screening tool for HBOC patients, especially for those with a family history of cancer" (Yoo et al., 2020).

BRCA testing has been demonstrated to be potentially beneficial, even when the testing is unselected and is population-based. Manchanda et al. (2020) examined the North London Ashkenazi-Jewish (AJ) population in a randomized controlled trial consisting of 1034 AJ women and men across two arms—one, a population-screening approach, and a second, a family history/clinical-criteria-based *BRCA* testing—to determine subsequent effects on psychological health and quality of life after providing genetic testing for three Jewish *BRCA* founder-mutations. Based on the results of the study, the researchers drew the conclusion that "Population-based AJ *BRCA* testing does not adversely affect long-term psychological wellbeing or quality-of-life, decreases anxiety and could identify up to 150% additional *BRCA* carriers" (Manchanda et al., 2020). However, these results on the anxiety and health-anxiety of this population may be contested, for validated questionnaires were used to measure the psychological wellbeing of the participants at baseline/1-year/2-year/3-year follow-ups. Moreover, the participants were recruited through self-referral, which may affect the internal validity of the trials.

VI. Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

NCCN guidelines titled *Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic Version 3.2024* address general hereditary cancer testing. Note that NCCN recommendations have been developed to be inclusive of individuals of all sexual and gender identities to the greatest extent possible. In this guideline, the terms males and females refer to sex assigned at birth. The NCCN list the following scenarios as "clinically indicated" for genetic testing:

1. "Individual with any blood relative with a known pathogenic/likely pathogen variant in a cancer susceptibility gene" [including *BRCA1/2*]
2. "Individuals meeting the criteria below but tested negative with previous limited testing, (eg, single gene and/or absent deletion duplication analysis) and are interested in pursuing multi-gene testing"
3. "A pathogenic/likely pathogenic variant identified on tumor genomic testing that has clinical implications if also identified in the germline"
4. "To aid in systemic therapy and surgical decision-making"
5. "Individual who meets Li-Fraumeni syndrome (LFS) testing criteria or Cowden syndrome/PTEN hamartoma tumor syndrome testing criteria or Lynch syndrome"
6. *Personal or family history of cancer*
    a. **Testing for high-penetrance breast cancer susceptibility genes** (Specifically *BRCA1, BRCA2, CDH1, PALB2, PTEN, STK11* and *TP53*)**:**

i. Personal history of breast cancer with specific features:
   a. By Age of diagnosis and family history
      (a) Diagnosed at age ≤50y
      (b) Diagnosed at any age:
         - Treatment indications:
            (i) To aid in systematic treatment decisions using PARP inhibitors for breast cancer in the metastatic setting
            (ii) To aid in adjuvant treatment decisions with Olaparib for high-risk, HER-2 negative breast cancer
         - Pathology/histology:
            (iii) Triple-negative breast cancer
            (iv) Multiple primary breast cancers (synchronous or metasynchronous)
            (v) Lobular breast cancer with personal or family history of diffuse gastric cancer
         - Family history:
            (vi) ≥1 close blood relative with ANY:
               - Breast cancer at age ≤50y
               - Male breast cancer
               - Ovarian cancer
               - Pancreatic cancer
               - Prostate cancer with metastatic or high- or very-high-risk group
            (vii) ≥3 total diagnoses of breast and/or prostate cancer (any grade) on the same side of the family including the patient with breast cancer.
         - Ancestry:
            (viii) Ashkenazi Jewish Ancestry
         - Male breast cancer
ii. Family history of cancer only
   a. Individuals affected with breast cancer (not meeting testing criteria listed above) or individual unaffected with breast cancer with a first- or second-degree blood relative meeting any of the criteria listed above (except unaffected individuals whose relatives meet criteria only for systemic therapy decision-making).
   b. Individuals affected or unaffected with breast cancer who otherwise do not meet the criteria above but have a probability >5% of a BRCA1/2 P/LP variant based on prior probability models (eg, Tyrer-Cuzick, BRCAPro,

CanRisk)

b. **Testing for ovarian cancer susceptibility genes**
   i. Personal history of epithelial ovarian cancer (including fallopian tube cancer or peritoneal cancer) at any age
   ii. Family history of cancer only
      a. An individual unaffected with ovarian cancer with a first- or second-degree blood relative with epithelial ovarian cancer (including fallopian tube cancer or peritoneal cancer) at any age
      b. An individual unaffected with ovarian cancer who otherwise does not meet the criteria above but has a probability >5% of a BRCA1/2 pathogenic variant based on prior probability models (eg, Tyrer-Cuzick, BRCAPro, CanRisk)

c. **Testing for pancreatic cancer susceptibility genes**
   i. Exocrine pancreatic cancer
      a. All individuals diagnosed with exocrine pancreatic cancer
      b. First-degree relatives of individuals diagnosed with exocrine pancreatic cancer

d. **Testing for high-penetrance prostate cancer susceptibility genes**
   i. Personal history of prostate cancer with specific features:
      a. By tumor characteristics (any age)
         (a) Metastatic
         (b) Histology
            (i) High- or very-high-risk group
      b. By family history and ancestry
         (a) ≥1 close blood relative with:
            (i) breast cancer at age ≤50 y
            (ii) triple-negative breast cancer at any age
            (iii) male breast cancer at any age
            (iv) ovarian cancer any age
            (v) pancreatic cancer any age
            (vi) metastatic, or high- or very-high risk group at any age
         (b) ≥3 close blood relatives with prostate cancer (any grade) and/or breast cancer on the same side of the family including the patient with prostate cancer
         (c) Ashkenazi Jewish ancestry
      c. Family history of cancer only

(a) An affected (not meeting testing criteria listed above) or unaffected individual with a first-degree blood relative meeting any of the criteria listed above (except unaffected individuals whose relatives meet criteria only for systemic therapy decision-making)

e. **Testing for Li-Fraumeni Syndrome**
   i. Individual from a family with a known TP53 pathogenic/likely pathogenic variant
   ii. Classic Li-Fraumeni syndrome (LFS) criteria:
      a. Combination of an individual diagnosed at age <45 years with a sarcoma AND
      b. A first-degree relative diagnosed at age <45 years with cancer AND
      c. An additional first- or second-degree relative in the same lineage with cancer diagnosed at age <45 years, or a sarcoma at any age
   iii. Chompret criteria:
      a. Individual with a tumor from LFS tumor spectrum (eg, soft tissue sarcoma, osteosarcoma, CNS tumor, breast cancer, adrenocortical carcinoma), before 46 years of age, AND at least one first- or second-degree relative with any of the aforementioned cancers (other than breast cancer if the proband has breast cancer) before the age of 56 years or with multiple primaries at any age OR
      b. Individual with multiple tumors (except multiple breast tumors), two of which belong to LFS tumor spectrum with the initial cancer occurring before the age of 46 years OR
      c. Individual with adrenocortical carcinoma, or choroid plexus carcinoma or rhabdomyosarcoma of embryonal anaplastic subtype, at any age of onset, regardless of family history OR
      d. Breast cancer before 31 years of age
   iv. Personal or family history of pediatric hypodiploid acute lymphoblastic leukemia
   v. In individuals with cancer with a pathogenic/likely pathogenic *TP53* variant identified on tumor-only genomic testing, germline testing should be considered for: (1) Those meeting one or more of the other LFS testing criterion above after reevaluation of personal and family history (2) those diagnosed age <30 years with any cancer (3) those with clinical scenario not meeting these criteria but warranting germline evaluation per clinician discretion.

f. **Testing for Cowden Syndrome (CS)/PTEN Hamartoma Tumor Syndrome (PHTS):**
   i. Individual from a family with a known PTEN pathogenic/likely pathogenic variant
   ii. Individual with a personal history of Bannayan-Riley-Ruvalcaba syndrome (BRRS)
   iii. Individual meeting clinical diagnostic criteria for CS/PHTS
   iv. Individual not meeting clinical diagnostic criteria for CS/PHTS with a personal history of:
      a. Adult Lhermitte-Duclos disease (cerebellar tumors); or
      b. Autism spectrum disorder and macrocephaly; or
      c. Two or more biopsy-proven trichilemmomas; or
      d. Two or more major criteria (one must be macrocephaly); or
      e. Three major criteria, without macrocephaly; or
      f. One major and ≥3 minor criteria; or
      g. ≥4 minor criteria
   v. Individual with a relative with a clinical diagnosis of CS/PHTS or BRRS for whom testing has not been performed
      a. The individual must have the following:
         (a) Any one major criterion or
            (i) Breast cancer
            (ii) Endometrial cancer
            (iii) Follicular thyroid cancer
            (iv) Multiple GI hamartomas or ganglioneuromas
            (v) Macrocephaly (megalocephaly) (ie, ≥97%, 58 cm in adult female, 60 cm in adult male)
            (vi) Macular pigmentation of glans penis
            (vii) Mucocutaneous lesions
               1) One biopsy-proven trichilemmoma
               2) Multiple palmoplantar keratoses
               3) Multifocal or extensive oral mucosal papillomatosis
               4) Multiple cutaneous facial papules (often verrucous)
         (b) Two minor criteria
            (i) Autism spectrum disorder
            (ii) Colon cancer
            (iii) ≥3 esophageal glycogenic acanthoses
            (iv) Lipomas

(v) Intellectual disability (ie, IQ ≤75)
(vi) Papillary or follicular variant of papillary thyroid cancer
(vii) Thyroid structural lesions (eg, adenoma, nodule[s], goiter)
(viii) Renal cell carcinoma
(ix) Single GI hamartoma or ganglioneuroma
(x) Testicular lipomatosis
(xi) Vascular anomalies (including multiple intracranial developmental venous anomalies)

vi. PTEN pathogenic/likely pathogenic variant detected by tumor genomic testing on any tumor type in the absence of germline analysis

The NCCN states that general hereditary cancer testing may be considered in the following scenario (with appropriate pre-test education and access to post-test management):

- An individual of Ashkenazi Jewish ancestry without additional risk factors.
- Personal history of serious endometrial cancer.

The NCCN states that testing for high-penetrance breast cancer susceptibility genes may be considered in the following scenarios (with appropriate pre-test education and access to post-test management):

1. Personal history of breast cancer < 60 y not meeting any of the above criteria may approach a 2.5% probability of having a pathogenic/likely pathogenic variant, based on recent data. It is cautioned that many of those PVs will be in moderate penetrance genes, which are over-represented in older affected individuals. Access to an experienced genetic counseling team to discuss management options is particularly important in this setting.
2. Personal history of breast cancer diagnosed at any age with ≥1 close blood relative with intermediate-risk prostate cancer with intraductal/cribriform histology.
3. Individuals affected or unaffected with breast cancer who otherwise do not meet any of the above criteria but with a 2.5%-5% probability of *BRCA 1/2* pathogenic variant/likely pathogenic variant based on prior probability models (e.g. Tyrer-Cuzick, BRCAPro, CanRisk).

The NCCN states that when testing for high-penetrance breast cancer susceptibility genes, there is "a low probability (<2.5%) that testing will have findings of documented high-penetrance genes in the following scenarios:

1. Female diagnosed with breast cancer at age >60 y, with no close relative with breast, ovarian, pancreatic, or prostate cancer
2. Diagnosed with localized prostate cancer with Gleason Score <7 and no close relative with breast, ovarian, pancreatic, or prostate cancer" (NCCN, 2024b).

The NCCN suggests that prior to genetic testing,

- "The probability of pathogenic or likely pathogenic variant detection associated with these criteria will vary based on family structure, which includes size of the family, age of the family members, early death, adoption, and number of male and female relatives. Individuals with unknown or limited family history/structure, such as fewer than 2 female first- or second-degree relatives having lived beyond age 45 in either lineage, may have an underestimated probability of familial P/LP variant detection. The estimated likelihood of P/LP variant detection may be low in families with many unaffected and/or male relatives.
- Patients who have received an allogeneic bone marrow transplant or with active or recent hematologic malignancies should not have molecular genetic testing via blood, saliva, or buccal samples (due to unreliable test results from contamination by donor DNA) until other technologies are available. If available, DNA should be extracted from a fibroblast culture. If this source of DNA is not possible, buccal samples can be considered, subject to the risk of donor DNA contamination or malignant cells from the hematologic malignancy.
- If more than one family member is affected with cancers highly associated with a particular inherited cancer susceptibility syndrome, consider initial testing of a family member with youngest age at diagnosis, bilateral disease, multiple primary cancers, or other cancers associated with the syndrome, or most closely related to the proband/patient. If there are no available family members with cancer that is a cardinal feature of the syndrome in question, consider testing first- or second-degree family members affected with other cancers thought to be related to the gene in question (eg, prostate or pancreas with *BRCA1/2*).
- Testing for unaffected family members when no affected member is available should be considered. Significant limitations of interpreting test results should be discussed.
- In children <18 y, genetic testing is generally not recommended when results would not impact medical management.
- Likely pathogenic variants are usually clinically managed similarly to pathogenic variants, while patients with variants of uncertain significance (VUS) and likely

benign variants should be managed based on the cancers present in the family.
- Choice of multi-gene testing" (NCCN, 2024b).

Furthermore, in the situation where the presence of a pathogenic or likely pathogenic variant is unknown, the NCCN recommends that "the testing of the unaffected individual (or of unaffected family members) should only be considered when no affected family member is available for testing. In such cases, the unaffected individual or unaffected close relative with the highest likelihood of testing positive for the pathogenic or likely pathogenic variant should be tested," though "A negative test result in such cases, however, is considered indeterminate and does not provide the same level of information as when there is known pathogenic or likely pathogenic variant in the family." The NCCN also remarks that "testing multiple family members may be indicated" when testing unaffected individuals "(in the absence of having tested affected family members)" to aid in interpreting results (NCCN, 2024b).

The NCCN also recommends assessing *BRCA1/2* in all patients with recurrent or metastatic breast cancer to identify candidates for PARP inhibitor therapy (NCCN, 2024a).

Regarding *BRCA* in ovarian cancer, the NCCN recommends testing for *BRCA1/2* mutations prior to initiating treatment for persistent/recurrent ovarian cancer since "germline and/or somatic *BRCA1/2* status informs maintenance therapy." The NCCN notes that *BRCA* testing may be done prior to this stage (NCCN, 2024c).

BRCA testing was also mentioned in guidelines for pancreatic adenocarcinoma. The NCCN recommends tumor/somatic gene profiling for those with "locally advanced/metastatic disease who are candidates for anti-cancer therapy to identify uncommon mutations, including testing for mutations in *BRAF, BRCA1/2, HER2, KRAS,* and *PALB2* genes, fusions in *ALK, NRG1, NTRK, ROS1, FGFR2, RET* genes, microsatellite instability (MSI), mismatch repair deficiency *(dMMR*) , or tumor mutational burden (TMB) via an FDA-approved and/or validated next-generation sequencing (NGS)-based assay" (NCCN, 2024d).

The NCCN also published guidelines regarding *BRCA* in prostate cancer. Germline genetic testing, which should include *BRCA1/2* among other genes, such as *ATM, PALB2,* and *CHEK2* was recommended for initial patients with prostate cancer and any of the following: "a positive family history; high-risk, very-high-risk, regional, or metastatic prostate cancer, regardless of family history; Ashkenazi Jewish ancestry; [and] a personal history of breast cancer." The NCCN also notes that "germline genetic testing should be considered in patients with a personal history of prostate cancer and 1) intermediate-risk prostate cancer and intraductal/cribriform histology or 2) a

personal history of exocrine pancreatic cancer, breast cancer, colorectal, gastric, melanoma, pancreatic cancer, upper tract urothelial cancer, glioblastoma, biliary tract cancer, and small intestinal cancer" Moreover, the NCCN asserts that germline testing should include "*MLH1, MSH2, MSH6, and PMS2* (for Lynch syndrome) and homologous recombination genes *BRCA1, BRCA2, ATM, PALB2, and CHEK2*", urging that cancer predisposition next-generation sequencing be considered (NCCN, 2024e).

Regarding somatic tumor testing in risk groups,

- "Tumor testing for somatic homologous recombination gene mutations (eg, *BRCA1, BRCA2, ATM, PALB2, FANCA, RAD51D, CHEK2, CDK12*) can be considered in patients with regional (N1) prostate cancer and is recommended for those with metastatic disease.
- Tumor testing for MSI or dMMR can be considered in patients with regional or metastatic castration-naïve prostate cancer and is recommended in the metastatic castrate-resistant prostate cancer (CRPC) setting.
- Tumor mutational burden (TMB) testing may be considered in patients with metastatic CRPC.
- Multigene molecular testing can be considered for patients with low-, intermediate-, and high-risk prostate cancer and life expectancy ≥10 years.
- The Decipher molecular assay is recommended to inform adjuvant treatment if adverse features are found post-radical prostatectomy and can be considered as part of counseling for risk stratification in patients with PSA resistance/recurrence after radical prostatectomy (category 2B)" (NCCN, 2024e).

The NCCN published information on *TP53* as a pathogenic/likely pathogenic variant, noting that testing for Li-Fraumeni syndrome should occur when the individual is from a family with a known *TP53* pathogenic/likely pathogenic variant. They specifically note that when this gene is "included as part of a multi-gene panel, an individual does not need to meet these testing criteria [for Li-Fraumeni syndrome]- if "testing criteria on other testing criteria pages are met" (NCCN, 2024b).

The U.S. Preventive Services Task Force (USPSTF)

In 2019, the USPSTF updated their 2014 recommendation (Moyer, 2014). In it, they state that "the USPSTF recommends that primary care clinicians assess women with a personal or family history of breast, ovarian, tubal, or peritoneal cancer or who have an ancestry associated with breast cancer susceptibility 1 and 2 (*BRCA1*/2) gene mutations with an appropriate brief familial risk assessment tool. Women with a positive result on the risk assessment tool should receive genetic counseling and, if indicated after counseling, genetic testing." This recommendation is intended for women with a "personal or family history of breast, ovarian, tubal, or peritoneal cancer or an ancestry

associated with *BRCA1*/2 gene mutation" (USPSTF, 2019).

Moreover, they do not recommend (i.e. issue a D recommendation) routine screening, genetic testing, or genetic counseling for women who have no family or personal history of breast cancer or whose ancestry or ethnicity is not associated with a higher risk for potentially pathogenic *BRCA1* or *BRCA2* gene mutations (USPSTF, 2019).

The American College of Obstetricians and Gynecologists (ACOG)

The ACOG released guidelines in 2019 for Hereditary Cancer Syndromes and Risk Assessments. The ACOG recommends:

- Evaluating a patient's risk of hereditary breast and ovarian cancer syndrome should be a routine part of obstetric and gynecologic practice. Initial risk evaluation should include a personal medical history and family history.
- Genetic testing is recommended when the results of a detailed risk assessment that is performed as part of genetic counseling suggest the presence of an inherited cancer syndrome for which specific genes have been identified and when the results of testing are likely to influence medical management.
- The two main genetic testing options for hereditary breast and ovarian cancer syndrome are *BRCA* mutation testing and multigene panel testing that includes both *BRCA* and other genetic mutations. Multigene panel testing may be useful when more than one gene may be associated with an inherited cancer syndrome or when a patient has a personal or family history that is consistent with an inherited cancer susceptibility, but single-gene testing has not identified a pathogenic variant (ACOG, 2019).

The American Society of Breast Surgeons (ASBS)

The American Society of Breast Surgeons have released guidelines on genetic testing for hereditary breast cancer. They are as follows:

1. "Breast surgeons, genetic counselors, and other medical professionals knowledgeable in genetic testing can provide patient education and counseling and make recommendations to their patients regarding genetic testing and arrange testing"
2. "Genetic testing should be made available to all patients with a personal history of breast cancer. Recent data support that genetic testing should be offered to each patient with breast cancer (newly diagnosed or with a personal history). If

genetic testing is performed, such testing should include *BRCA1*/*BRCA2* and *PALB2*, with other genes as appropriate for the clinical scenario and family history. For patients with newly diagnosed breast cancer, identification of a mutation may impact local treatment"

3. "Patients who had genetic testing previously may benefit from updated testing. Every patient being seen by a breast surgeon, who had genetic testing in the past and no pathogenic variant was identified, should be re-evaluated and updated testing considered. In particular, a patient who had negative germline *BRCA1* and 2 testing, who is from a family with no pathogenic variants, should be considered for additional testing.1 Genetic testing performed prior to 2014 most likely would not have had PALB2 or other potentially relevant genes included and may not have included testing for large genomic rearrangements in *BRCA1* or *BRCA2*"
4. "Genetic testing should be made available to patients without a history of breast cancer who meet NCCN guidelines. Unaffected patients should be informed that testing an affected relative first, whenever possible, is more informative than undergoing testing themselves. When it is not feasible to test the affected relative first, then the unaffected family member should be considered for testing if they are interested, with careful pre-test counseling to explain the limited value of "uninformative negative" results. It is also reasonable to order a multi-gene panel if the family history is incomplete (i.e., a case of adoption, patient is uncertain of exact type of cancer affecting family members, among others) or other cancers are found in the family history, as described above" (Manahan et al., 2019).

American Society of Clinical Oncology (ASCO)

ASCO recommends germline genetic testing for *BRCA1/2* for all women diagnosed with epithelial ovarian cancer. Somatic tumor testing for *BRCA1/2* should be performed in women that do not carry a germline pathogenic or likely pathogenic variant (Konstantinopoulos et al., 2020).

ASCO also published a guideline regarding PARP inhibitors for ovarian cancer. In recommendation 2.2, they recommend the use of "Myriad myChoice CDx" to determine *BRCA1/2* status for therapy decisions (Tew et al., 2020).

In 2024, ASCO published "Germline Testing in Patients With Breast Cancer: ASCO-Society of Surgical Oncology Guideline" with the following recommendations:

- "BRCA1/2 mutation testing should be offered to all newly diagnosed patients with breast cancer ≤65 years and select patients >65 years based on personal history, family history, ancestry, or eligibility for poly(ADP-ribose) polymerase (PARP) inhibitor therapy."
- "All patients with recurrent breast cancer who are candidates for PARP inhibitor therapy should be offered BRCA1/2 testing, regardless of family history. BRCA1/2 testing should be offered to women who develop a second primary cancer in the ipsilateral or contralateral breast."
- "For patients with prior history of breast cancer and without active disease, testing should be offered to patients diagnosed ≤65 years and selectively in patients diagnosed after 65 years, if it will inform personal and family risk."
- "Testing for high-penetrance cancer susceptibility genes beyond BRCA1/2 should be offered to those with supportive family histories; testing for moderate-penetrance genes may be offered if necessary to inform personal and family cancer risk."
- "Patients should be provided enough pretest information for informed consent; those with pathogenic variants should receive individualized post-test counseling. Variants of uncertain significance should not impact management, and patients with such variants should be followed for reclassification."
- "Referral to providers experienced in clinical cancer genetics may help facilitate patient selection and interpretation of expanded testing, and provide counseling of individuals without pathogenic germline variants but with significant family history" (Bedrosian et al., 2024).

National Institute for Health and Care Excellence (NICE)

NICE updated their guidelines on familial breast cancer in 2019. In it, they maintain their *BRCA*-related recommendations from 2013, which are as follows:

"Offer genetic testing in specialist genetic clinics to a relative with a personal history of breast and/or ovarian cancer if that relative has a combined *BRCA1* and *BRCA2* mutation carrier probability of 10% or more."

"Offer genetic testing in specialist genetic clinics to a person with no personal history of breast or ovarian cancer if their combined *BRCA1* and *BRCA2* mutation carrier probability is 10% or more and an affected relative is unavailable for testing."

"Offer genetic testing in specialist genetic clinics to a person with breast or ovarian

cancer if their combined *BRCA1* and *BRCA2* mutation carrier probability is 10% or more" (NICE, 2019).

European Expert Group

A group of 19 experts in *BRCA* testing were convened to publish this set of guidelines. These experts came from across Europe and Israel, and participants included clinical or medical geneticists (32%), oncologists (37%), and gynaecologists (26%).

The guidelines state that with the rise of next-generation sequencing, hotspot testing instead of complete sequencing is "not acceptable," albeit noting a possible exception of founder mutations representing >99% of pathogenic variants in a specific area.

A majority of experts (60%) voted that *BRCA* testing should be offered to all patients with metastatic breast cancer (Singer et al., 2019).

In 2021, the group published guidance on *BRCA* and HRR deficiency testing for recently diagnosed patients with ovarian cancer. Consensus statements pertaining to *BRCA1/2* include (not an all-inclusive list):

1. "Tumour *BRCA1/2* testing should be carried out at primary diagnosis.
2. Tumour *BRCA1/2* testing should be carried out at disease recurrence (if not carried out earlier)
3. Germline and/or tumour *BRCA* testing should be used (in either order) to determine *BRCA* status at primary diagnosis" (Vergote et al., 2022).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

The Center for Devices and Radiological Health of the Food and Drug Administration (FDA, 2018) granted premarket approval on January 12,2018 to BRACAnalysis CDx®,

an in vitro diagnostic device intended for the qualitative detection and classification of variants in the protein coding regions and intron/exon boundaries of the *BRCA1* and *BRCA2* genes using genomic DNA obtained from whole blood specimens collected in EDTA. Single nucleotide variants and small insertions and deletions (indels) are identified by polymerase chain reaction (PCR) and Sanger sequencing. Large deletions and duplications in *BRCA1* and *BRCA2* are detected using multiplex PCR. Another FDA-approved device is the "FoundationFocus CDxBRCA", which is a "is a next generation sequencing based in vitro diagnostic device for qualitative detection of *BRCA1* and *BRCA2* alterations in formalin-fixed paraffin-embedded (FFPE) ovarian tumor tissue." This test is intended to be used "as an aid in identifying ovarian cancer patients for whom treatment with Rubraca (rucaparib) is being considered" (FDA, 2016). A more recent FDA-approved device comes out of Myriad Genetics, Inc., the myChoice HRD CDx, which was approved on October 23, 2019. This test is a "next generation sequencing-based in vitro diagnostic test that assesses the qualitative detection and classification of single nucleotide variants, insertions and deletions, and large rearrangement variants in protein coding regions and intron/exon boundaries of the BRCA1 and BRCA2 genes and the determination of Genomic Instability Score (GIS)" based off tumor tissue specimens.

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81162	BRCA1 (BRCA1, DNA repair associated), BRCA2 (BRCA2, DNA repair associated) (e.g., hereditary breast and ovarian cancer) gene analysis; full sequence analysis and full duplication/deletion analysis (i.e., detection of large gene rearrangements)
81163	BRCA1 (BRCA1, DNA repair associated), BRCA2 (BRCA2, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; full sequence analysis
81164	BRCA1 (BRCA1, DNA repair associated), BRCA2 (BRCA2, DNA repair associated) (e.g., hereditary breast and ovarian cancer) gene analysis; full duplication/deletion analysis (i.e., detection of large gene rearrangements)
81165	BRCA1 (BRCA1, DNA repair associated) (e.g., hereditary breast and ovarian cancer) gene analysis; full sequence analysis

CPT	Code Description
81166	BRCA1 (BRCA1, DNA repair associated) (e.g., hereditary breast and ovarian cancer) gene analysis; full duplication/deletion analysis (i.e., detection of large gene rearrangements)
81167	BRCA2 (BRCA2, DNA repair associated) (e.g., hereditary breast and ovarian cancer) gene analysis; full duplication/deletion analysis (i.e., detection of large gene rearrangements)
81212	BRCA1 (BRCA1, DNA repair associated), BRCA2 (BRCA2, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; 185delAG, 5385insC, 6174delT variants
81215	BRCA1 (BRCA1, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; known familial variant
81216	BRCA2 (BRCA2, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; full sequence analysis
81217	BRCA2 (BRCA2, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; known familial variant
81432	Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer); genomic sequence analysis panel, must include sequencing of at least 10 genes, always including BRCA1, BRCA2, CDH1, MLH1, MSH2, MSH6, PALB2, PTEN, STK11, and TP53
81433	Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer); duplication/deletion analysis panel, must include analyses for BRCA1, BRCA2, MLH1, MSH2, and STK11
81479	Unlisted molecular pathology procedure
0102U	Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with MRNA analytics to resolve variants of unknown significance when indicated (17 genes [sequencing and deletion/duplication]) Proprietary test: BreastNext® Lab/Manufacturer: Ambry Genetics®
0103U	Hereditary ovarian cancer (eg, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with MRNA analytics to resolve variants of unknown significance when indicated (24 genes [sequencing and deletion/duplication], EPCAM [deletion/duplication only]) Proprietary test: OvaNext® Lab/Manufacturer: Ambry Genetics®

CPT	Code Description
0129U	Hereditary breast cancer–related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis and deletion/duplication analysis panel (ATM, BRCA1, BRCA2, CDH1, CHEK2, PALB2, PTEN, and TP53) Proprietary test: BRCAplus Lab/Manufacturer: Ambry Genetics
0172U	Oncology (solid tumor as indicated by the label), somatic mutation analysis of BRCA1 (BRCA1, DNA repair associated), BRCA2 (BRCA2, DNA repair associated) and analysis of homologous recombination deficiency pathways, DNA, formalin-fixed paraffin-embedded tissue, algorithm quantifying tumor genomic instability score Proprietary test: myChoice® CDx Lab/Manufacturer: Myriad Genetics Laboratories, Inc
0474U	Hereditary pan-cancer (eg, hereditary sarcomas, hereditary endocrine tumors, hereditary neuroendocrine tumors, hereditary cutaneous melanoma), genomic sequence analysis panel of 88 genes with 20 duplications/deletions using nextgeneration sequencing (NGS), Sanger sequencing, blood or saliva, reported as positive or negative for germline variants, each gene Proprietary test: GeneticsNow® Comprehensive Germline Panel Lab/Manufacturer: GoPath Diagnostics, Inc
0475U	Hereditary prostate cancerrelated disorders, genomic sequence analysis panel using next-generation sequencing (NGS), Sanger sequencing, multiplex ligation-dependent probe amplification (MLPA), and array comparative genomic hybridization (CGH), evaluation of 23 genes and duplications/deletions when indicated, pathologic mutations reported with a genetic risk score for prostate cancer Proprietary test: ProstateNowTM Prostate Germline Panel Lab/Manufacturer: GoPath Diagnostics, Inc

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

ACOG. (2019). Hereditary Cancer Syndromes and Risk Assessment. *The American College of Obstetricians and Gynecologists*. https://www.acog.org/clinical/clinical-guidance/committee-opinion/articles/2019/12/hereditary-cancer-syndromes-and-risk-assessment

Ambry. (2023). *Ambry Genetics*. https://www.ambrygen.com/providers/oncology/test-menu#breast

Bedrosian, I., Somerfield, M. R., Achatz, M. I., Boughey, J. C., Curigliano, G., Friedman, S., Kohlmann, W. K., Kurian, A. W., Laronga, C., Lynce, F., Norquist, B. S., Plichta, J. K., Rodriguez, P., Shah, P. D., Tischkowitz, M., Wood, M., Yadav, S., Yao, K., & Robson, M. E. (2024). Germline Testing in Patients With Breast Cancer: ASCO–Society of Surgical Oncology Guideline. *Journal of Clinical Oncology*, *42*(5), 584-604. https://doi.org/10.1200/jco.23.02225

Buchanan, A. H., Voils, C. I., Schildkraut, J. M., Fine, C., Horick, N. K., Marcom, P. K., Wiggins, K., & Skinner, C. S. (2017). Adherence to Recommended Risk Management among Unaffected Women with a BRCA Mutation. *J Genet Couns*, *26*(1), 79-92. https://doi.org/10.1007/s10897-016-9981-6

FDA. (2016). *FoundationFocus CDxBRCA* https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm?db=pma&id=389050

FDA. (2018). *Premarket Approval (PMA) 	BRACAnalysis CDx* https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P140020s012

Friebel, T. M., Domchek, S. M., & Rebbeck, T. R. (2014). Modifiers of cancer risk in BRCA1 and BRCA2 mutation carriers: systematic review and meta-analysis. *J Natl Cancer Inst*, *106*(6), dju091. https://doi.org/10.1093/jnci/dju091

Illumina. (2021). *AmpliSeq for Illumina BRCA Plus, Extended Hereditary Breast and Ovarian Research Panel*. Illumina, Inc. Retrieved 2/12 from https://www.illumina.com/products/by-brand/ampliseq/community-panels/brca-plus-extended-hereditary-breast-ovarian.html

Isaacs, C., & Peshkin, B. N. (2024, January 29, 2024). *Cancer risks and management of BRCA1/2 carriers without cancer*. https://www.uptodate.com/contents/cancer-risks-and-management-of-brca1-2-carriers-without-cancer

Konstantinopoulos, P. A., Norquist, B., Lacchetti, C., Armstrong, D., Grisham, R. N., Goodfellow, P. J., Kohn, E. C., Levine, D. A., Liu, J. F., Lu, K. H., Sparacio, D., & Annunziata, C. M. (2020). Germline and Somatic Tumor Testing in Epithelial Ovarian Cancer: ASCO Guideline. *Journal of Clinical Oncology*, JCO.19.02960. https://doi.org/10.1200/JCO.19.02960

Kuchenbaecker, K. B., Hopper, J. L., Barnes, D. R., Phillips, K. A., Mooij, T. M., Roos-Blom, M. J., Jervis, S., van Leeuwen, F. E., Milne, R. L., Andrieu, N., Goldgar, D. E., Terry, M. B., Rookus, M. A., Easton, D. F., Antoniou, A. C., McGuffog, L., Evans, D. G., Barrowdale, D., Frost, D., . . . Olsson, H. (2017). Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. *Jama*, *317*(23), 2402-2416. https://doi.org/10.1001/jama.2017.7112

Lang, G. T., Shi, J. X., Hu, X., Zhang, C. H., Shan, L., Song, C. G., Zhuang, Z. G., Cao, A. Y., Ling, H., Yu, K. D., Li, S., Sun, M. H., Zhou, X. Y., Huang, W., & Shao, Z. M. (2017). The spectrum of BRCA mutations and characteristics of BRCA-associated breast cancers in China: Screening of 2,991 patients and 1,043 controls by next-generation sequencing. *Int J Cancer*, *141*(1), 129-142. https://doi.org/10.1002/ijc.30692

Manahan, E. R., Kuerer, H. M., Sebastian, M., Hughes, K. S., Boughey, J. C., Euhus, D. M., Boolbol, S. K., & Taylor, W. A. (2019). Consensus Guidelines on Genetic Testing for Hereditary Breast Cancer from the American Society of Breast Surgeons. *Ann Surg Oncol*, *26*(10), 3025-3031. https://doi.org/10.1245/s10434-019-07549-8

Manchanda, R., Burnell, M., Gaba, F., Desai, R., Wardle, J., Gessler, S., Side, L., Sanderson, S., Loggenberg, K., Brady, A. F., Dorkins, H., Wallis, Y., Chapman, C., Jacobs, C., Legood, R., Beller, U., Tomlinson, I., Menon, U., & Jacobs, I. (2020). Randomised trial of population-based BRCA testing in Ashkenazi Jews: long-term outcomes. *Bjog*, *127*(3), 364-375. https://doi.org/10.1111/1471-0528.15905

Meric-Bernstam, F., Gutierrez-Barrera, A. M., Litton, J., Mellor-Crummey, L., Ready, K., Gonzalez-Angulo, A. M., Lu, K. H., Hortobagyi, G. N., & Arun, B. K. (2013). Genotype in BRCA-associated breast cancers. *Breast J*, *19*(1), 87-91. https://doi.org/10.1111/tbj.12056

Moyer, V. A. (2014). Risk assessment, genetic counseling, and genetic testing for BRCA-related cancer in women: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med*, *160*(4), 271-281. https://doi.org/10.7326/m13-2747

Myriad Genetics, I. (2024). *What is Myriad myChoice® CDx?* Myriad Genetics, Inc. Retrieved 4/4/2024 from https://myriad.com/products-services/precision-medicine/mychoice-cdx/

NCCN. (2024a, March 11, 2024). *Breast Cancer, Version 2.2024*. Retrieved 4/4/2024 from https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf

NCCN. (2024b, February 12, 2024). *Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic Version 3.2024*. NCCN. Retrieved 4/4/2024 from https://www.nccn.org/professionals/physician_gls/pdf/genetics_bop.pdf

NCCN. (2024c, May 13, 2024). *Ovarian Cancer Including Fallopian Tube Cancer and Peritoneal Cancer Version 2.2024 — May 13, 2024*. NCCN. Retrieved 4/4/2024 from https://www.nccn.org/professionals/physician_gls/pdf/ovarian.pdf

NCCN. (2024d, April 30, 2024). *Pancreatic adenocarcinoma Version 2.2024 — April 30, 2024*. Retrieved 4/4/2024 from https://www.nccn.org/professionals/physician_gls/pdf/pancreatic.pdf

NCCN. (2024e, May 17, 2024). *Prostate Cancer Version 4.2024 — May 17, 2024*. NCCN. Retrieved 4/4/2024 from https://www.nccn.org/professionals/physician_gls/pdf/prostate.pdf

NICE. (2019). *Familial breast cancer: classification, care and managing breast cancer and related risks in people with a family history of breast cancer*.

https://www.nice.org.uk/guidance/cg164/chapter/Recommendations#genetic-testing

Palmer, J. R., Polley, E. C., Hu, C., John, E. M., Haiman, C., Hart, S. N., Gaudet, M., Pal, T., Anton-Culver, H., Trentham-Dietz, A., Bernstein, L., Ambrosone, C. B., Bandera, E. V., Bertrand, K. A., Bethea, T. N., Gao, C., Gnanaolivu, R. D., Huang, H., Lee, K. Y., . . . Couch, F. J. (2020). Contribution of Germline Predisposition Gene Mutations to Breast Cancer Risk in African American Women. *J Natl Cancer Inst*, *112*(12), 1213-1221. https://doi.org/10.1093/jnci/djaa040

Pan, Z., & Xie, X. (2017). BRCA mutations in the manifestation and treatment of ovarian cancer. *Oncotarget*, *8*(57), 97657-97670. https://doi.org/10.18632/oncotarget.18280

Paul, A., & Paul, S. (2014). The breast cancer susceptibility genes (BRCA) in breast and ovarian cancers. *Front Biosci (Landmark Ed)*, *19*, 605-618. https://doi.org/10.2741%2F4230

Petrucelli, N., Daly, M., & Pal, T. (2016). *BRCA1- and BRCA2-Associated Hereditary Breast and Ovarian Cancer* [Text]. https://pubmed.ncbi.nlm.nih.gov/20301425/

Roy, R., Chun, J., & Powell, S. N. (2012). BRCA1 and BRCA2: different roles in a common pathway of genome protection. *Nat Rev Cancer*, *12*(1), 68-78. https://doi.org/10.1038/nrc3181

Singer, C. F., Balmaña, J., Bürki, N., Delaloge, S., Filieri, M. E., Gerdes, A.-M., Grindedal, E. M., Han, S., Johansson, O., Kaufman, B., Krajc, M., Loman, N., Olah, E., Paluch-Shimon, S., Plavetic, N. D., Pohlodek, K., Rhiem, K., Teixeira, M., & Evans, D. G. (2019). Genetic counselling and testing of susceptibility genes for therapeutic decision-making in breast cancer—an European consensus statement and expert recommendations. *European Journal of Cancer*, *106*, 54-60. https://doi.org/10.1016/j.ejca.2018.10.007

Tew, W. P., Lacchetti, C., Ellis, A., Maxian, K., Banerjee, S., Bookman, M., Jones, M. B., Lee, J. M., Lheureux, S., Liu, J. F., Moore, K. N., Muller, C., Rodriguez, P., Walsh, C., Westin, S. N., & Kohn, E. C. (2020). PARP Inhibitors in the Management of Ovarian Cancer: ASCO Guideline. *J Clin Oncol*, *38*(30), 3468-3493. https://doi.org/10.1200/jco.20.01924

Tomao, F., Musacchio, L., Di Mauro, F., Boccia, S. M., Di Donato, V., Giancotti, A., Perniola, G., Palaia, I., Muzii, L., & Benedetti Panici, P. (2019). Is BRCA mutational status a predictor of platinum-based chemotherapy related hematologic toxicity in high-grade serous ovarian cancer patients? *Gynecol Oncol*, *154*(1), 138-143. https://doi.org/10.1016/j.ygyno.2019.04.009

USPSTF. (2019, August 20). *BRCA-Related Cancer: Risk Assessment, Genetic Counseling, and Genetic Testing*. Retrieved 02/27/2019 from https://www.uspreventiveservicestaskforce.org/uspstf/document/RecommendationStatementFinal/brca-related-cancer-risk-assessment-genetic-counseling-and-genetic-testing

Vergote, I., González-Martín, A., Ray-Coquard, I., Harter, P., Colombo, N., Pujol, P., Lorusso, D., Mirza, M. R., Brasiuniene, B., Madry, R., Brenton, J. D., Ausems, M. G. E. M., Büttner, R., Lambrechts, D., Vergote, I., Ausems, M., Brasiuniene, B., Brenton, J., Büttner, R., . . . Witteveen, E. (2022). European experts consensus: BRCA/homologous recombination deficiency testing in first-line ovarian cancer. *Annals of Oncology*, *33*(3), 276-287. https://doi.org/10.1016/j.annonc.2021.11.013

Walsh, C. S. (2015). Two decades beyond BRCA1/2: Homologous recombination, hereditary cancer risk and a target for ovarian cancer therapy. *Gynecol Oncol*, *137*(2), 343-350. https://doi.org/10.1016/j.ygyno.2015.02.017

Yoo, J., Lee, G. D., Kim, J. H., Lee, S. N., Chae, H., Han, E., Kim, Y., & Kim, M. (2020). Clinical Validity of Next-Generation Sequencing Multi-Gene Panel Testing for Detecting Pathogenic Variants in Patients With Hereditary Breast-Ovarian Cancer Syndrome. *Ann Lab Med*, *40*(2), 148-154. https://doi.org/10.3343/alm.2020.40.2.148

Yoshida, K., & Miki, Y. (2004). Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage. *Cancer Sci*, *95*(11), 866-871. https://pubmed.ncbi.nlm.nih.gov/15546503/

Genetic Testing for CHARGE Syndrome

Policy Number: AHS – M2070 – Genetic Testing for CHARGE Syndrome	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

CHARGE (coloboma, heart defects, atresia choanae, growth retardation, genital abnormalities, and ear abnormalities) syndrome is a multiple congenital anomaly condition affected by mutations in the *CHD7* gene (Hsu et al., 2014). The majority of these mutations result in a wide range of congenital anomalies that include colobomas (congenital absence of pieces of tissue in eye structures that may cause defects in the iris, retina, or optic nerve); heart defects; choanal atresia (an obliteration or blockage of the posterior nasal aperture due to a persistent oronasal membrane that prevents joining of the nose and oropharynx); retarded growth and development; genital hypoplasia; ear anomalies; and deafness (Guercio & Martyn, 2007; Isaacson, 2022; Jongmans et al., 2006).

II. Related Policies

Policy Number	Policy Title
AHS-M2145	General Genetic Testing, Germline Disorders

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) To confirm a diagnosis in a patient with signs/symptoms of CHARGE (coloboma, heart defects, atresia choanae, growth retardation, genital abnormalities, and ear abnormalities) syndrome when a definitive diagnosis cannot be made with clinical criteria, genetic testing for the presence of *CHD7* variants **MEETS COVERAGE CRITERIA**.

2) For asymptomatic individuals who have a first-degree relative (see Note 1) diagnosed with CHARGE syndrome who have a known likely pathogenic or

pathogenic variant, genetic testing restricted to the known familial *CHD7* variant **MEETS COVERAGE CRITERIA**.

3) For individuals seeking prenatal or pre-implantation screening, genetic testing for the presence of *CHD7* variants **MEETS COVERAGE CRITERIA**.

4) For individuals with clinical features of CHARGE syndrome who have already tested negative for likely pathogenic or pathogenic variants in *CHD7*, screening for variants in *ZEB2, KMT2D* and *EFTUD2* **MEETS COVERAGE CRITERIA.**

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

5) For all other situations not discussed above, genetic testing for CHARGE syndrome **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: First-degree relatives include parents, full siblings, and children of the individual.

IV. Table of Terminology

Term	Definition
ATP	Adenosine triphosphate
CHARGE	Coloboma, heart defects, atresia choanae, growth retardation, genital abnormalities, and ear abnormalities
CHD7	*Chromodomain helicase deoxyribonucleic acid binding protein 7*
CHH	Congenital hypogonadotropic hypogonadism
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CN	Cranial nerve
CN I	Cranial nerve absent or reduced sense of smell
CN IX, X	Cranial nerve swallowing problem
CN V	Cranial nerve weak chewing/swallowing
CN VII	Cranial nerve facial palsy
CN VIII	Cranial nerve sensorineural hearing loss and balance/ vestibular problems
DNA	Deoxyribonucleic acid
EFTUD2	*Elongation factor Tu GTP binding domain containing 2*
ENT	Ear, nose, and throat

EP300	*E1A binding protein p300*
FDA	Food and Drug Administration
GI	Gastrointestinal
GnRH	*Gonadotropin-releasing hormone*
HH	Hypogonadotropic hypogonadism
KMT2D	*Lysine methyltransferase 2D*
LDTs	Laboratory-developed tests
MLPA	Multiplex ligation-dependent probe amplification
NGS	Next-generation sequencing
NIPT	Non-invasive prenatal test
NORD	National Organization for Rare Disorders
PUF60	*Poly(U) binding splicing factor 60*
RERE	*Arginine-glutamic acid dipeptide repeats*
SNP	Single nucleotide polymorphism
SWI-SNF	Switch/sucrose non-fermentable
TBX1	*T-box transcription factor 1*
ZEB2	*Zinc finger E-box binding homeobox 2*

V. Scientific Background

CHARGE (coloboma, heart defects, atresia choanae, growth retardation, genital abnormalities, and ear abnormalities) syndrome is a relatively common cause of congenital anomalies affecting approximately 1 in 8,500 to 10,000 births (Longman, 2018). First described by Hall (1979) and Hittner et al. (1979), CHARGE syndrome was diagnosed clinically (Blake et al., 1998; Pagon et al., 1981) until causative mutations were identified in the *CHD7* (Chromodomain-helicase-DNA-binding protein 7/ATP-dependent helicase CHD7) gene (Vissers et al., 2004). Due to the variability associated with *CHD7* mutations, genetic analysis may be helpful for genotypic diagnostics but will not necessarily assist in phenotypic predictions (Bergman et al., 2011). Most cases of CHARGE syndrome occur through spontaneous mutation of the *CHD7* gene; however, the disorder can also be passed from parent to offspring in an autosomal dominant fashion (Usman & Sur, 2024).

The *CHD7* gene contains 38 exons that encode for the 300-kDa CHD7 chromatin remodeler protein (Bilan et al., 2012). The CHD7 protein is a member of the SWI-SNF superfamily of ATP-dependent chromatin remodelers that bind to DNA and modulate gene expression (Asad et al., 2016; Marfella & Imbalzano, 2007). CHD7 has an important, dosage-dependent role in the development of several craniofacial tissues (Sperry et al., 2014) and has also been found to assist with orchestrating neural crest

and central nervous system development (Bajpai et al., 2010; He et al., 2016; Van Nostrand et al., 2014; Whittaker et al., 2017). Further, *CHD7* plays a role in additional gene expression programs and cellular interactions during embryogenesis; this likely occurs through the dysregulation of co-transcriptional alternative splicing (Belanger et al., 2018; Berube-Simard & Pilon, 2018; Schulz et al., 2014).

It is worth noting that the CHARGE syndrome acronym does not cover all disorders that may result from this disease; a diagnosis may include additional sensory deficits and birth defects, including cranial nerve dysfunction and feeding and gastrointestinal (GI) dysfunction (Blake & Hudson, 2017). It is notable that more than 90% of patients experience feeding and GI dysfunction; this is known to cause significant morbidity and mortality in the CHARGE syndrome patient population (Blake & Hudson, 2017; Hefner & Fassi, 2017). Further, many CHARGE syndrome patients exhibit clival pathology, such as coronal clefts; this is now considered a useful diagnostic criteria for patients (Mahdi & Whitehead, 2018). Nonetheless, the range of mutations in the *CHD7* gene results in a broad phenotype that may involve almost all organ and sensory systems in the body, therefore causing significant variabilities in severity and comorbidity (de Geus et al., 2017). Hence, no single feature is universally present or sufficient for the clinical diagnosis of CHARGE syndrome.

Clinical Validity

The initial clinical CHARGE syndrome diagnostic criteria (Blake et al., 1998) was first adapted to include supplemental clinical abnormalities (Verloes, 2005). More recently, the diagnostic criteria were updated to incorporate results of molecular testing (Hale et al., 2016a). Most individuals (90-95%) fulfilling the clinical criteria for a CHARGE syndrome diagnosis have a *CHD7* variant that is detectable by Sanger sequencing or next-generation sequencing (NGS) (Bergman et al., 2011; Janssen et al., 2012). However, since the inclusion of *CHD7*, variants have been described in 14-17% of mildly affected individuals who would not meet the clinical criteria for a CHARGE syndrome diagnosis (Bergman et al., 2011). This has resulted in the addition of *CHD7* to NGS gene panels for developmental delay, colobomata, heart defects (Corsten-Janssen et al., 2014), and other congenital malformations (van Ravenswaaij-Arts & Martin, 2017). The clinical validity of genetic testing that relies on identifying *CHD7* gene mutations may create issues in the future; van Ravenswaaij-Arts and Martin (2017) stated that individuals with a missense variant of the *CHD7* gene will less often fulfill clinical criteria for a CHARGE syndrome diagnosis, since there may be a decreased prevalence of congenital heart defects and choanal atresia with a missense variant. However, this type of variant is overrepresented in families with parent to child transmission of CHARGE syndrome (van Ravenswaaij-Arts & Martin, 2017).

Despite the availability of molecular diagnostic tools, "the cause of CHARGE syndrome remains unclear in approximately 5-10% of typical CHARGE patients and in 40-60% of suspected cases" (Janssen et al., 2012). Other genetic conditions such as 22q11.2 deletion (DiGeorge) syndrome, Kallmann syndrome, and Kabuki syndrome are known to have an overlapping phenotypic spectrum with CHARGE syndrome (Janssen et al., 2012), which may complicate diagnosis based strictly on clinical criteria. Additionally, it is challenging to distinguish younger patients with Kabuki syndrome from those with CHARGE syndrome since they lack the facial gestalt of Kabuki syndrome but show similar organ malformations to those of CHARGE syndrome patients (Pauli et al., 2017).

A more recent study utilized whole exome sequencing to genetically analyze 28 individuals exhibiting CHARGE syndrome features. Pathogenic variants in *CHD7*, other genes (*RERE, KMT2D, EP300, PUF60)*, and no pathogenic variants were found in 53.6%, 14.3%, and 28.6% of participants, respectively (Moccia et al., 2018). Based on these results, it was suggested that "the phenotypic features of CHARGE syndrome overlap with multiple other rare single-gene syndromes" (Moccia et al., 2018).

In a study by Gonçalves et al. (2019), mutations in the *CHD7* gene were observed in patients with isolated congenital hypogonadotropic hypogonadism (CHH), a condition that is characterized by the lack of normal pubertal development resulting from deficient gonadotropin-releasing hormone (GnRH). This demonstrates a limitation to clinical validity in *CHD7* genetic testing for CHARGE syndrome. The variable phenotypic expression is related to the type of mutation, as CHARGE syndrome patients seem to have "typically highly deleterious protein-truncating mutations, whereas *CHD7* mutations in isolated CHH are typically missense" (Gonçalves et al., 2019).

A study conducted by Qin et al. (2020) also found five neonatal patients to have drastically different clinical CHARGE syndrome phenotypes, with postnatal dyspnea as the most prominent symptom in the study cohort. The study found three novel genetic variants (c.2828_2829delAG, c.4667dupC, and c.7873C > T) and two reported variants (c.4667dupC and c.1480C > T) using whole exome sequencing that contributed to CHARGE syndrome clinical presentations. In accordance with this data, researchers concluded that though prenatal diagnosis of CHARGE syndrome may continue to be a challenge, "fetal *de novo* mutations screening by non-invasive prenatal test (NIPT) with maternal plasma is highly efficient for diagnosis. Detection of mutations in E1 and E38 may also provide clues for predicting severity of CHARGE syndrome by NIPT with maternal plasma" (Qin et al., 2020).

Another study was completed with data from 145 participants, all of whom were previously clinically diagnosed with CHARGE syndrome. Researchers surveyed these

participants to determine if they had completed genetic testing to confirm a CHARGE syndrome diagnosis. Of the total survey participants, 68% had never received genetic testing; of the 46 patients who did complete genetic testing, 74% tested positive for a *CHD7* mutation (Hartshorne et al., 2011).

Clinical Utility and Validity

Patients with CHARGE syndrome experience a wide spectrum of comorbidities, some more severe than others, and the complex management of these comorbidities can often lead to more issues. The clinical utility of making a definite diagnosis of CHARGE syndrome is high since a confirmed CHARGE diagnosis will lead to changes in clinical management, including well-defined clinical assessment and treatment recommendations (de Geus et al., 2017; Trider et al., 2017). No consensus on the utility of genetic testing in patients who present with a clear clinical diagnosis exists. However, testing may be useful in patients who do not have the classical CHARGE characteristics and may be at risk for the long-term complications of CHARGE syndrome (Blake et al., 2011). For instance, many patients with CHARGE syndrome will often have more than one dysfunctional cranial nerve (CN), which can manifest as an absent or reduced sense of smell (CN I), weak chewing/swallowing (CN V), facial palsy (CN VII), sensorineural hearing loss (CN VIII), balance/vestibular problems (CN VIII), and swallowing problems (CN IX, X) (Hudson et al., 2017). Testing is recommended in all suspected cases of CHARGE syndrome, especially in patients who partially meet the clinical criteria (Bergman et al., 2011; Hale et al., 2016a; Trider et al., 2017).

Hefner and Fassi (2017) state that a CHARGE syndrome diagnosis "should be considered in patients with any of the major diagnostic features: coloboma, choanal atresia, semicircular canal anomalies, or cranial nerve anomalies." These features are also common in 22q11.2 deletion (DiGeorge) and Kabuki syndromes, and genetic testing may be used to distinguish between these conditions; further, genetic counseling is an important step in a CHARGE syndrome diagnosis (Hefner & Fassi, 2017). This will prove to be critical in establishing a multidisciplinary care team for potential developmental concerns of a CHARGE syndrome child, such as combined deafness-blindness (Hudson et al., 2017). As CHARGE patients grow up, they may have feeding difficulties or orofacial anomalies that may need to be attended to by ENT specialists, cardiovascular malformations that may involve pediatric cardiologists, or concomitant hypogonadotropic hypogonadism (HH) that may require the help of pediatric endocrinologists, supporting the high clinical utility of *CHD7* testing of CHARGE syndrome (Dijk et al., 2019).

VI. Guidelines and Recommendations

The CHARGE Syndrome Foundation

The CHARGE Syndrome Foundation states that CHARGE syndrome is marked by key features such as coloboma, cranial nerve abnormalities, choanal atresia, heart defects, characteristic external ears, esophageal defects, small/absent semicircular canals, genitourinary abnormalities, and *CHD7* gene mutations, and that its "diagnosis should be made by a Medical Geneticist. Diagnosis is based on key features, ideally with DNA testing for *CHD7* mutations." Though "It does not usually run in families," the "Recurrence risk to unaffected parents is 1-2%" and "If a parent has CHARGE Syndrome, the risk to a baby is 50/50" (CHARGE Syndrome Foundation, 2024).

The National Organization for Rare Disorders (NORD)

NORD states that "Diagnosis can be confirmed by molecular genetic testing identifying the variants in the *CHD7* gene associated with the condition. If no disease-causing variants are found, a SNP chromosomal microarray should be done, because in a few patients, there has been a submicroscopic change in the chromosome 8q12.2 region. If both these tests are negative, whole genome exome sequencing should be done, since other genetic disorders share some clinical features with CHARGE syndrome, and new variants in the *ZEB2, KMT2D* and *EFTUD2* genes have been found in children previously diagnosed as having CHARGE syndrome" (National Organization for Rare Disorders, 2024).

Other Recommendations

Guidelines by professional societies and organizations about genetic testing for CHARGE syndrome are limited; however, recommendations by subject matter experts in the field are included below.

A comprehensive guideline and clinical checklist were developed by the Atlantic Canadian CHARGE syndrome team. This checklist includes diagnostic criteria such as clinical diagnoses and genetic testing; genetic consultation for *CHD7* analysis and array comparative genomic hybridization is also recommended. Further, the guideline notes that although "there is no consensus on genetic testing in the presence of a clear clinical diagnosis," multiple guidelines recommend genetic testing in "all suspected cases of CHARGE syndrome and especially for patients who partially meet the clinical criteria" (Trider et al., 2017).

According to guidelines published by researchers at The Children's Mercy Hospitals and Clinics in Kansas City, Missouri, a previously unknown missense mutation in exon 31 of *CHD7* can cause a diagnosis of CHARGE syndrome. This mutation can be inherited, showing that family history should be considered as a major diagnostic criterion for CHARGE syndrome (Hughes et al., 2014). Moreover, because orofacial

clefting is often observed with a diagnosis of CHARGE syndrome, it is also suggested that patients with this anomaly be tested for CHARGE syndrome (Hughes et al., 2014).

Guidelines published by de Geus et al. (2017) provide a comprehensive overview of all other published recommendations for CHARGE syndrome and introduce guidelines for cranial imaging. A summary of their recommendations is included in the table below (de Geus et al., 2017).

Recommendation	References
CHARGE is a clinical diagnosis	(Bergman et al., 2011; Blake et al., 1998; Harris et al., 1997; Issekutz et al., 2005; Verloes, 2005)
***CHD7* testing can confirm uncertain diagnosis in mildly affected patients**	(Bergman et al., 2011)
***CHD7* testing may be performed according to a flow diagram**	(Bergman et al., 2011)
A genome-wide array should be performed in patients with CHARGE syndrome but without a *CHD7* mutation	(Corsten-Janssen et al., 2013)
Clinical genetics consultation is indicated, including options for prenatal diagnosis	(Bergman et al., 2011; Lalani et al., 2012)
Patients diagnosed with hypogonadotropic hypogonadism and anosmia should be screened for clinical features consistent with CHARGE syndrome	(Jongmans et al., 2009)
Olfactory bulb hypoplasia and semicircular canal aplasia should be considered major signs for CHARGE syndrome	(Asakura et al., 2008; Sanlaville et al., 2006)
If a parent has any features of CHARGE syndrome, molecular genetic testing is appropriate if a *CHD7* pathogenic variant has been identified in the proband	(Jongmans et al., 2008)
***CHD7* analysis should be performed in patients with a 22q11.2 deletion**	(Corsten-Janssen et al., 2013)

phenotype without *TBX1* haploinsufficiency	
***CHD7* analysis should be performed in patients with Kallmann syndrome who have at least two additional CHARGE features or semicircular canal anomalies**	(Bergman et al., 2012; Costa-Barbosa et al., 2013; Jongmans et al., 2009)
***CHD7* should be included in massive parallel sequencing gene panels for diagnostics in syndromic heart defects**	(Corsten-Janssen et al., 2014)
***CHD7* analysis should not be performed routinely in patients with only atrial septal defect or conotruncal heart defects**	(Corsten-Janssen et al., 2014)
***CHD7* analysis should not be performed in septo-optic dysplasia patients without features of CHARGE**	(Gregory et al., 2013)
MLPA analysis is indicated if no causal *CHD7* is mutation is found	(Wincent et al., 2008; Wincent et al., 2009)
MLPA analysis is not indicated if no *CHD7* mutation is found	(Bergman et al., 2008)

Guidelines for clinical diagnosis have also been published by Hale et al. (2016a), which include the identification of a pathogenic *CHD7* variant as major criteria for a CHARGE syndrome diagnosis. In a response to comments received on their publication by (Blake et al., 2011), Hale and colleagues reaffirmed the appropriateness of *CHD7* testing under the right circumstances. They state "there are specific (and extremely useful) guidelines for when to test for *CHD7* sequence variants in individuals with CHARGE features (Bergman et al., 2011). Accurate and meaningful genetic information can lead to improved understanding of etiology, provide accurate recurrence risks, and help pave the way toward better clinical care. We advocate incorporating *CHD7* sequence variant information into the diagnostic algorithm, when it is available, since this information can improve understanding of disease causation, pathogenesis, and treatment options. In cases when *CHD7* variant testing is not available, the diagnosis can still be made based on appropriate clinical assessments" (Hale et al., 2016b).

Bergman et al. (2011) asserted that *CHD7* testing can confirm uncertain diagnoses in mildly affected patients. Moreover, a clinical genetics consultation is also indicated, including options for prenatal diagnosis.

Corsten-Janssen et al. (2014) published recommendations which state that:

- *CHD7* should be included in massive parallel sequencing gene panels for diagnostics in syndromic heart defects
- *CHD7* analysis should be performed in patients with a 22q11.2 deletion phenotype without *TBX1* haploinsufficiency
- Genome-wide array should be performed in patients with CHARGE syndrome but without a *CHD7* mutation

Jongmans et al. (2008) and Jongmans et al. (2009) recommended that:

- Patients diagnosed with hypogonadotropic hypogonadism and anosmia should be screened for clinical features consistent with CHARGE syndrome
- If a parent has any features of CHARGE syndrome, molecular genetic testing is appropriate if a *CHD7* pathogenic variant has been identified in the proband
- *CHD7* analysis should be performed in patients with Kallmann syndrome who have at least two additional CHARGE features or semicircular canal anomalies

Usman and Sur (2024) compiled guidelines for the diagnosis of CHARGE syndrome that state that the "only gene associated with CHARGE syndrome is *CHD7*, encrypting the chromodomain helicase DNA binding protein. This sequencing detects pathogenic variants in maximum individuals with typical CHARGE syndrome with the following criteria of having the three primary characteristics or four major and three minor characteristics." The major criteria are the 4C's: coloboma, cranial nerve abnormalities, choanal atresia, and typical CHARGE ear. The minor criteria are heart defects, cleft lip or palate, genital abnormalities, hypotonia, kidney abnormalities, esophageal atresia, poor growth, typical CHARGE face, and typical CHARGE hand. The authors summarize the outline of diagnosis as:

- "Clinical diagnosis: It is a combination of major and minor diagnostic characteristics, having the three primary features or four major and three minor characteristics."
- "Laboratory analysis: It includes having the blood workup done, such as complete blood count (CBC), serum electrolytes, renal function test, luteinizing hormone-releasing hormone, Human chorionic gonadotropin (hCG), blood urea nitrogen (BUN), creatinine, growth hormone levels, and immunologic studies."
- "Genetic analysis: Prenatal screening for *CHD7* variants is restricted to familial cases, via amniocentesis chorionic or villus sampling at 10–12 and 18–20 weeks' gestation."
- "Imaging studies: Involves a skeletal survey, abdominal ultrasound, barium swallow, echocardiography, chest x-ray, cranial ultrasound in neonates, and head computed tomography (CT) scan and magnetic resonance imaging (MRI)" (Usman & Sur, 2024).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81403	Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)
81404	Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81407	Molecular pathology procedure, level 8 (eg, analysis of 26-50 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of >50 exons, sequence analysis of multiple genes on one platform)
81479	Unlisted molecular pathology procedure

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Asad, Z., Pandey, A., Babu, A., Sun, Y., Shevade, K., Kapoor, S., Ullah, I., Ranjan, S., Scaria, V., Bajpai, R., & Sachidanandan, C. (2016). Rescue of neural crest-derived phenotypes in a zebrafish CHARGE model by Sox10 downregulation. *Hum Mol Genet*, *25*(16), 3539-3554. https://doi.org/10.1093/hmg/ddw198

Asakura, Y., Toyota, Y., Muroya, K., Kurosawa, K., Fujita, K., Aida, N., Kawame, H., Kosaki, K., & Adachi, M. (2008). Endocrine and radiological studies in patients with molecularly confirmed CHARGE syndrome. *J Clin Endocrinol Metab*, *93*(3), 920-924. https://doi.org/10.1210/jc.2007-1419

Bajpai, R., Chen, D. A., Rada-Iglesias, A., Zhang, J., Xiong, Y., Helms, J., Chang, C. P., Zhao, Y., Swigut, T., & Wysocka, J. (2010). CHD7 cooperates with PBAF to control multipotent neural crest formation. *Nature*, *463*(7283), 958-962. https://doi.org/10.1038/nature08733

Belanger, C., Berube-Simard, F. A., Leduc, E., Bernas, G., Campeau, P. M., Lalani, S. R., Martin, D. M., Bielas, S., Moccia, A., Srivastava, A., Silversides, D. W., & Pilon, N. (2018). Dysregulation of cotranscriptional alternative splicing underlies CHARGE syndrome. *Proc Natl Acad Sci U S A*, *115*(4), E620-E629. https://doi.org/10.1073/pnas.1715378115

Bergman, J. E., de Ronde, W., Jongmans, M. C., Wolffenbuttel, B. H., Drop, S. L., Hermus, A., Bocca, G., Hoefsloot, L. H., & van Ravenswaaij-Arts, C. M. (2012). The results of CHD7 analysis in clinically well-characterized patients with Kallmann syndrome. *J Clin Endocrinol Metab*, *97*(5), E858-862. https://doi.org/10.1210/jc.2011-2652

Bergman, J. E., de Wijs, I., Jongmans, M. C., Admiraal, R. J., Hoefsloot, L. H., & van Ravenswaaij-Arts, C. M. (2008). Exon copy number alterations of the CHD7 gene are not a major cause of CHARGE and CHARGE-like syndrome. *Eur J Med Genet*, *51*(5), 417-425. https://doi.org/10.1016/j.ejmg.2008.03.003

Bergman, J. E., Janssen, N., Hoefsloot, L. H., Jongmans, M. C., Hofstra, R. M., & van Ravenswaaij-Arts, C. M. (2011). CHD7 mutations and CHARGE syndrome: the clinical implications of an expanding phenotype. *J Med Genet*, *48*(5), 334-342. https://doi.org/10.1136/jmg.2010.087106

Berube-Simard, F. A., & Pilon, N. (2018). Molecular dissection of CHARGE syndrome highlights the vulnerability of neural crest cells to problems with alternative splicing and other transcription-related processes. *Transcription*, 1-8. https://doi.org/10.1080/21541264.2018.1521213

Bilan, F., Legendre, M., Charraud, V., Maniere, B., Couet, D., Gilbert-Dussardier, B., & Kitzis, A. (2012). Complete screening of 50 patients with CHARGE syndrome for anomalies in the CHD7 gene using a denaturing high-performance liquid

chromatography-based protocol: new guidelines and a proposal for routine diagnosis. *J Mol Diagn*, *14*(1), 46-55. https://doi.org/10.1016/j.jmoldx.2011.08.003

Blake, K., van Ravenswaaij-Arts, C. M., Hoefsloot, L., & Verloes, A. (2011). Clinical utility gene card for: CHARGE syndrome. *Eur J Hum Genet*, *19*(9). https://doi.org/10.1038/ejhg.2011.45

Blake, K. D., Davenport, S. L., Hall, B. D., Hefner, M. A., Pagon, R. A., Williams, M. S., Lin, A. E., & Graham, J. M., Jr. (1998). CHARGE association: an update and review for the primary pediatrician. *Clin Pediatr (Phila)*, *37*(3), 159-173. https://doi.org/10.1177/000992289803700302

Blake, K. D., & Hudson, A. S. (2017). Gastrointestinal and feeding difficulties in CHARGE syndrome: A review from head-to-toe. *Am J Med Genet C Semin Med Genet*, *175*(4), 496-506. https://doi.org/10.1002/ajmg.c.31586

CHARGE Syndrome Foundation. (2024). *CHARGE Syndrome Fact Sheet*. CHARGE Syndrome Foundation. https://www.chargesyndrome.org/for-families/new-diagnosis/factsheet/

Corsten-Janssen, N., du Marchie Sarvaas, G. J., Kerstjens-Frederikse, W. S., Hoefsloot, L. H., van Beynum, I. M., Kapusta, L., & van Ravenswaaij-Arts, C. M. (2014). CHD7 mutations are not a major cause of atrioventricular septal and conotruncal heart defects. *Am J Med Genet A*, *164A*(12), 3003-3009. https://doi.org/10.1002/ajmg.a.36747

Corsten-Janssen, N., Saitta, S. C., Hoefsloot, L. H., McDonald-McGinn, D. M., Driscoll, D. A., Derks, R., Dickinson, K. A., Kerstjens-Frederikse, W. S., Emanuel, B. S., Zackai, E. H., & van Ravenswaaij-Arts, C. M. (2013). More Clinical Overlap between 22q11.2 Deletion Syndrome and CHARGE Syndrome than Often Anticipated. *Mol Syndromol*, *4*(5), 235-245. https://doi.org/10.1159/000351127

Costa-Barbosa, F. A., Balasubramanian, R., Keefe, K. W., Shaw, N. D., Al-Tassan, N., Plummer, L., Dwyer, A. A., Buck, C. L., Choi, J. H., Seminara, S. B., Quinton, R., Monies, D., Meyer, B., Hall, J. E., Pitteloud, N., & Crowley, W. F., Jr. (2013). Prioritizing genetic testing in patients with Kallmann syndrome using clinical phenotypes. *J Clin Endocrinol Metab*, *98*(5), E943-953. https://doi.org/10.1210/jc.2012-4116

de Geus, C. M., Free, R. H., Verbist, B. M., Sival, D. A., Blake, K. D., Meiners, L. C., & van Ravenswaaij-Arts, C. M. A. (2017). Guidelines in CHARGE syndrome and the missing link: Cranial imaging. *Am J Med Genet C Semin Med Genet*, *175*(4), 450-464. https://doi.org/10.1002/ajmg.c.31593

Dijk, D. R., Bocca, G., & van Ravenswaaij-Arts, C. M. (2019). Growth in CHARGE syndrome: optimizing care with a multidisciplinary approach. *J Multidiscip Healthc*, *12*, 607-620. https://doi.org/10.2147/jmdh.S175713

Gonçalves, C. I., Patriarca, F. M., Aragüés, J. M., Carvalho, D., Fonseca, F., Martins, S., Marques, O., Pereira, B. D., Martinez-de-Oliveira, J., & Lemos, M. C. (2019). High

frequency of CHD7 mutations in congenital hypogonadotropic hypogonadism. *Scientific Reports*, *9*(1), 1597. https://doi.org/10.1038/s41598-018-38178-y

Gregory, L. C., Gevers, E. F., Baker, J., Kasia, T., Chong, K., Josifova, D. J., Caimari, M., Bilan, F., McCabe, M. J., & Dattani, M. T. (2013). Structural pituitary abnormalities associated with CHARGE syndrome. *J Clin Endocrinol Metab*, *98*(4), E737-743. https://doi.org/10.1210/jc.2012-3467

Guercio, J. R., & Martyn, L. J. (2007). Congenital malformations of the eye and orbit. *Otolaryngol Clin North Am*, *40*(1), 113-140, vii. https://doi.org/10.1016/j.otc.2006.11.013

Hale, C. L., Niederriter, A. N., Green, G. E., & Martin, D. M. (2016a). Atypical phenotypes associated with pathogenic CHD7 variants and a proposal for broadening CHARGE syndrome clinical diagnostic criteria. *Am J Med Genet A*, *170A*(2), 344-354. https://doi.org/10.1002/ajmg.a.37435

Hale, C. L., Niederriter, A. N., Green, G. E., & Martin, D. M. (2016b). Response to correspondence to Hale et al. atypical phenotypes associated with pathogenic CHD7 variants and a proposal for broadening CHARGE syndrome clinical diagnostic criteria. *Am J Med Genet A*, *170*(12), 3367-3368. https://doi.org/10.1002/ajmg.a.37629

Hall, B. D. (1979). Choanal atresia and associated multiple anomalies. *J Pediatr*, *95*(3), 395-398. https://www.ncbi.nlm.nih.gov/pubmed/469662

Harris, J., Robert, E., & Kallen, B. (1997). Epidemiology of choanal atresia with special reference to the CHARGE association. *Pediatrics*, *99*(3), 363-367.

Hartshorne, T. S., Stratton, K. K., & van Ravenswaaij-Arts, C. M. (2011). Prevalence of genetic testing in CHARGE syndrome. *J Genet Couns*, *20*(1), 49-57. https://doi.org/10.1007/s10897-010-9328-7

He, D., Marie, C., Zhao, C., Kim, B., Wang, J., Deng, Y., Clavairoly, A., Frah, M., Wang, H., He, X., Hmidan, H., Jones, B. V., Witte, D., Zalc, B., Zhou, X., Choo, D. I., Martin, D. M., Parras, C., & Lu, Q. R. (2016). Chd7 cooperates with Sox10 and regulates the onset of CNS myelination and remyelination. *Nat Neurosci*, *19*(5), 678-689. https://doi.org/10.1038/nn.4258

Hefner, M. A., & Fassi, E. (2017). Genetic counseling in CHARGE syndrome: Diagnostic evaluation through follow up. *Am J Med Genet C Semin Med Genet*, *175*(4), 407-416. https://doi.org/10.1002/ajmg.c.31589

Hittner, H. M., Hirsch, N. J., Kreh, G. M., & Rudolph, A. J. (1979). Colobomatous microphthalmia, heart disease, hearing loss, and mental retardation--a syndrome. *J Pediatr Ophthalmol Strabismus*, *16*(2), 122-128. https://www.ncbi.nlm.nih.gov/pubmed/458518

Hudson, A., Trider, C.-L., & Blake, K. (2017). CHARGE Syndrome. *Pediatrics in Review*, *38*(1), 56-59. https://doi.org/10.1542/pir.2016-0050

Hughes, S. S., Welsh, H. I., Safina, N. P., Bejaoui, K., & Ardinger, H. H. (2014). Family history and clefting as major criteria for CHARGE syndrome. *Am J Med Genet A*, *164a*(1), 48-53. https://doi.org/10.1002/ajmg.a.36192

Isaacson, G. C. (2022, 01/10/2022). *Congenital anomalies of the nose*. UpToDate, Inc. https://www.uptodate.com/contents/congenital-anomalies-of-the-nose

Issekutz, K. A., Graham, J. M., Jr., Prasad, C., Smith, I. M., & Blake, K. D. (2005). An epidemiological analysis of CHARGE syndrome: preliminary results from a Canadian study. *Am J Med Genet A*, *133a*(3), 309-317. https://doi.org/10.1002/ajmg.a.30560

Janssen, N., Bergman, J. E., Swertz, M. A., Tranebjaerg, L., Lodahl, M., Schoots, J., Hofstra, R. M., van Ravenswaaij-Arts, C. M., & Hoefsloot, L. H. (2012). Mutation update on the CHD7 gene involved in CHARGE syndrome. *Hum Mutat*, *33*(8), 1149-1160. https://doi.org/10.1002/humu.22086

Jongmans, M. C., Admiraal, R. J., van der Donk, K. P., Vissers, L. E., Baas, A. F., Kapusta, L., van Hagen, J. M., Donnai, D., de Ravel, T. J., Veltman, J. A., Geurts van Kessel, A., De Vries, B. B., Brunner, H. G., Hoefsloot, L. H., & van Ravenswaaij, C. M. (2006). CHARGE syndrome: the phenotypic spectrum of mutations in the CHD7 gene. *J Med Genet*, *43*(4), 306-314. https://doi.org/10.1136/jmg.2005.036061

Jongmans, M. C., Hoefsloot, L. H., van der Donk, K. P., Admiraal, R. J., Magee, A., van de Laar, I., Hendriks, Y., Verheij, J. B., Walpole, I., Brunner, H. G., & van Ravenswaaij, C. M. (2008). Familial CHARGE syndrome and the CHD7 gene: a recurrent missense mutation, intrafamilial recurrence and variability. *Am J Med Genet A*, *146A*(1), 43-50. https://doi.org/10.1002/ajmg.a.31921

Jongmans, M. C., van Ravenswaaij-Arts, C. M., Pitteloud, N., Ogata, T., Sato, N., Claahsen-van der Grinten, H. L., van der Donk, K., Seminara, S., Bergman, J. E., Brunner, H. G., Crowley, W. F., Jr., & Hoefsloot, L. H. (2009). CHD7 mutations in patients initially diagnosed with Kallmann syndrome--the clinical overlap with CHARGE syndrome. *Clin Genet*, *75*(1), 65-71. https://doi.org/10.1111/j.1399-0004.2008.01107.x

Lalani, S. R., Hefner, M. A., Belmont, J. W., & Davenport, S. L. (2012). CHARGE syndrome In R. A. Pagon, M. P. Adam, & H. H. Ardinger (Eds.), *GeneReviews (R)*. University of Washington, SEattle.

Longman. (2018). *Obstetric Imaging: Fetal Diagnosis and Care*.

Mahdi, E. S., & Whitehead, M. T. (2018). Clival Malformations in CHARGE Syndrome. *AJNR Am J Neuroradiol*, *39*(6), 1153-1156. https://doi.org/10.3174/ajnr.A5612

Marfella, C. G., & Imbalzano, A. N. (2007). The Chd family of chromatin remodelers. *Mutat Res*, *618*(1-2), 30-40. https://doi.org/10.1016/j.mrfmmm.2006.07.012

Moccia, A., Srivastava, A., Skidmore, J. M., Bernat, J. A., Wheeler, M., Chong, J. X., Nickerson, D., Bamshad, M., Hefner, M. A., Martin, D. M., & Bielas, S. L. (2018). Genetic analysis of CHARGE syndrome identifies overlapping molecular biology. *Genet Med*, *20*(9), 1022-1029. https://doi.org/10.1038/gim.2017.233

National Organization for Rare Disorders, I. (2024). *CHARGE Syndrome*. National Organization for Rare Disorders, Inc. https://rarediseases.org/rare-diseases/charge-syndrome/

Pagon, R. A., Graham, J. M., Jr., Zonana, J., & Yong, S. L. (1981). Coloboma, congenital heart disease, and choanal atresia with multiple anomalies: CHARGE association. *J Pediatr*, *99*(2), 223-227. https://www.ncbi.nlm.nih.gov/pubmed/6166737

Pauli, S., Bajpai, R., & Borchers, A. (2017). CHARGEd with neural crest defects. *Am J Med Genet C Semin Med Genet*, *175*(4), 478-486. https://doi.org/10.1002/ajmg.c.31584

Qin, Z., Su, J., Li, M., Yang, Q., Yi, S., Zheng, H., Zhang, Q., Chen, F., Yi, S., Lu, W., Li, W., Huang, L., Xu, J., Shen, Y., & Luo, J. (2020). Clinical and Genetic Analysis of CHD7 Expands the Genotype and Phenotype of CHARGE Syndrome. *Front Genet*, *11*, 592. https://doi.org/10.3389/fgene.2020.00592

Sanlaville, D., Etchevers, H. C., Gonzales, M., Martinovic, J., Clement-Ziza, M., Delezoide, A. L., Aubry, M. C., Pelet, A., Chemouny, S., Cruaud, C., Audollent, S., Esculpavit, C., Goudefroye, G., Ozilou, C., Fredouille, C., Joye, N., Morichon-Delvallez, N., Dumez, Y., Weissenbach, J., . . . Attie-Bitach, T. (2006). Phenotypic spectrum of CHARGE syndrome in fetuses with CHD7 truncating mutations correlates with expression during human development. *J Med Genet*, *43*(3), 211-217. https://doi.org/10.1136/jmg.2005.036160

Schulz, Y., Wehner, P., Opitz, L., Salinas-Riester, G., Bongers, E. M., van Ravenswaaij-Arts, C. M., Wincent, J., Schoumans, J., Kohlhase, J., Borchers, A., & Pauli, S. (2014). CHD7, the gene mutated in CHARGE syndrome, regulates genes involved in neural crest cell guidance. *Hum Genet*, *133*(8), 997-1009. https://doi.org/10.1007/s00439-014-1444-2

Sperry, E. D., Hurd, E. A., Durham, M. A., Reamer, E. N., Stein, A. B., & Martin, D. M. (2014). The chromatin remodeling protein CHD7, mutated in CHARGE syndrome, is necessary for proper craniofacial and tracheal development. *Dev Dyn*, *243*(9), 1055-1066. https://doi.org/10.1002/dvdy.24156

Trider, C. L., Arra-Robar, A., van Ravenswaaij-Arts, C., & Blake, K. (2017). Developing a CHARGE syndrome checklist: Health supervision across the lifespan (from head to toe). *Am J Med Genet A*, *173*(3), 684-691. https://doi.org/10.1002/ajmg.a.38085

Usman, N., & Sur, M. (2024). CHARGE Syndrome. In *StatPearls*. https://www.ncbi.nlm.nih.gov/pubmed/32644625

Van Nostrand, J. L., Brady, C. A., Jung, H., Fuentes, D. R., Kozak, M. M., Johnson, T. M., Lin, C. Y., Lin, C. J., Swiderski, D. L., Vogel, H., Bernstein, J. A., Attie-Bitach, T., Chang, C. P., Wysocka, J., Martin, D. M., & Attardi, L. D. (2014). Inappropriate p53 activation during development induces features of CHARGE syndrome. *Nature*, *514*(7521), 228-232. https://doi.org/10.1038/nature13585

van Ravenswaaij-Arts, C., & Martin, D. M. (2017). New insights and advances in CHARGE syndrome: Diagnosis, etiologies, treatments, and research discoveries. *Am J Med Genet C Semin Med Genet*, *175*(4), 397-406. https://doi.org/10.1002/ajmg.c.31592

Verloes, A. (2005). Updated diagnostic criteria for CHARGE syndrome: a proposal. *Am J Med Genet A*, *133A*(3), 306-308. https://doi.org/10.1002/ajmg.a.30559

Vissers, L. E., van Ravenswaaij, C. M., Admiraal, R., Hurst, J. A., de Vries, B. B., Janssen, I. M., van der Vliet, W. A., Huys, E. H., de Jong, P. J., Hamel, B. C., Schoenmakers, E. F., Brunner, H. G., Veltman, J. A., & van Kessel, A. G. (2004). Mutations in a new member of the chromodomain gene family cause CHARGE syndrome. *Nat Genet*, *36*(9), 955-957. https://doi.org/10.1038/ng1407

Whittaker, D. E., Riegman, K. L., Kasah, S., Mohan, C., Yu, T., Sala, B. P., Hebaishi, H., Caruso, A., Marques, A. C., Michetti, C., Smachetti, M. E., Shah, A., Sabbioni, M., Kulhanci, O., Tee, W. W., Reinberg, D., Scattoni, M. L., Volk, H., McGonnell, I., . . . Basson, M. A. (2017). The chromatin remodeling factor CHD7 controls cerebellar development by regulating reelin expression. *J Clin Invest*, *127*(3), 874-887. https://doi.org/10.1172/JCI83408

Wincent, J., Holmberg, E., Stromland, K., Soller, M., Mirzaei, L., Djureinovic, T., Robinson, K., Anderlid, B., & Schoumans, J. (2008). CHD7 mutation spectrum in 28 Swedish patients diagnosed with CHARGE syndrome. *Clin Genet*, *74*(1), 31-38. https://doi.org/10.1111/j.1399-0004.2008.01014.x

Wincent, J., Schulze, A., & Schoumans, J. (2009). Detection of CHD7 deletions by MLPA in CHARGE syndrome patients with a less typical phenotype. *Eur J Med Genet*, *52*(4), 271-272. https://doi.org/10.1016/j.ejmg.2009.02.005

Genetic Testing for Connective Tissue Disorders

Policy Number: AHS – M2144 – Genetic Testing for Connective Tissue Disorders	Prior Policy Name and Number, as applicable: • AHS-M2144 Marfan Syndrome
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

More than 200 heritable connective tissue disorders exist and include Marfan Syndrome (MFS), Ehlers-Danlos syndrome (EDS), Epidermolysis bullosa (EB), and Loeys-Dietz syndrome (LDS) (NIH, 2016). Every disorder impacts connective tissue differently, including several with vascular implications, and clinical severity varies within each disorder.

II. Related Policies

Policy Number	Policy Title
AHS-M2145	General Genetic Testing, Germline Disorders

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals who have consulted with a cardiology specialist prior to genetic testing, *FBN1* mutation testing for Marfan Syndrome **MEETS COVERAGE CRITERIA** in the following situations:
    a) When Marfan syndrome is suspected based on clinical features, but a definitive diagnosis cannot be made using established clinical diagnostic criteria (see Note 1).

b) For an asymptomatic individual who has an affected first-degree blood relative (i.e., parent, sibling, child) with a known mutation.

c) For the prenatal diagnosis or preimplantation genetic diagnosis (PGD) of Marfan syndrome in the offspring of patients with known disease-causing variants.

2) Genetic testing for Loeys-Dietz Syndrome (*TGFBR1* or *TGFBR2* mutation) **MEETS COVERAGE CRITERIA** in the following situations:

a) To confirm or establish a diagnosis of LDS in an individual with vascular characteristics of LDS (see Note 2).

b) For an asymptomatic individual who has an affected first-degree blood relative (i.e., parent, sibling, child) with a known mutation.

c) For individuals suspected of having Marfan Syndrome who have tested negative for *FBN1*.

3) For individuals with characteristics of vascular Ehlers-Danlos Syndrome (vEDS) (see Note 3), genetic panel testing for *COL3A1* and *COL1A1* mutations to confirm or establish a diagnosis of vEDS **MEETS COVERAGE CRITERIA.**

4) For individuals with characteristics of hypermobile Ehlers-Danlos syndrome (hEDS) (see Note 5), genetic testing to confirm or establish a diagnosis **DOES NOT MEET COVERAGE CRITERIA.**

5) All other gene testing for Marfan Syndrome or other connective tissue disorders, including Ehlers-Danlos Syndrome, **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Clinical Diagnostic Criteria for Marfan Syndrome is as follows:

Revised Ghent nosology — The 2010 revised Ghent nosology puts greater weight on aortic root dilatation/dissection and ectopia lentis as the cardinal clinical features of MFS and on testing for mutations in *FBN1* (Loeys et al., 2010; Wright & Connolly, 2023).

- In the absence of family history of MFS, the presence of one of any of the following criteria is diagnostic for MFS:
  - Aortic criterion (aortic diameter Z ≥2 or aortic root dissection) and ectopia lentis*
  - Aortic criterion (aortic diameter Z ≥2 or aortic root dissection) and a causal *FBN1* mutation
  - Aortic criterion (aortic diameter Z ≥2 or aortic root dissection) and a systemic score ≥7 points*

- Ectopia lentis and a causal *FBN1* mutation that has been identified in an individual with aortic aneurysm

- In the presence of family history of MFS (as defined by the above criteria), the presence of one of any of the following criteria is diagnostic for MFS:
  - Ectopia lentis
  - Systemic score ≥7 points*
  - Aortic criterion (aortic diameter Z ≥2 above 20 years old, Z ≥3 below 20 years, or aortic root dissection) *

For criteria with an asterisk (*), the diagnosis of MFS can be made only in the absence of discriminating features of Shprintzen-Goldberg syndrome, Loeys-Dietz syndrome, or vascular Ehlers-Danlos syndrome **and** after *TGFBR1/2*, collagen biochemistry, or *COL3A1* testing if indicated.

Systemic score — The revised Ghent nosology includes the following scoring system for systemic features (Loeys et al., 2010; Wright & Connolly, 2023):

- Wrist AND thumb sign: 3 points
- Wrist OR thumb sign: 1 point
- Pectus carinatum deformity: 2 points
- Pectus excavatum or chest asymmetry: 1 point
- Hindfoot deformity: 2 points
- Plain pes planus: 1 point
- Pneumothorax: 2 points
- Dural ectasia: 2 points
- Protrusio acetabuli: 2 points
- Reduced upper segment/lower segment ratio AND increased arm span/height AND no severe scoliosis: 1 point
- Scoliosis or thoracolumbar kyphosis: 1 point
- Reduced elbow extension (≤170 degrees with full extension): 1 point
- Facial features (at least three of the following five features: dolichocephaly, malar hypoplasia, enophthalmos, downslanting palpebral fissures, retrognathia): 1 point
- Skin striae: 1 point
- Myopia >3 diopters: 1 point
- Mitral valve prolapse: 1 point

Note 2: Clinical features of Loeys-Dietz Syndrome: aortic/arterial aneurysms/tortuosity, arachnodactyly, bicuspid aortic valve and patent ductus arteriosus, blue sclerae, camptodactyly, cerebral, thoracic or abdominal arterial aneurysms and/or dissections, cleft palate/bifid uvula, club feet, craniosynostosis, easy bruising, joint hypermobility, ocular hypertelorism, pectus carinatum or pectus

excavatum, scoliosis, talipes equinovarus, thin skin with atrophic scars, velvety and translucent skin, widely spaced eyes (Loeys & Dietz, 2018).

Note 3: Clinical features of Vascular EDS (vEDS) from The 2017 International Classification For The Ehlers-Danlos Syndromes (Malfait et al, 2017):

- Inheritance
  Autosomal dominant
- Major criteria
  1. Family history of vEDS with documented causative variant in *COL3A1*
  2. Arterial rupture at a young age
  3. Spontaneous sigmoid colon perforation in the absence of known diverticular disease or other bowel pathology
  4. Uterine rupture during the third trimester in the absence of previous C-section and/or severe peripartum perineum tears
  5. Carotid-cavernous sinus fistula (CCSF) formation in the absence of trauma
- Minor criteria
  6. Bruising unrelated to identified trauma and/or in unusual sites such as cheeks and back
  7. Thin, translucent skin with increased venous visibility
  8. Characteristic facial appearance
  9. Spontaneous pneumothorax
  10. Acrogeria
  11. Talipes equinovarus
  12. Congenital hip dislocation
  13. Hypermobility of small joints
  14. Tendon and muscle rupture
  15. Keratoconus
  16. Gingival recession and gingival fragility
  17. Early onset varicose veins (under age 30 and nulliparous if female)
- Minimal criteria suggestive for vEDS:
  A family history of the disorder, arterial rupture or dissection in individuals less than 40 years of age, unexplained sigmoid colon rupture, or spontaneous pneumothorax in the presence of other features consistent with vEDS should all lead to diagnostic studies to determine if the individual has vEDS. Testing for vEDS should also be considered in the presence of a combination of the other "minor" clinical features listed above.

Note 4: For 5 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

Note 5: Clinical features of Hypermobile EDS (hEDS) from The 2017 International Classification For The Ehlers-Danlos Syndromes (Malfait et al, 2017):

- Inheritance
  Autosomal dominant
- Molecular basis
  Unknown
- Clinical diagnosis

Clinical spectrum ranging from asymptomatic joint hypermobility, through "non-syndromic" hypermobility with secondary manifestations, to hEDS (*see "A Framework for the Classification of Joint Hypermobility and Related Conditions" by Castori et al., this issue*).

The clinical diagnosis of hEDS needs the simultaneous presence of criteria 1 AND 2 AND 3:

Criterion 1: Generalized Joint Hypermobility (GJH)

Criterion 2: Two or More Among the Following Features (A–C) MUST Be Present (for Example: A and B; A and C; B and C; A and B and C)

Feature A: systemic manifestations of a more generalized connective tissue disorder (a total of five must be present)

1. Unusually soft or velvety skin
2. Mild skin hyperextensibility
3. Unexplained striae such as striae distensae or rubrae at the back, groins, thighs, breasts and/or abdomen in adolescents, men or prepubertal women without a history of significant gain or loss of body fat or weight
4. Bilateral piezogenic papules of the heel
5. Recurrent or multiple abdominal hernia(s) (e.g., umbilical, inguinal, crural)
6. Atrophic scarring involving at least two sites and without the formation of truly papyraceous and/or hemosideric scars as seen in classical EDS
7. Pelvic floor, rectal, and/or uterine prolapse in children, men, or nulliparous women without a history of morbid obesity or other known predisposing medical condition
8. Dental crowding and high or narrow palate
9. Arachnodactyly, as defined in one or more of the following: (i) positive wrist sign (Steinberg sign) on both sides; (ii) positive thumb sign (Walker sign) on both sides
10. Arm span-to-height ≥1.05
11. Mitral valve prolapse (MVP) mild or greater based on strict echocardiographic criteria
12. Aortic root dilatation with Z-score > +2

Feature B: positive family history, with one or more first degree relatives independently meeting the current diagnostic criteria for hEDS.

Feature C: musculoskeletal complications (must have at least one)

1. 1.Musculoskeletal pain in two or more limbs, recurring daily for at least 3 months
2. 2.Chronic, widespread pain for ≥3 months
3. Recurrent joint dislocations or frank joint instability, in the absence of trauma (a or b)
   a. Three or more atraumatic dislocations in the same joint or two or more atraumatic dislocations in two different joints occurring at different times
   b. Medical confirmation of joint instability at two or more sites not related to trauma

Criterion 3: All the Following Prerequisites MUST Be Met

1. Absence of unusual skin fragility, which should prompt consideration of other types of EDS
2. Exclusion of other heritable and acquired connective tissue disorders, including autoimmune rheumatologic conditions. In patients with an acquired connective tissue disorder (e.g., lupus, rheumatoid arthritis, etc.), additional diagnosis of hEDS requires meeting both Features A and B of Criterion 2. Feature C of Criterion 2 (chronic pain and/or instability) cannot be counted towards a diagnosis of hEDS in this situation.
3. Exclusion of alternative diagnoses that may also include joint hypermobility by means of hypotonia and/or connective tissue laxity. Alternative diagnoses and diagnostic categories include, but are not limited to, neuromuscular disorders (e.g., myopathic EDS, Bethlem myopathy), other HCTD (e.g., other types of EDS, Loeys–Dietz syndrome, Marfan syndrome), and skeletal dysplasias (e.g., OI). Exclusion of these considerations may be based upon history, physical examination, and/or molecular genetic testing, as indicated.

IV. Table of Terminology

Term	Definition
AAP	American Academy of Pediatrics
AATS	American Association for Thoracic Surgery
ACC	American College of Cardiology
ACCF	American College of Cardiology Foundation
ACMG	American College of Medical Genetics
ACR	American College of Radiology
ACTA2	*Actin alpha 2, smooth muscle gene*
AD	Autosomal dominant

ADAMTS2	*ADAM metallopeptidase with thrombospondin type 1 motif 2 gene*
aEDS	Arthrochalasia Ehlers-Danlos syndrome
AHA	American Heart Association
AngII	Angiotensin II
AR	Autosomal recessive
ARBs	Angiotensin receptor blockers
ASA	American Stroke Association
ATR1	Angiotensin II receptor type 1
B3GALT6	*Beta-1,3-galactosyltransferase 6* gene
B4GALT7	*Beta-1,4-galactosyltransferase 7* gene
BAV	Bicuspid aortic valve
BCS	Brittle cornea syndrome
C	Carboxy
C1R	Complement C1r
C1S	Complement C1s
CCS	Canadian Cardiovascular Society
CCSF	Carotid-cavernous sinus fistula
cEDS	Classical Ehlers-Danlos syndrome
CHST14	*Carbohydrate sulfotransferase 14* gene
clEDS	Classical-like Ehlers-Danlos syndrome
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare and Medicaid Services
CNV	Copy number variant
COL12A1	*Collagen type XII alpha 1 chain* gene
COL1A1	*Collagen type I alpha 1 chain* gene
COL1A2	*Collagen type I alpha 2 chain* gene
COL1A2 NMD	*Collagen type I alpha 2* gene nonsense-mediated mRNA decay
COL3A1	*Collagen type III alpha 1 chain* gene
CPD	Clinical provisional diagnosis
CT	Computerized tomography
cvEDS	Cardiac-valvular Ehlers-Danlos syndrome
CVS	Chorionic villus sampling
D4ST1	Dermatan 4-sulfotransferase-1 protein
dEDS	Dermatosparaxis Ehlers-Danlos syndrome
DNA	Deoxyribonucleic acid
DSE	*Dermatan sulfate epimerase* gene
EB	Epidermolysis bullosa
EDS	Ehlers-Danlos syndrome

EFEMP2	*EGF containing fibulin extracellular matrix protein 2* gene
EGF	*Epidermal growth factor*
ELN	*Elastin gene*
EM	Electron microscopy
FBN1	*Fibrillin-1* gene
FBN2	*Fibrillin-2* gene
FDA	Food and Drug Administration
FKBP14	*FKBP (FK506 binding protein) prolyl isomerase 14* gene
FKBP22	*FKBP (FK506 binding protein) prolyl isomerase 22* gene
FLNA	*Filamin A* gene
GAG	Glycosaminoglycan
GJH	Generalized joint hypermobility
HCTD	Heritable connective tissue disorders
hEDS	Hypermobile Ehlers-Danlos syndrome
HP	Hydroxylysyl-pyridinoline
HPLC	High-performance liquid chromatography
IFM	Immunofluorescence mapping
kEDS	Kyphoscoliotic
KRT14	*Keratin 14* gene
KRT5	*Keratin 5* gene
LDS	Loeys-Dietz syndrome
LDTs	Laboratory-developed tests
LH1	Lysyl hydroxylase 1
LOX	Lysyl oxidase gene
LP	Lysyl-pyridinoline
MCC	Meets coverage criteria
mcEDS	Musculocontractural Ehlers-Danlos syndrome
mEDS	Myopathic Ehlers-Danlos syndrome
MFS	Marfan syndrome
MLPA	Multiplex ligation-dependent probe amplification
MRI	Magnetic resonance imaging
MVP	Mitral valve prolapse
MYH11	*Myosin heavy chain 11* gene
MYLK	*Myosin light chain kinase* gene
NGS	Next-generation sequencing
NORD	National Organization for Rare Disorders
OI	Osteogenesis imperfecta
PCR	Polymerase chain reaction

pEDS	Periodontal Ehlers-Danlos syndrome
PGD	Preimplantation genetic diagnosis
PGT-M	Preimplantation genetic testing for monogenic diseases
PLEC	*Plectin gene*
PLOD1	*Procollagen-Lysine,2-Oxoglutarate 5-Dioxygenase 1* gene
PRDM5	*PR/SET Domain 5* gene
PRKG1	*Protein kinase cGMP-dependent 1* gene
RCT	Randomized controlled trial
RNA	Ribonucleic acid
SCA	Society of Cardiovascular Anesthesiologists
SCAI	Society for Cardiovascular Angiography and Interventions
SDS-PAGE	Sodium dodecyl sulphate–polyacrylamide gel electrophoresis
SIR	Society of Interventional Radiology
SKI	SKI proto-oncogene
SLC2A10	*Solute Carrier Family 2 Member 10* gene
SLC39A13	*Solute Carrier Family 39 Member 13* gene
SMAD3	*Mothers against decapentaplegic homolog 3* gene
SMAD4	*Mothers against decapentaplegic homolog 4* gene
SMC	Smooth muscle cell
spEDS	Spondylodysplastic Ehlers-Danlos syndrome
STS	Society of Thoracic Surgeons
SVM	Society for Vascular Medicine
TAD	Thoracic aortic disease
TAAD	Thoracic aortic aneurysm and dissection
TEM	Transmission electron microscopy
TGFB	*Transforming growth factor beta gene*
TGFB2	*Transforming Growth Factor B 2 Ligand* gene
TGFB3	*Transforming Growth Factor B 3 Ligand* gene
TGFBR	*Transforming growth factor beta receptor* gene
TGFBR1	*Transforming Growth Factor B Receptor I* gene
TGFBR2	*Transforming Growth Factor B Receptor II* gene
TGF-β	Transforming Growth Factor-B
TNXA	*Tenascin XA* (Pseudogene)
TNXB	*Tenascin XB* gene
vEDS	Vascular Ehlers-Danlos Syndrome
VUS	Variant of unknown significance
WES	Whole exome sequencing
WGS	Whole genome sequencing
ZIP13	Zrt- and Irt-like protein 13

ZNF469	*Zinc finger protein 469* gene

V. Scientific Background

Connective tissue helps to bind and support other types of tissue in the body. Unfortunately, many types of connective tissue afflictions exist, including more than 200 heritable connective tissue disorders (NIH, 2016) such as Marfan Syndrome (MFS), Ehlers-Danlos syndrome (EDS), Epidermolysis bullosa (EB), and Loeys-Dietz syndrome (LDS). Each disorder affects connective tissue in a different manner. Symptoms may include joint issues, bone growth problems, blood vessel damage, cranial structural problems, skin problems, and height issues (NIH, 2016).

Marfan Syndrome (MFS) was first described more than 100 years ago by a Parisian professor of pediatrics, Antoine-Bernard Marfan. He was the first to report the association of long slender digits with other skeletal abnormalities in a 5-year-old girl (Radke & Baumgartner, 2014). MFS is a fairly common condition with an incidence of about 1 in 3000 to 5000 individuals. MFS is a systemic disorder of connective tissue with significant clinical variability across a broad phenotypic continuum, ranging from mild isolated features to severe and rapidly progressive neonatal multiorgan disease (Faivre et al., 2007). Ocular findings include myopia, ectopia lentis, and an increased risk for retinal detachment, glaucoma, and early cataracts. Skeletal system symptoms include "bone overgrowth and joint laxity, disproportionately long extremities for the size of the trunk, overgrowth of the ribs, and scoliosis." The major cause of death in MFS results from cardiovascular system problems, including aortic root dilatation and rupture, mitral or tricuspid valve prolapse, and enlargement of the proximal pulmonary artery. Severe and prolonged regurgitation of the mitral or aortic valve can lead to left ventricular dysfunction and heart failure. Patients presenting with one isolated symptom are rare. However, with careful management, life expectancy approximates that of the general population (Dietz, 2017; Pyeritz, 2017; Wright & Connolly, 2023).

MFS primarily affects connective tissue, particularly the fibrillin component of the extracellular matrix. Fibrillins are large glycoproteins that form extracellular microfibrils that provide elasticity and structural support to tissues, modulate elastic fiber biogenesis and homeostasis, and regulate the bioavailability and activity of different growth factors (Davis & Summers, 2012; Grewal & Gittenberger-de Groot, 2018). Fibrillin-1 is an important matrix component of both elastic and nonelastic tissues (Wright & Connolly, 2023). Mutations can lead to impaired fibrillin-1 protein function, causing extracellular matrix integrity to fail (Grewal & Gittenberger-de Groot, 2018). These fibrillin-1 problems also cause smooth muscle cell (SMC) contractile dysfunction and dysregulation of the tensile strength of aortic tissue, which is a common finding in many cardiovascular conditions (Nataatmadja et al., 2003). Recent studies indicate a

role for SMC phenotype in the pathogenesis of MFS. Early phenotypic switch resulting from *FBN1* mutation appears to be associated with initiation of important metabolic changes in SMCs that contribute to subsequent pathology (Dale et al., 2017). Mutation in *FBN1* has been shown to dysregulate the transforming growth factor-β (TGF-β) pathway, as matrix sequestration of cytokines is crucial to their regulated activation and signaling (Bin Mahmood et al., 2017; Neptune et al., 2003).

EDS is a term that encompasses several rare genetic connective tissue disorders. Each disorder is characterized by specific features, including "skin hyperextensibility, joint hypermobility, and tissue fragility," and affects approximately 1 in 5000 individuals (Pauker & Stoler, 2023). EDS hypermobile type (hEDS) is the most common type of EDS. Unfortunately, the genetic basis for hEDS is still unknown, meaning that a genetic test to confirm diagnosis is not available for this subtype. As of 2017, an international forum has classified EDS into 13 different subtypes. The table below has been modified from Malfait et al. (2017) and lists all EDS types:

Clinical EDS Subtype	**Abbreviation**	**Inheritance Pattern**	**Genetic Bases**	**Protein**
Classical EDS	cEDS	AD (autosomal dominant)	Major: *COL5A1, COL5A1* Rare: *COL1A1*	Type V collagen Type I collagen
Classical-like EDS	clEDS	AR (autosomal recessive)	*TNXB*	Tenascin XB
Cardiac-valvula	cvEDS	AR	*COL1A2* (biallelic mutations that lead to *COL1A2* NMD and absence of pro α2(I) collagen chains)	Type I collagen
Vascular EDS	vEDS	AD	Major: *COL3A1* Rare: *COL1A1*	Type III collagen Type I collagen
Hypermobile EDS	hEDS	AD	Unknown	Unknown

Arthrochalasia EDS	aEDS	AD	*COL1A1, COL1A2*	Type I collagen
Dermatosparaxis EDS	dEDS	AR	*ADAMTS2*	ADAMTS-2
Kyphoscoliotic EDS	kEDS	AR	*PLOD1* *FKBP14*	LH1 FKBP22
Brittle Cornea syndrome	BCS	AR	*ZNF469* *PRDM5*	ZNF469 PRDM5
Spondylodysplastic EDS	spEDS	AR	*B4GALT7* *B3GALT6* *SLC39A13*	β4GalT7 β3GalT6 ZIP13
Musculocontractural EDS	mcEDS	AR	*CHST14* *DSE*	D4ST1 DSE
Myopathic EDS	mEDS	AD or AR	*COL12A1*	Type XII collagen
Periodontal EDS	pEDS	AD	*C1R* *C1S*	C1r C1s

This naming convention has also been adopted by The Ehlers Danlos Society (EDS, 2017), who previously used Villefranche nosology to classify EDS types. Unfortunately, no cure for EDS currently exists, and treatments may include physical therapy, braces, counseling, and pain medication (Pauker & Stoler, 2023).

Vascular EDS (vEDS) is characterized by "arterial aneurysm, dissection and rupture, bowel rupture, and rupture of the gravid uterus" and affects 1 in 50,000 to 200,000 individuals (Byers et al., 2017). These arterial aneurysms may be life threatening. As noted in the table above, this disorder is due to mutations in the *COL3A1* or *COL1A1* genes, with a sequence analysis of *COL3A1* thought to identify approximately 98% of vEDS cases (Malfait et al., 2017). A diagnosis depends on clinical features, including family history. Aneurysms occur in other types of EDS, including classical EDS (cEDS), due to vascular fragility (Malfait, 2018). Johansen et al. (2020) published a recent cross-sectional study with data collected from 18 patients with genetically verified vEDS and 34 patients with genetically verified LDS. The median age at diagnosis was 34 years. "Most respondents (87%) had cardiovascular surveillance visits, 58% yearly or more often, and still 29% had no antihypertensive medications (Johansen et al., 2020)."

LDS was first described in 2005 and is now considered an autosomal dominant connective tissue disorder characterized by "aortic aneurysms and generalized arterial tortuosity, hypertelorism, and bifid/broad uvula or cleft palate" (MacCarrick et al., 2014). LDS was initially characterized by mutations in the transforming growth factor β receptor I (*TGFBR1*) and transforming growth factor β receptor II (*TGFBR2*) genes;

however, additional genes have been identified, including the mothers against decapentaplegic homolog 3 (*SMAD3*) gene, the transforming growth factor β 2 ligand (*TGFB2*) gene, and the transforming growth factor β 3 ligand (*TGFB3*) gene (MacCarrick et al., 2014; Wright & Connolly, 2023). If a mutation is identified in all three genes, transforming growth factor-β (TGF-β) signaling is affected and patients typically exhibit similar craniofacial, cutaneous, cardiovascular, and skeletal features. Vascular involvement in LDS has recently been studied by Jud and Hafner (2019) who published a case study which followed a woman with a history of ectasias of the aortic arch, abdominal aorta, carotid bulbs, and common femoral arteries, as well as an asymptomatic aneurysm in superior mesenteric artery. In comparing surgical outcomes between those with LDS versus MFS, it was found that LDS patients had a greater likelihood of reoperation for aortic arch aneurysms than MFS patients, and that those with mutations in *TGFBR1* had higher rates of reoperation than those with *TGFBR2* mutations (Seike et al., 2020).

Epidermolysis bullosa (EB) is a group of hereditary diseases characterized by mucosa and skin fragility due to mutations that affect skin structural proteins, causing the skin to easily blister. Four major types of EB have been identified and include EB simplex, junctional EB, dystrophic EB, and Kindler syndrome (Murrell, 2024). Unfortunately, there is currently no effective therapeutic option for this disorder, and treatment largely focuses on wound management. All of the major EB types may result from mutations in the keratin 5 (*KRT5*) or keratin 14 (*KRT14*) gene (Coulombe et al., 1991; NIH, 2020). These two genes work together to encourage strength in the epidermis. Mutations prevent the keratin from assembling in necessary networks, leading to fragility. Further, a rare type of EB, known as Ogna, has been associated with mutations in the *PLEC* gene, leading to issues in the attachment of the epidermis to other layers of the skin (NIH, 2020). Ryan et al. (2016) note that ventricular dysfunction and aortic dilation have been identified in patients with recessive dystrophic EB.

Clinical Utility and Validity

More than 90% of patients with the typical Marfan phenotype have mutations involving the gene encoding the connective tissue protein fibrillin-1 (*FBN1*). Out of a sample of 93 patients with MFS, 85 (91%) were found to have a *FBN1* mutation. The eight remaining patients did not display any drastically different clinical features or family history, and the authors suggest that *FBN1* mutations that go undetected are due to technical limitations (Loeys et al., 2004). Most patients have a family history of MFS, but up to 25% have a mutation *de novo*. Mutations are in one of five categories: nonsense, frameshift (deletion, insertion), splicing errors, a missense mutation that substitutes or creates cysteine residues, or a missense mutation affecting a conserved *EGF* sequence. Although the phenotypic variability is wide, mutations involving exon

skipping tend to result in more severe disease. Genetic findings have importance in the diagnosis, risk stratification, and clinical management of patients, as well as identifying potentially affected relatives (Wright & Connolly, 2023).

Becerra-Munoz et al. (2018) conducted a prospective cohort study to summarize variants in *FBN1* and establish a genotype-phenotype correlation. Genotype-phenotype correlations have identified that patients with MFS and truncating variants in *FBN1* presented a higher proportion of aortic events compared to a more benign course in patients with missense mutations. A total of 84 patients fulfilled the Ghent diagnostic criteria, and of these 84, 44 had missense mutations and 35 had truncating mutations. However, of the 44 with missense mutations, only six had suffered an aortic event (such as aortic aneurysm) whereas 20 of the 35 with a truncating mutation had suffered an aortic event (Becerra-Munoz et al., 2018). Up to 10% of patients with the Marfan phenotype have no identifiable mutation in the *FBN1* gene. Rather, mutations are identified in TGF-beta receptor 1 (*TGFBR1*) and *TGFBR2* genes. It has been proposed that patients with the Marfan phenotype and *TGFBR1* or *TGFBR2* mutations be classified as having LDS to properly address the potential for more aggressive vascular disease than seen in MFS (Wright & Connolly, 2023).

The diagnosis of MFS is now established by an *FBN1* pathogenic variant known to be associated with Marfan syndrome AND one of the following: aortic root enlargement (Z-score ≥2.0), ectopia lentis, demonstration of aortic root enlargement (Z-score ≥2.0) and ectopia lentis OR a defined combination of features throughout the body yielding a systemic score ≥7 (Dietz, 2017). These features are summarized in the 2010 Ghent nosology, which is slightly altered for patients under 20 years old (Wright & Connolly, 2023). Due to the identification of *FBN1* as the genetic basis for MFS and its subsequent effects, the understanding of MFS as a structural disorder has become one of a developmental abnormality with broad effects on the morphogenesis and function of multiple organ systems. Importantly, this also introduced new biological targets for treatment strategies in MFS (Dietz et al., 2005; Jensen & Handford, 2016).

Current clinical studies have elucidated a medical regimen for patients with MFS to help control the progression of cardiovascular manifestations and resulting mortality. The standard of care for medical management includes the use of β-blockers with supplementation or replacement by angiotensin receptor blockers (ARBs). However, the best course of treatment is a subject of ongoing research (Bin Mahmood et al., 2017; Hiratzka et al., 2010). However, a Cochrane review concluded, "Based on only one, low-quality RCT comparing long-term propranolol to no treatment in people with Marfan Syndrome, we could draw no definitive conclusions for clinical practice." The authors concluded that further, high-quality, randomized trials were needed to evaluate the long-term efficacy of beta-blocker treatment in people

with Marfan syndrome (Koo et al., 2017). Sellers et al. (2018) recently reported, "Despite promising preclinical and pilot clinical data, a recent large-scale study using antihypertensive angiotensin II (AngII) receptor type 1 (ATR1) blocker losartan has failed to meet expectations at preventing MFS-associated aortic root dilation, casting doubts about optimal therapy." Their mouse study suggested that "increased protective endothelial function, rather than ATR1 inhibition or blood pressure lowering, might be of therapeutic significance in preventing aortic root disease in MFS (Sellers et al., 2018)."

Johansen et al. (2020); Ritelli et al. (2020); Shalhub et al. (2020) analyzed vEDS data from 11 institutions between the year 2000 and 2015. Data used for this study included family history, clinical features, diagnostic criteria, demographics, and molecular testing results. A total of 173 individuals were identified for the purposes of this study, with 11 excluded because pathogenic *COL3A1* variants were not identified. Of the remaining individuals, 86 had been diagnosed with a pathogenic *COL3A1* variants, and 76 were diagnosed with only clinical criteria. "Compared with the cohort with pathogenic *COL3A1* variants, the clinical diagnosis only cohort had a higher number of females (80.3% vs 52.3%; P < .001), mitral valve prolapse (10.5% vs 1.2%; P = .009), and joint hypermobility (68.4% vs 40.7%; P < .001). Additionally, they had a lower frequency of easy bruising (23.7% vs 64%; P < .001), thin translucent skin (17.1% vs 48.8%; P < .001), intestinal perforation (3.9% vs 16.3%; P = .01), spontaneous pneumothorax/hemothorax (3.9% vs 14%, P.03), and arterial rupture (9.2% vs 17.4%; P = .13) (Shalhub et al., 2020)." This study highlights the importance of genetic testing for a vEDS diagnosis as the symptoms of vEDS overlap with many other disorders and a correct diagnosis is necessary for efficient disease treatment. Further, not all *COL3A1* variants are pathogenic, meaning that genetic results must be interpreted by a trained professional.

Using a next-generation sequencing (NGS) multigene panel, Mariath et al. (2019) identified 11 disease-causing variants of EB in a Brazilian population with an efficiency of 94.3%. Other studies that they have included have calculated efficiencies of 83.5% for a panel with 21 genes, 90% with 49 genes, and 97.7% in 21 genes, where all identified mutations were only in five genes. This conveys the clinical utility of gene variants in EB that could be translated to other connective tissue disorder mutations. In a study done with children with inherited EB, the accuracy of several diagnostic techniques, which included electron microscopy (EM), immunofluorescence mapping (IFM), and clinical provisional diagnosis (CPD) was evaluated. It was found that IFM, EM, and CPD yielded an accuracy of 75%, 75%, and 81.5%, respectively (Saunderson et al., 2019). All genetic components, tissue specimen, and clinical history are all necessary for a confirmed EB diagnosis.

Li et al. (2021) conducted a study in northwestern China to determine the genotype-phenotype correlation for thoracic aortic aneurysm and dissection via NGS. They screened 15 genes from 212 patients to find that 67 (31.60%) patients in this cohort had a (likely) pathogenic variant, "42 (19.81%) had a variant of uncertain significance (VUS), and 103 (48.58%) had no variant (likely benign/benign/negative)," with 135 reportable variants. With *FBN1*, a gene implicated in MFS, they found that "patients with truncating and splicing mutations are more prone to developing severe aortic dissection than those with missense mutations, especially frameshift mutations (82.76% vs. 42.86%)," and "the positive rate of genetic testing was higher in TAAD [thoracic aortic aneurysm and dissection] patients with family history than in those without (76.74% vs. 18.94%)".

Chen et al. (2021) investigated how genetic testing could aid in avoiding the occurrence of MFS among Chinese families. Using data from 11 families, as well as variant classification and interpretation through pedigree analysis, the researchers were able to support two families who agreed to pre-implantation genetic testing for monogenic diseases (PGT-M) as part of the *in vitro* fertilization process. They were able to identify 11 potential-disease causing *FBN1* variants and found that "nine variants were classified as likely pathogenic/pathogenic variants. Among 11 variants, eight variants were missense and seven of them were located in the Ca-binding *EGF*-like motifs. Moreover, half of them substituted conserved Cysteine residues." They also found one splice site variant, one frameshift variant, one synonymous variant, and two *de novo* variants. All variants were detected by polymerase chain reaction (PCR). Ultimately, the two MFS families were able to give birth to a baby without the *FBN1* mutation, as the healthy embryo was selected using haplotype analysis "to deduce the embryo's genotype by using single nucleotide polymorphisms." This demonstrated the tangible benefits of genetic testing for eliminating MFS and the development of comorbid conditions among future generations.

Damseh et al. (2022) conducted a retrospective study using the 2017 EDS classification criteria on 72 pediatric patients who were referred for evaluation of EDS. From this initial cohort, 18 patients met the clinical criteria for an EDS subtype diagnosis, and 15 were confirmed molecularly. 75% (n=54) of the patients also had clinical features that belonged to EDS and other joint hypermobility syndromes, but not a complete qualification of EDS clinical criteria. From those 54 patients, it was discovered that 12 patients (22%) had a molecular genetic diagnosis of EDS. An EDS genetic panel, microarray, whole exome sequencing, single gene sequencing, familial variant testing, and other genetic panels were utilized to confirm genetic based diagnoses of EDS. Of the 15 patients who met clinical criteria and had a positive molecular diagnosis and 12 that did not meet clinical criteria but had a positive molecular diagnosis, 41% had classical EDS, 26% had arthrochalasia EDS, 11% had kyphoscoliotic EDS, and 22% had

vascular EDS. The researchers ultimately "observed a correlation between generalized joint hypermobility, poor healing, easy bruising, atrophic scars, skin hyperextensibility, and developmental dysplasia of the hip with a positive molecular result." This study aided in expanding the scope of the 2017 EDS classifications into the pediatric population and effecting changes to clinical decision making and treatment.

Veatch et al. (2022) utilized clinical exam data and genetic testing results to understand the phenotypic and genotypic correlation for hereditary connective tissue diseases from 2016-2020. From a cohort of 100 unrelated individuals, the researchers isolated six likely pathogenic, and 35 classified "potentially pathogenic variants of unknown clinical significance." They found that those with potentially pathogenic variants and pathogenic/likely pathogenic variants of the same genes exhibited similar symptoms, as those with "connective tissue symptoms had suggestive evidence of increased odds of having skin (odds ratio 2.18, 95% confidence interval 1.12 to 4.24) and eye symptoms (odds ratio 1.89, 95% confidence interval 0.98 to 3.66) requiring further studies." Ultimately, the symptoms were broken up into classes of minimal skeletal symptoms (e.g., limb asymmetry, scoliosis, pes planus), more skeletal than connective tissue (e.g., joint hypermobility, dental defects, repeated ligament and cartilage disease), nervous, or gastrointestinal (e.g., irritable bowel syndrome, food intolerance) symptoms, and more nervous system (e.g., migraines, neuropathy) symptoms. Comprehending the spectrum of phenotypic heterogeneity could guide consequential clinical decision making for surveilling and counseling patients with hereditary connective tissue disorders and their current and future families (Veatch et al., 2022).

VI. Guidelines and Recommendations

American College of Cardiology (ACC)

The ACC released guidelines on thoracic aortic disease jointly with the American Association for Thoracic Surgery, American College of Radiology, American Stroke Association, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of Thoracic Surgeons, and Society for Vascular Medicine. The MFS-specific guidelines are listed below:

- An echocardiogram is recommended at the time of diagnosis of Marfan syndrome to determine the aortic root and ascending aortic diameters and six months thereafter to determine the rate of enlargement of the aorta.
- Annual imaging is recommended for patients with Marfan syndrome if stability of the aortic diameter is documented. If the maximal aortic diameter is 4.5 cm or greater, or if the aortic diameter shows significant growth from baseline, more frequent imaging should be considered.

- If a mutant gene (*FBN1*, *TGFBR1*, *TGFBR2*, *COL3A1*, *ACTA2*, *MYH11*) associated with aortic aneurysm and/or dissection is identified in a patient, first-degree relatives should undergo counseling and testing.
- Sequencing of other genes known to cause familial thoracic aortic aneurysms and/or dissection (*TGFBR1*, *TGFBR2*, *MYH11*) may be considered in patients with a family history and clinical features associated with mutations in these genes (Hiratzka et al., 2010).
- Aortic imaging is recommended in patients with LDS or a who have a confirmed genetic mutation known to predispose an individual to aortic aneurysms and aortic dissections (*TGFBR1, TGFBR2, FBN1, ACTA2*, or *MYH11*)

American Heart Association (Malfait et al.)

The AHA published a guideline regarding genetic testing for inherited cardiovascular diseases. The AHA notes that genetic testing plays a major role in diagnosing both Loeys-Dietz Syndrome and Marfan Syndrome, as well as confirming diagnoses of familial thoracic aortic aneurysm and dissection. A confirmed diagnosis may then affect timing of treatment or extent of screening for family members of the proband.

The AHA cites an ACMG list of "Genes Associated With Cardiovascular Disorders in Which Secondary/Incidental Findings Are Reportable". *COL3A1* is listed for Ehlers-Danlos Syndrome and *FBN1, TGFBR1, TGFBR2, SMAD3, ACTA2, MYH11* are listed for Marfan syndrome, Loeys-Dietz syndromes, and familial thoracic aortic aneurysms and dissections.

The AHA then lists another ACMG list of "Lists of Genes to Be Considered for Testing From Guidelines and Statements". Regarding heritable thoracic aortic aneurysm(s) or dissection(s), the genes *ACTA2, COL3A1, FBN1, MYH11, SMAD3, TGFB2, TGFBR1, TGFBR2, MYLK, LOX, PRKG1* are listed as having "definitive or strong evidence", and the genes *EFEMP2, ELN, FBN2, FLNA, NOTCH1, SLC2A10, SMAD4, SKI*, are considered as "potentially diagnostic" (Musunuru et al., 2020).

American College of Medical Genetics (ACMG)

The ACMG recommends the following diagnostic evaluations for a MFS diagnosis: a physical exam, family history, echocardiogram, dilated eye exam, CT or MRI, and the consideration of *FBN1* gene sequencing (Pyeritz, 2012). The ACMG notes that, since *FBN1* mutations may cause conditions other than MFS (such as EDS and LDS), clinical features must be used to diagnose MFS properly. The ACMG further notes *SMAD3*, *ACTA2*, and *MYH11* as potential genes of interest in identifying MFS, in addition to *FBN1*, *TGFBR1*, and *TGFBR2* (Pyeritz, 2012).

Regarding LDS, the ACMG notes that "LDS strongly resembles the vascular form of Ehlers–Danlos syndrome, especially in terms of thin skin" (Pyeritz, 2012). Further, a diagnostic evaluation of LDS includes the following: a "physical exam, family history, echocardiogram, dilated eye exam (to exclude MFS), magnetic resonance angiography of the head, neck thorax, abdomen and pelvis, and TGFBR1 and *TGFBR2* gene sequencing (Pyeritz, 2012)." Specifically, the ACMG states that "In a patient found to have consistent facial features, bifid uvula, and arterial tortuosity, the diagnosis [of LDS] can be confirmed with *TGFBR* testing (Pyeritz, 2012)."

Regarding EDS hypermobile type, the ACMG recommends the following diagnostic evaluation: a physical exam, family history, echocardiogram and dilated eye exam (to exclude MFS). The guidelines also specifically state that "Diagnosis is based on clinical evaluation and family history. A small subset of individuals with the hypermobile form of EDS have an insertion or deletion in the *TNXB* gene" (Pyeritz, 2012).

ACMG also published a statement titled "Recommendations for reporting of secondary findings in clinical exome and genome sequencing". In it, *COL3A1* is listed for Ehlers-Danlos Syndrome, vascular type, and *FBN1, TGFBR1, TGFBR2, SMAD3, ACTA2,* and *MYH11* were listed as relevant genes for aortopathies (Miller et al., 2022; Miller et al., 2021).

American Academy of Pediatrics (AAP)

The AAP has released guidelines on the management of supervision of children with MFS. However, they allude to genetic testing of *FBN1*, stating it is "best reserved" for patients with "strong clinical suspicion" of MFS. The AAP states that younger patients (18 and under) should be evaluated periodically instead of undergoing genetic testing (Tinkle & Saal, 2013).

The Marfan Foundation

The Marfan Foundation has released recommendations on certain aspects of testing for MFS. The Foundation mentions several situations in which genetic testing may be useful, such as patients with features of multiple disorders, patients with a clinical symptom characteristic of MFS (such as ectopia lentis), children of parents affected by MFS, or adults with MFS that are considering having children. Prenatal testing may be performed, either a chorionic villus sampling (CVS) at 10-11 weeks or amniocentesis at 16-18 weeks. However, the parent's mutation must be confirmed before proceeding with either prenatal test (Foundation, 2024).

Screening of first-degree relatives of patients with MFS is also warranted. Aortic imaging may be performed if the mutation has not been identified (Foundation, 2015).

The Ehlers Danlos Society and the International Consortium on the Ehlers-Danlos Syndromes

These guidelines state that Ehlers-Danlos syndrome "Molecular diagnostic strategies should rely on NGS technologies, which offer the potential for parallel sequencing of multiple genes. Targeted resequencing of a panel of genes, for example, *COL5A1*, *COL5A2*, *COL1A1* and *COL1A2*, is a time- and cost-effective approach for the molecular diagnosis of the genetically heterogeneous EDS. When no mutation (or in case of an autosomal recessive condition only one mutation) is identified, this approach should be complemented with a copy number variant (CNV) detection strategy to identify large deletions or duplications, for example Multiplex Ligation-dependent Probe Amplification (MLPA), qPCR, or targeted array analysis. Alternatively, or in a second phase, whole exome sequencing (Nataatmadja et al.) or whole genome sequencing (WGS) and RNA sequencing techniques can be used, with data-analysis initially focusing on the genes of interest for a given EDS subtype. In absence of the identification of a causal mutation, this approach allows to expand the analysis to other genes within the genome. This is particularly interesting in view of the clinical overlap between EDS subtypes and with other HCTDs, and the observation that in an important proportion of EDS-patients, no pathogenic variants are identified in any of the known EDS-associated genes (Malfait et al., 2017)."

For cEDS, the following guidelines were given:

- "Molecular screening by means of targeted resequencing of a gene panel that includes at least the *COL5A1, COL5A2, COL1A1*, and *COL1A2* genes, or by WES or WGS, is indicated. When no mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications.
  Absence of these confirmatory findings does not exclude the diagnosis, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques; however, alternative diagnoses should be considered in the absence of (a) *COL5A1, COL5A2, COL1A1*, or *COL1A2* mutation(s)" (Malfait et al., 2017).

For classical-like EDS (clEDS), the following guidelines were given:

- "Molecular analysis of the *TNXB* gene should be used as the standard confirmatory test. Difficulties in DNA testing are related to the presence of a pseudogene (*TNXA*), which is more than 97% identical to the 3′ end of *TNXB* (exons 32–44). With the only exception of exon 35, which partially shows a TNXB-specific sequence, exon and intron sequences in this region are identical or

almost identical in both the gene and the pseudogene. This has implications both for sequencing and deletion/duplication analysis.

- For sequence analysis of *TNXB*, two approaches are recommended.
  - Sanger sequencing of the entire *TNXB* gene.
  - Next-generation sequencing of *TNXB* + Sanger sequencing of the pseudogene region."
- If no or only one causative mutation is identified by classic sequencing, additional methods that allow detection of large deletions/duplications should be added. So far no method is able to specifically detect TNXB CNVs in the highly homologous exons 32–34 and 36–44. CNV analysis of exon 35 is currently used to detect deletions in this region, including the 30 kb deletion
- Absence of these confirmatory findings does not exclude the diagnosis, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques; however, alternative diagnoses should be considered in the absence of a TNXB mutation (Malfait et al., 2017)."

For cardiac-valvular EDS (cvEDS), the following recommendations were given:

- "Molecular screening by Sanger sequencing of *COL1A2*, or targeted resequencing of a gene panel that includes *COL1A2* is indicated. When no mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications.
- In case of unavailability of genetic testing, SDS PAGE demonstrates total absence of (pro-) α2(I) collagen chains.
- Whereas absence of these confirmatory biochemical findings allows to exclude the diagnosis of cvEDS, absence of these confirmatory genetic findings does not exclude the diagnosis, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques (Malfait et al., 2017)."

For vEDS, the following guidelines were given:

- "Molecular screening by Sanger sequencing of *COL3A1*, or targeted resequencing of a gene panel that includes *COL3A1* and *COL1A1* (the latter to identify the above-listed arginine-to-cysteine substitution mutations) is indicated. When no mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications.
- Absence of these confirmatory findings does not exclude the diagnosis, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques; however, alternative diagnoses should

be considered in the absence of a *COL3A1* or *COL1A1* mutation (Malfait et al., 2017)."

For hypermobile EDS (hEDS), the following guidelines were given:

- "The diagnosis of hEDS remains clinical as there is yet no reliable or appreciable genetic etiology to test for in the vast majority of patients (Malfait et al., 2017)."

For arthrochalasia EDS (aEDS), the following guideline were given:

- "Molecular screening by Sanger sequencing of *COL1A1* and *COL1A2*, or targeted resequencing of a gene panel that includes these genes, is indicated. When no mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications.
- In case of unavailability of genetic testing, SDS PAGE of the pepsin-digested collagen in the medium or cell layer of cultured dermal fibroblasts demonstrates the presence of a mutant pNα1(I) or pNα2(I) chain (precursor procollagen chains in which the carboxy (C)-but not the amino (N)-propetide is cleaved off).
- TEM of skin specimens shows loosely and randomly organized collagen fibrils with a smaller and more variable diameter, and an irregular outline. These findings may support the diagnosis, but cannot confirm it.
- Absence of a causative mutation in *COL1A1* or *COL1A2* that leads to complete or partial deletion of the exon 6 of either gene excludes the diagnosis of aEDS (Malfait et al., 2017)."

For dermatosparaxis EDS (dEDS), the following guidelines were given:

- "Molecular screening by Sanger sequencing of targeted resequencing of a gene panel that includes *ADAMTS2* is indicated. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications.
- In case of unavailability of genetic testing, SDS, PAGE demonstrates presence of pNα1(I) and pNα2(I) chains of type I procollagen extracted from dermis in the presence of protease inhibitors or detected in fibroblast cultures.
- TEM shows collagen fibrils in affected skin specimens with a hieroglyphic pattern. These ultrastructural findings are usually typical but may be almost indistinguishable from those observed in aEDS. As such, they are not sufficient to confirm the diagnosis.
- Absence of these confirmatory findings does not exclude the diagnosis of dEDS, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques; however, alternative diagnoses

should be considered in the absence of ADAMTS2 mutations (Malfait et al., 2017)."

For kyphoscoliotic (kEDS), the following recommendations were given:

- Laboratory confirmation of kEDS should start with the quantification of deoxypyridinoline (Dpyr or LP for lysyl-pyridinoline) and pyridinoline (Pyr or HP for hydroxylysyl-pyridinoline) cross-links in urine quantitated by means of high-performance liquid chromatography (HPLC). An increased Dpyr/Pyr ratio is a highly sensitive and specific test for kEDS caused by biallelic PLOD1 mutations (kEDS-PLOD1), but is normal for biallelic FKBP14 mutations (kEDS-FKBP14).
- The normal ratio of Dpyr/Pyr cross-links is approximately 0.2, whereas in kEDS-PLOD1 the ratio is significantly increased (approximately 10–40 times increase, range 2–9). This method is fast and cost-effective and it can also be used to determine the pathogenic status of a VUS in PLOD1.
- SDS–PAGE may detect faster migration of underhydroxylated collagen chains and their derivatives in kEDS-*PLOD1* but not in kEDS-*FKBP14*. However, abnormalities in migration can be subtle.
- Molecular analysis for kEDS-*PLOD1* may start with MLPA analysis of *PLOD1*, for the evaluation of the common intragenic duplication in PLOD1 caused by an Alu-Alu recombination between introns 9 and 16 (the most common mutant allele) [Hautala et al., 1993].
- Molecular screening by means of targeted resequencing of a gene panel that includes *PLOD1* and *FKBP14*, is indicated when MLPA of *PLOD1* fails to identify the common duplication. Such a gene panel my also include other genes associated with phenotypes that clinically overlap with kEDS, such as *ZNF469, PRDM5, B4GALT7, B3GALT6, SLC39A13, CHST14* and *DSE*. Alternatively, WES may be performed. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications in these genes.
- TEM on skin specimens has shown variable diameters and abnormal contours of the collagen fibrils and irregular interfibrillar space, but these abnormalities are not unique to this condition. As such, whereas TEM on a skin biopsy can support diagnosis, it cannot confirm it.
- Whereas absence of an abnormal urinary LP/HP ratio excludes the diagnosis of kEDS-PLOD1, absence of the confirmatory genetic findings does not exclude the diagnosis of kEDS, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques and/or other, yet to be discovered, genes, may be associated with this phenotype; however, alternative diagnoses should be considered in the absence of *PLOD1* or *FKBP14* mutations (Malfait et al., 2017)."

For brittle cornea syndrome (BCS), the following guidelines were given:

- "Molecular screening by means of targeted resequencing of a gene panel that includes ZNF469 and PRDM5 is indicated. Such a gene panel my also include other genes associated with phenotypes that clinically overlap with BCS, such as *PLOD1, FKBP14, B4GALT7, B3GALT6, SLC39A13, CHST14*, and *DSE*. Alternatively, WES may be performed. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications in these genes.
- Absence of these confirmatory findings does not exclude the diagnosis, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques, and other, yet unknown genes, might be associated with BCS (Malfait et al., 2017)."

For spondylodysplastic EDS (spEDS), the following guidelines were given:

- Molecular screening by means of targeted resequencing of a gene panel that includes *B4GALT7, B3GALT6*, and *SLC39A13* is indicated. Such a gene panel my also include other genes associated with phenotypes that clinically overlap with spEDS, such as *PLOD1, FKBP14, ZNF469, PRDM5, CHST14,* and *DSE*. Alternatively, WES may be performed. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications in these genes.
- For definite proof of GAG deficiency (*B4GALT7* and *B3GALT6* mutations), biochemical methods to assess GAG synthesis in patients' cultured fibroblasts are currently available in many specialized laboratories.
- The laboratory measurement of urinary pyridinolines, lysyl-pyridinoline (LP) and hydroxylysyl-pyridinoline (HP) quantitated by HPLC allows the detection of an increased ratio LP/HP to approximately 1, (compared to a normal value of approximately 0.2) in patients with mutations in *SLC39A13*. This fast and cost-effective method can also be used to determine the pathogenic status of a VUS (see also "verification of diagnosis" in kEDS-PLOD1).
- Absence of confirmatory genetic findings does not exclude the diagnosis of spEDS, as specific types of mutations (eg deep intronic mutations) may go undetected by standard diagnostic molecular techniques, and still other, yet to be discovered, genes may be associated with these phenotypes. In case no *B4GALT7*, *B3GALT6*, or *SCL39A13* mutations are identified, alternative diagnoses should however be considered (Malfait et al., 2017)."

For musculocontractural EDS (mcEDS), the following guidelines were given:

- "Molecular screening by means of targeted resequencing of a gene panel that includes *CHST14* and *DSE* is indicated. Such a gene panel my also include other genes associated with phenotypes that clinically overlap with mcEDS, such as *PLOD1, FKBP14, ZNF469, PRDM5*, *B4GALT7, B3GALT6* and *SLC39A13*. Alternatively, WES may be performed. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications in these genes.
- Absence of these confirmatory genetic findings does not exclude the diagnosis of mcEDS, as specific types of mutations (e.g., deep intronic mutations) may go undetected by standard diagnostic molecular techniques. In case no CHST14 or DSE mutations are identified, alternative diagnoses should be considered (Malfait et al., 2017)."

For myopathic EDS (mEDS), the following guidelines were given:

- "Molecular screening by means of targeted resequencing of a gene panel that includes *COL12A1* is indicated. Such a gene panel my also include other genes associated with phenotypes that clinically overlap with mEDS, such as *COL6A1*, *COL6A2*, *COL6A3*. Alternatively, WES may be performed. When no, or only one, causative mutation is identified, this approach should be complemented with a CNV detection strategy to identify large deletions or duplications in these genes.
- Absence of these confirmatory findings does not exclude the diagnosis, as specific types of mutations (eg deep intronic mutations) may go undetected by standard diagnostic molecular techniques, and other, yet to be discovered, genes may be associated with this phenotype (Malfait et al., 2017)."

For periodontal EDS (pEDS), the following guidelines were given:

- "Identification of known or compatible mutations by sequence analysis of C1R and C1S. Large deletions or null mutations that completely remove C1r or C1s protein function do not cause pEDS.
- At present it cannot be stated whether absence of a C1R or C1S mutations excludes the diagnosis because the experience with the molecular diagnosis is limited (Malfait et al., 2017)."

Canadian Cardiovascular Society (CCS)

The CCS has published recommendations for MFS stating a strong recommendation for clinical and genetic screening for anyone with suspected MFS "to clarify the nature of the disease and provide a basis for individual genetic counseling" (Boodhwani et al., 2014).

The CCS also published recommendations for non-Marfan genetic forms of aortic disease such as thoracic aortic disease (TAD). These guidelines state that "We recommend screening for TAD-associated genes in non-BAV aortopathy index cases to clarify the origin of disease and improve clinical and genetic counselling (Boodhwani et al., 2014)." These guidelines also state that individuals with a known LDS mutation (such as *TGFBR1/2*, *TGFB*, *SMAD3*, *ACTA2*, or *MYH11*) should receive complete aortic imaging when diagnosed and 6 months after diagnosis.

International Group of Specialists with a Broad Aggregate Experience in the Care of Individuals with Vascular EDS

Recommendations made by this group of vEDS specialists recommend to "identify causative variants in *COL3A1* prior to [the] application of diagnosis" of vEDS (Byers et al., 2017).

National Organization for Rare Disorders (NORD)

NORD has posted recommendations on EB stating that "When EB is suspected, a skin biopsy should be obtained and sent to an appropriate laboratory to confirm the diagnosis with transmission electron microscopy (TEM) and/or immunofluorescent antibody/antigen mapping. Molecular genetic testing for mutations in most of the genes known to be associated with the various types of EB is clinically available" (NORD, 2024).

On the diagnosis of EDS, the NORD has stated that diagnosis is generally made using patient histories and clinical findings, and that genetic testing can help in the diagnosis of some subtypes. Electron microscopic analysis could also aid in revealing the collagen abnormalities seen in EDS. "The clinical evaluation of individuals with suspected or diagnosed EDS typically includes assessments to detect and determine the extent of skin and joint hyperextensibility." The NORD also posted recommendations of utilizing computerized tomography (Musunuru et al.) scanning, magnetic resonance imaging (MRI), and echocardiography to observe any mitral valve prolapse and aortic dilatation. On kEDS, NORD has written of confirmatory tests using "either a urine sample and extrapolated ratio of deoxypyridinoline to pyridinoline cross-links, or on a skin biopsy sample and measurement of lysyl hydroxylase enzyme activity from skin fibroblast cells" (NORD, 2021)

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state

coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, please visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81405	Molecular pathology procedure, Level 6 Genes: ***TGFBR1*** (transforming growth factor, beta receptor 1) (eg, Marfan syndrome), full gene sequence ***TGFBR2*** (transforming growth factor, beta receptor 2) (eg, Marfan syndrome), full gene sequence
81408	Molecular pathology procedure, Level 9 Genes: ***FBN1*** (fibrillin 1) (eg, Marfan syndrome), full gene sequence
81410	Aortic dysfunction or dilation (eg, Marfan syndrome, Loeys Dietz syndrome, Ehler Danlos syndrome type IV, arterial tortuosity syndrome); genomic sequence analysis panel, must include sequencing of at least 9 genes, including FBN1, TGFBR1, TGFBR2, COL3A1, MYH11, ACTA2, SLC2A10, SMAD3, and MYLK
81411	Aortic dysfunction or dilation (eg, Marfan syndrome, Loeys Dietz syndrome, Ehler Danlos syndrome type IV, arterial tortuosity syndrome); duplication/deletion analysis panel, must include analyses for TGFBR1, TGFBR2, MYH11, and COL3A1

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Becerra-Munoz, V. M., Gomez-Doblas, J. J., Porras-Martin, C., Such-Martinez, M., Crespo-Leiro, M. G., Barriales-Villa, R., de Teresa-Galvan, E., Jimenez-Navarro, M., & Cabrera-Bueno, F. (2018). The importance of genotype-phenotype correlation in the clinical management of Marfan syndrome. *Orphanet J Rare Dis*, *13*(1), 16. https://doi.org/10.1186/s13023-017-0754-6

Bin Mahmood, S. U., Velasquez, C. A., Zafar, M. A., Saeyeldin, A. A., Brownstein, A. J., Ziganshin, B. A., Elefteriades, J. A., & Mukherjee, S. K. (2017). Medical management of aortic disease in Marfan syndrome. *Ann Cardiothorac Surg*, *6*(6), 654-661. https://doi.org/10.21037/acs.2017.11.09

Boodhwani, M., Andelfinger, G., Leipsic, J., Lindsay, T., McMurtry, M. S., Therrien, J., & Siu, S. C. (2014). Canadian Cardiovascular Society position statement on the management of thoracic aortic disease. *Can J Cardiol*, *30*(6), 577-589. https://doi.org/10.1016/j.cjca.2014.02.018

Byers, P. H., Belmont, J., Black, J., De Backer, J., Frank, M., Jeunemaitre, X., Johnson, D., Pepin, M., Robert, L., Sanders, L., & Wheeldon, N. (2017). Diagnosis, natural history, and management in vascular Ehlers-Danlos syndrome. *Am J Med Genet C Semin Med Genet*, *175*(1), 40-47. https://doi.org/10.1002/ajmg.c.31553

Chen, S., Fei, H., Zhang, J., Chen, Y., Huang, H., Lu, D., & Xu, C. (2021). Classification and Interpretation for 11 FBN1 Variants Responsible for Marfan Syndrome and Pre-implantation Genetic Testing (PGT) for Two Families Successfully Blocked Transmission of the Pathogenic Mutations. *Front Mol Biosci*, *8*, 749842. https://doi.org/10.3389/fmolb.2021.749842

Coulombe, P. A., Hutton, M. E., Letai, A., Hebert, A., Paller, A. S., & Fuchs, E. (1991). Point mutations in human keratin 14 genes of epidermolysis bullosa simplex patients: genetic and functional analyses. *Cell*, *66*(6), 1301-1311. https://doi.org/10.1016/0092-8674(91)90051-y

Dale, M., Fitzgerald, M. P., Liu, Z., Meisinger, T., Karpisek, A., Purcell, L. N., Carson, J. S., Harding, P., Lang, H., Koutakis, P., Batra, R., Mietus, C. J., Casale, G., Pipinos, I., Baxter, B. T., & Xiong, W. (2017). Premature aortic smooth muscle cell differentiation contributes to matrix dysregulation in Marfan Syndrome. *PLoS One*, *12*(10), e0186603. https://doi.org/10.1371/journal.pone.0186603

Damseh, N., Dupuis, L., O'Connor, C., Oh, R. Y., Wang, Y. W., Stavropoulos, D. J., Schwartz, S. B., & Mendoza-Londono, R. (2022). Diagnostic outcomes for molecular genetic testing in children with suspected Ehlers-Danlos syndrome. *Am J Med Genet A*, *188*(5), 1376-1383. https://doi.org/10.1002/ajmg.a.62672

Davis, M. R., & Summers, K. M. (2012). Structure and function of the mammalian fibrillin gene family: implications for human connective tissue diseases. *Mol Genet Metab*, *107*(4), 635-647. https://doi.org/10.1016/j.ymgme.2012.07.023

Dietz, H. (2017). Marfan Syndrome. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews((R))*. University of Washington, SeattleUniversity of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved. https://www.ncbi.nlm.nih.gov/pubmed/20301510

Dietz, H. C., Loeys, B., Carta, L., & Ramirez, F. (2005). Recent progress towards a molecular understanding of Marfan syndrome. *Am J Med Genet C Semin Med Genet*, *139c*(1), 4-9. https://doi.org/10.1002/ajmg.c.30068

Faivre, L., Collod-Beroud, G., Loeys, B., Child, A., Binquet, C., Gautier, E., Callewaert, B., Arbustini, E., Mayer, K., Arslan-Kirchner, M., Kiotsekoglou, A., Comeglio, P., Marziliano, N., Dietz, H., Halliday, D., Beroud, C., Bonithon-Kopp, C., Claustres, M., Muti, C., . . . Boileau, C. (2007). Effect of Mutation Type and Location on Clinical Outcome in 1,013 Probands with Marfan Syndrome or Related Phenotypes and FBN1 Mutations: An International Study. *Am J Hum Genet*, *81*(3), 454-466. https://www.ncbi.nlm.nih.gov/pubmed/17701892

Foundation, M. (2015). *Overview of Cardiac Management in Marfan Syndrome*. The Marfan Foundation. https://marfan.org/wp-content/uploads/2021/10/Cardiac-Management-2015.pdf

Foundation, M. (2024). *Genetic Testing and Marfan Syndrome*. The Marfan Foundation,. https://marfan.org/resource-library/genetic-testing-and-marfan-syndrome/

Grewal, N., & Gittenberger-de Groot, A. C. (2018). Pathogenesis of aortic wall complications in Marfan syndrome. *Cardiovasc Pathol*, *33*, 62-69. https://doi.org/10.1016/j.carpath.2018.01.005

Hiratzka, L. F., Bakris, G. L., Beckman, J. A., Bersin, R. M., Carr, V. F., Casey, D. E., Jr., Eagle, K. A., Hermann, L. K., Isselbacher, E. M., Kazerooni, E. A., Kouchoukos, N. T., Lytle, B. W., Milewicz, D. M., Reich, D. L., Sen, S., Shinn, J. A., Svensson, L. G., & Williams, D. M. (2010). 2010 ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM guidelines for the diagnosis and management of patients with Thoracic Aortic Disease. *Circulation*, *121*(13), e266-369. https://doi.org/10.1161/CIR.0b013e3181d4739e

Jensen, S. A., & Handford, P. A. (2016). New insights into the structure, assembly and biological roles of 10-12 nm connective tissue microfibrils from fibrillin-1 studies. *Biochem J*, *473*(7), 827-838. https://doi.org/10.1042/bj20151108

Johansen, H., Velvin, G., & Lidal, I. (2020). Adults with Loeys-Dietz syndrome and vascular Ehlers-Danlos syndrome: A cross-sectional study of health burden perspectives. *Am J Med Genet A*, *182*(1), 137-145. https://doi.org/10.1002/ajmg.a.61396

Jud, P., & Hafner, F. (2019). Vascular Involvement in Loeys-Dietz Syndrome. *Mayo Clin Proc*, *94*(6), 1117-1119. https://doi.org/10.1016/j.mayocp.2018.12.030

Koo, H. K., Lawrence, K. A., & Musini, V. M. (2017). Beta-blockers for preventing aortic dissection in Marfan syndrome. *Cochrane Database Syst Rev*, *11*, Cd011103. https://doi.org/10.1002/14651858.CD011103.pub2

Li, J., Yang, L., Diao, Y., Zhou, L., Xin, Y., Jiang, L., Li, R., Wang, J., Duan, W., & Liu, J. (2021). Genetic testing and clinical relevance of patients with thoracic aortic aneurysm and dissection in northwestern China. *Mol Genet Genomic Med*, *9*(10), e1800. https://doi.org/10.1002/mgg3.1800

Loeys, B., De Backer, J., Van Acker, P., Wettinck, K., Pals, G., Nuytinck, L., Coucke, P., & De Paepe, A. (2004). Comprehensive molecular screening of the FBN1 gene favors locus homogeneity of classical Marfan syndrome. *Hum Mutat*, *24*(2), 140-146. https://doi.org/10.1002/humu.20070

Loeys, B., & Dietz, H. (2018, 03/01/2018). *Loeys-Dietz Syndrome*. University of Washington, Seattle. Retrieved 01/08/2019 from https://www.ncbi.nlm.nih.gov/books/NBK1133/

Loeys, B. L., Dietz, H. C., Braverman, A. C., Callewaert, B. L., De Backer, J., Devereux, R. B., Hilhorst-Hofstee, Y., Jondeau, G., Faivre, L., Milewicz, D. M., Pyeritz, R. E., Sponseller, P. D., Wordsworth, P., & De Paepe, A. M. (2010). The revised Ghent nosology for the Marfan syndrome. *Journal of Medical Genetics*, *47*(7), 476. https://doi.org/10.1136/jmg.2009.072785

MacCarrick, G., Black, J. H., 3rd, Bowdin, S., El-Hamamsy, I., Frischmeyer-Guerrerio, P. A., Guerrerio, A. L., Sponseller, P. D., Loeys, B., & Dietz, H. C., 3rd. (2014). Loeys-Dietz syndrome: a primer for diagnosis and management. *Genet Med*, *16*(8), 576-587. https://doi.org/10.1038/gim.2014.11

Malfait, F. (2018). Vascular aspects of the Ehlers-Danlos Syndromes. *Matrix Biol*, *71-72*, 380-395. https://doi.org/10.1016/j.matbio.2018.04.013

Malfait, F., Francomano, C., Byers, P., Belmont, J., Berglund, B., Black, J., Bloom, L., Bowen, J. M., Brady, A. F., Burrows, N. P., Castori, M., Cohen, H., Colombi, M., Demirdas, S., De Backer, J., De Paepe, A., Fournel-Gigleux, S., Frank, M., Ghali, N., . . . Tinkle, B. (2017). The 2017 international classification of the Ehlers-Danlos syndromes. *Am J Med Genet C Semin Med Genet*, *175*(1), 8-26. https://doi.org/10.1002/ajmg.c.31552

Mariath, L. M., Santin, J. T., Frantz, J. A., Doriqui, M. J. R., Kiszewski, A. E., & Schuler-Faccini, L. (2019). An overview of the genetic basis of epidermolysis bullosa in Brazil: discovery of novel and recurrent disease-causing variants. *Clin Genet*, *96*(3), 189-198. https://doi.org/10.1111/cge.13555

Miller, D. T., Lee, K., Abul-Husn, N. S., Amendola, L. M., Brothers, K., Chung, W. K., Gollob, M. H., Gordon, A. S., Harrison, S. M., Hershberger, R. E., Klein, T. E., Richards, C. S., Stewart, D. R., & Martin, C. L. (2022). ACMG SF v3.1 list for reporting of secondary findings in clinical exome and genome sequencing: A policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *24*(7), 1407-1414. https://doi.org/10.1016/j.gim.2022.04.006

Miller, D. T., Lee, K., Gordon, A. S., Amendola, L. M., Adelman, K., Bale, S. J., Chung, W. K., Gollob, M. H., Harrison, S. M., Herman, G. E., Hershberger, R. E., Klein, T. E., McKelvey, K., Richards, C. S., Vlangos, C. N., Stewart, D. R., Watson, M. S., & Martin, C. L. (2021).

Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2021 update: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *23*(8), 1391-1398. https://doi.org/10.1038/s41436-021-01171-4

Murrell, D. F. (2024, Jan. 8). *Overview of the management of epidermolysis bullosa*. https://www.uptodate.com/contents/overview-of-the-management-of-epidermolysis-bullosa

Musunuru, K., Hershberger Ray, E., Day Sharlene, M., Klinedinst, N. J., Landstrom Andrew, P., Parikh Victoria, N., Prakash, S., Semsarian, C., Sturm Amy, C., & null, n. (2020). Genetic Testing for Inherited Cardiovascular Diseases: A Scientific Statement From the American Heart Association. *Circulation: Genomic and Precision Medicine*, *13*(4), e000067. https://doi.org/10.1161/HCG.0000000000000067

Nataatmadja, M., West, M., West, J., Summers, K., Walker, P., Nagata, M., & Watanabe, T. (2003). Abnormal extracellular matrix protein transport associated with increased apoptosis of vascular smooth muscle cells in marfan syndrome and bicuspid aortic valve thoracic aortic aneurysm. *Circulation*, *108 Suppl 1*, Ii329-334. https://doi.org/10.1161/01.cir.0000087660.82721.15

Neptune, E. R., Frischmeyer, P. A., Arking, D. E., Myers, L., Bunton, T. E., Gayraud, B., Ramirez, F., Sakai, L. Y., & Dietz, H. C. (2003). Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. *Nat Genet*, *33*(3), 407-411. https://doi.org/10.1038/ng1116

NIH. (2016). *What are heritable disorders of connective tissue?* https://www.niams.nih.gov/health-topics/heritable-disorders-connective-tissue

NIH. (2020). Epidermolysis bullosa simplex. https://ghr.nlm.nih.gov/condition/epidermolysis-bullosa-simplex#genes

NORD. (2021, 09/27/21). *Ehlers Danlos Syndromes*. Retrieved January 20 from https://rarediseases.org/rare-diseases/ehlers-danlos-syndrome/

NORD. (2024, 01/22/24). *Epidermolysis Bullosa*. https://rarediseases.org/rare-diseases/epidermolysis-bullosa/

Pauker, S., & Stoler, J. (2023, Oct. 10). *Clinical manifestations and diagnosis of Ehlers-Danlos syndromes*. https://www.uptodate.com/contents/clinical-manifestations-and-diagnosis-of-ehlers-danlos-syndromes

Pyeritz, R. E. (2012). Evaluation of the adolescent or adult with some features of Marfan syndrome. *Genet Med*, *14*(1), 171-177. https://doi.org/10.1038/gim.2011.48

Pyeritz, R. E. (2017). Etiology and pathogenesis of the Marfan syndrome: current understanding. *Ann Cardiothorac Surg*, *6*(6), 595-598. https://doi.org/10.21037/acs.2017.10.04

Radke, R. M., & Baumgartner, H. (2014). Diagnosis and treatment of Marfan syndrome: an update. *Heart*, *100*(17), 1382-1391. https://doi.org/10.1136/heartjnl-2013-304709

Ritelli, M., Rovati, C., Venturini, M., Chiarelli, N., Cinquina, V., Castori, M., & Colombi, M. (2020). Application of the 2017 criteria for vascular Ehlers-Danlos syndrome in 50 patients ascertained according to the Villefranche nosology. *Clin Genet*, *97*(2), 287-295. https://doi.org/10.1111/cge.13653

Ryan, T. D., Lucky, A. W., King, E. C., Huang, G., Towbin, J. A., & Jefferies, J. L. (2016). Ventricular dysfunction and aortic dilation in patients with recessive dystrophic epidermolysis bullosa. *Br J Dermatol*, *174*(3), 671-673. https://doi.org/10.1111/bjd.14168

Saunderson, R. B., Vekic, D. A., Mallitt, K., Mahon, C., Robertson, S. J., & Wargon, O. (2019). A retrospective cohort study evaluating the accuracy of clinical diagnosis compared with immunofluorescence and electron microscopy in children with inherited epidermolysis bullosa. *Br J Dermatol*, *180*(5), 1258-1259. https://doi.org/10.1111/bjd.17648

Seike, Y., Matsuda, H., Inoue, Y., Sasaki, H., Morisaki, H., Morisaki, T., & Kobayashi, J. (2020). The differences in surgical long-term outcomes between Marfan syndrome and Loeys-Dietz syndrome. *J Thorac Cardiovasc Surg*. https://doi.org/10.1016/j.jtcvs.2020.07.089

Sellers, S. L., Milad, N., Chan, R., Mielnik, M., Jermilova, U., Huang, P. L., de Crom, R., Hirota, J. A., Hogg, J. C., Sandor, G. G., Van Breemen, C., Esfandiarei, M., Seidman, M. A., & Bernatchez, P. (2018). Inhibition of Marfan Syndrome Aortic Root Dilation by Losartan: Role of Angiotensin II Receptor Type 1-Independent Activation of Endothelial Function. *Am J Pathol*, *188*(3), 574-585. https://doi.org/10.1016/j.ajpath.2017.11.006

Shalhub, S., Byers, P. H., Hicks, K. L., Coleman, D. M., Davis, F. M., De Caridi, G., Weaver, K. N., Miller, E. M., Schermerhorn, M. L., Shean, K., Oderich, G., Ribeiro, M., Nishikawa, C., Charlton-Ouw, K., Behrendt, C. A., Debus, E. S., von Kodolitsch, Y., Zarkowsky, D., Powell, R. J., . . . Woo, K. (2020). A multi-institutional experience in vascular Ehlers-Danlos syndrome diagnosis. *J Vasc Surg*, *71*(1), 149-157. https://doi.org/10.1016/j.jvs.2019.04.487

Tinkle, B. T., & Saal, H. M. (2013). Health Supervision for Children With Marfan Syndrome. *Pediatrics*, *132*(4), e1059. https://doi.org/10.1542/peds.2013-2063

Veatch, O. J., Steinle, J., Hossain, W. A., & Butler, M. G. (2022). Clinical genetics evaluation and testing of connective tissue disorders: a cross-sectional study. *BMC Med Genomics*, *15*(1), 169. https://doi.org/10.1186/s12920-022-01321-w

Wright, M. J., & Connolly, H. M. (2023, April 29). *Genetics, clinical features, and diagnosis of Marfan syndrome and related disorders*. https://www.uptodate.com/contents/genetics-clinical-features-and-diagnosis-of-marfan-syndrome-and-related-disorders

Genetic Testing for Cystic Fibrosis

Policy Number: AHS – M2017 – Genetic Testing for Cystic Fibrosis	Prior Policy Name and Number, as applicable:
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Cystic fibrosis (CF) is an autosomal recessive genetic disease in which dysfunctional epithelial chloride channels lead to excessively thick mucus affecting multiple organ systems. Common complications include mucous plugging of the airway, lung inflammation, chronic pulmonary infections, intestinal malabsorption, pancreatic insufficiency, and infertility (Pritchard, 2016).

When pursuing genetic testing for cystic fibrosis, genetic counseling is strongly recommended.

For guidance on pre-implantation genetic testing, please see AHS-M2039-Pre-Implantation Genetic Testing. For guidance on prenatal screening and preconception screening for cystic fibrosis, please see AHS-M2179-Prenatal Screening (Genetic).

II. Related Policies

Policy Number	Policy Title
AHS-M2039	Pre-Implantation Genetic Testing
AHS-M2179	Prenatal Screening (Genetic)

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Common variant testing (see Note 1) or comprehensive gene sequencing for mutations in the *CFTR* gene **MEETS COVERAGE CRITERIA** in **any** of the following situations:
   a) In a newborn after an abnormal newborn screening result using immunoreactive trypsinogen.
   b) For infants with meconium ileus.
   c) For individuals with congenital absence of the vas deferens (CAVD).
   d) For individuals with idiopathic pancreatitis or bronchiectasis.

2) As an adjunct to sweat testing in an individual presenting with symptoms of cystic fibrosis, common variant testing (see Note 1) or comprehensive gene sequencing for mutations in the *CFTR* gene **MEETS COVERAGE CRITERIA** in **any** of the following situations:
   a) When there are no known familial mutations.
   b) When there are known familial mutations; testing must include the familial mutations.
   c) When only one familial mutation is known; testing should include the known familial mutation.

3) For individuals with congenital bilateral absence of the vas deferens (CBAVD), common variant testing (see Note 1) or comprehensive gene sequencing for mutations in the *CFTR* gene, including testing for the *CFTR* IVS8 5T/7T/9T variant, **MEETS COVERAGE CRITERIA**.

4) When the *CFTR* R117H mutation is detected on carrier screening, reflex genetic testing for the *CFTR* IVS8 5T/7T/9T variant **MEETS COVERAGE CRITERIA**.

5) For individuals for whom common variant testing (see Note 1) identified no mutations or only one mutation, but for whom the clinical suspicion of cystic fibrosis still remains, comprehensive sequencing of the *CFTR* gene **MEETS COVERAGE CRITERIA** in **any** of the following situations:
   a) For individuals that presented with symptoms of cystic fibrosis and who received panel testing as an adjunct to sweat testing.
   b) When a diagnosis of cystic fibrosis related CBAVD remains a consideration in individuals with CBAVD.

6) For all other situations not described above, genetic testing for mutations in the *CFTR* gene **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Common variant testing for *CFTR* mutations must include the American College of Medical Genetics' (ACMG) *CFTR* carrier screening variant set (n=100). Please see the "Guidelines and Recommendations" section of this policy for a table of ACMG's *CFTR* minimum variant set.

IV. Table of Terminology

Term	Definition
ACMG	American College of Medical Genetics
ACOG	American College of Obstetrics and Gynecology
CAVD	Congenital absence of the vas deferens
CBAVD	Congenital bilateral absence of the vas deferens
CF	Cystic fibrosis
CFCD	Cystic fibrosis carrier detection
CFF	Cystic Fibrosis Foundation
CFSPID	Cystic fibrosis screen positive inconclusive diagnosis
CFTR	Cystic fibrosis transmembrane conductance regulator
CFTR2	Clinical and functional translation of CFTR
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CLSI	Clinical and Laboratory Standards Institute
CMS	Centers For Medicare and Medicaid
CPIC	Clinical Pharmacogenetics Implementation Consortium
CRMS	Customer relationship management system
CVS	Chorionic villus sampling
DNA	Deoxyribonucleic acid
ECFS	European Cystic Fibrosis Society
FDA	Food and Drug Administration
FEV1	Forced expiratory volume in 1 second

ICM	Intestinal current measurement
IRT	Immunoreactive trypsinogen
IVA	Ivacaftor
LDT	Laboratory-developed test
LUM	Lumacaftor
MMWR	Morbidity And Mortality Weekly Report
MVCC	Mutation of varying clinical consequence
NBS	Newborn screening
NGS	Next generation sequencing
NICE	National Institute for Health and Care Excellence
NPD	Nasal potential difference
NSGC	National Society of Genetic Counselors
PCR	Polymerase chain reaction
PCRM	Physicians Committee for Responsible Medicine
PCS	Cystic fibrosis carrier screening
QALY	Quality adjusted life year
RT-PCR	Reverse transcription polymerase chain reaction
TDAS	XTAG Data Analysis Software
TDN	Therapeutics Development Network
TOP	Termination of pregnancy
UNK	Uncharacterized mutation
VUS	Variant of uncertain significance

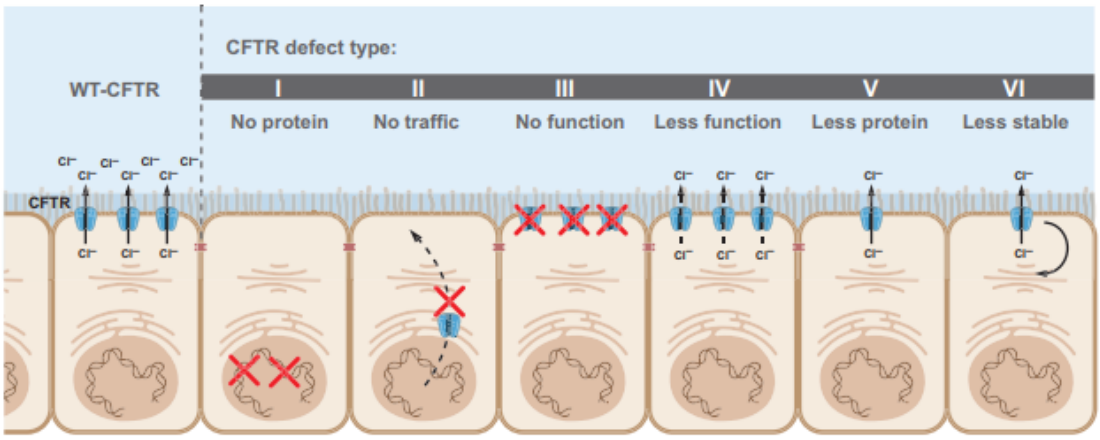
V. Scientific Background

Cystic fibrosis (CF) is a common life-limiting autosomal recessive genetic disorder caused by the mutation of a gene that encodes an epithelial chloride-conducting transmembrane channel called the cystic fibrosis transmembrane conductance regulator (CFTR). This gene regulates anion (negatively charged ion) transport and mucociliary clearance. Mucociliary clearance is the process by which particles and gases dissolved in the mucus are moved unidirectionally from the respiratory tract. The CFTR protein was first identified as a chloride channel but has been shown to facilitate or regulate the transport of other ions, such as sodium, thiocyanate, bicarbonate, as well as water absorption and excretion (Clancy & Jain, 2012; Cohen-Cymberknoh et al., 2011). The CFTR protein is present in the epithelia of various tissues, including that of the lungs, sweat glands, gastrointestinal tract, and pancreas (Barrett et al., 2012). CFTR

dysfunction mainly affects epithelial cells; although, there is evidence of a role in immune cells.

Mutations in the *CFTR* gene which impede protein production, stability, or activity result in less available functional protein (Clancy & Jain, 2012). Functional failure of CFTR results in defective mucociliary clearance, chronic infection, and abnormal inflammatory response leading to progressive, irreversible lung damage (Cohen-Cymberknoh et al., 2011). Other manifestations of dysfunctional CFTR include pancreatic insufficiency and meconium ileus (Barrett et al., 2012). The early identification and treatment of patients by multidisciplinary teams have resulted in improvements in both quality of life and clinical outcomes in patients with cystic fibrosis, with life expectancy reaching 50 or even 60 years (Clancy & Jain, 2012).

Most mutations of the *CFTR* gene are missense alterations, but other mutations, deletions and insertions have been described (Bell et al., 2015). Missense mutations occur when a single nucleotide is changed, resulting in a different amino acid which may affect the overall protein functionality. To date, over 2000 mutations have been identified in the *CFTR* gene (CF Foundation, 2023). *CFTR* mutations can be divided into six classes according to their effects on protein function as depicted in the figure below (Bell et al., 2015).



Mutation examples	G542X (a) W1282X (a) 1717-1G (b)	F508del N1303K A561E	G551D S549R G1349D	R117H R334W A455E	A455E 3272-26A>G 3849+10 kb C>T	c.120del23 rF508del
Corrective therapy	Rescue synthesis	Rescue traffic	Restore channel activity	Restore channel activity	Correct splicing	Promote stability
Drug	Read-through compounds	Correctors	Potentiators	Potentiators	AONs Correctors Potentiators	Stabilizers
Drug approved (Yes/No)	No	No	Yes	No	No	No

Class I, II, and III mutations are associated with no residual CFTR function and patients with these mutations have a severe phenotype, whereas individuals with class IV, V, and VI mutations have some residual function of CFTR protein and have a mild lung phenotype and pancreatic sufficiency (Bell et al., 2015; Wilschanski et al., 1995).

Due to the relatively high frequency in the U.S. population and studies demonstrating that early detection and care can improve outcomes, CF testing is included as part of the newborn screen in all states (Grosse et al., 2004). Newborn screening (NBS) for CF can include testing for immunoreactive trypsinogen (IRT), which is a pancreatic enzyme found at elevated levels in the blood of some individuals with CF, genetic testing for common CF mutations, or a combination of IRT and genetic testing. Positive newborn screens are typically followed by genetic testing (if not already done), and a sweat test, which measures the chloride concentration in sweat. The sweat test historically has been considered the "gold standard" for CF diagnosis. The presence of two disease-causing mutations also may be considered diagnostic of the disease, even in the absence of classic symptoms or in the case of a negative or inconclusive sweat test (Farrell et al., 2008).

In addition to NBS, CF carrier screening (PCS) has become commonplace in the U.S., particularly among pregnant individuals and couples planning a pregnancy. PCS has been found to markedly reduce CF birth rates with a shift towards milder mutations, but it was often avoided for cultural reasons necessitating the use of complementary PCS and NBS (Stafler et al., 2016).

A subset of 23 mutations accounts for most cystic fibrosis cases in the U.S. and were accepted by most guidelines as the primary genes to be screened for diagnosis of CF and carrier status. The most common CF-causing mutation is F508del, which is present in over 70% of known CF cases. The Clinical and Functional Translation of CFTR (CFTR2) project continually evaluates genotype and phenotype correlations and has confirmed many additional mutations as being causative of CF (CF Foundation, 2023). Information from over 88,000 patients with specific cystic fibrosis variants from the United States, Canada, and Europe was collected by the CFTR2 team from national CF Patient Registries and placed in the CFTR2 database and then compiled into the CFTR2 website that contains information about the 322 most common CFTR variants (CF Foundation, 2023).

Analysis of data from the CF Foundation Patient Registry found that patients of Hispanic, black, or Asian ancestry were less likely to have two identified *CFTR* variants and more likely to carry no mutations on the commonly used 23 mutation carrier screening panel (Schrijver et al., 2016). Analysis of the Exome Aggregation Consortium dataset also found that none of the current genetic screening panels or existing *CFTR* mutation databases covered most deleterious variants in any geographical population outside of Europe. Both clinical annotation and mutation coverage by commercially available targeted screening panels for CF are strongly biased toward detection of reproductive risk in persons of European descent (Lim et al., 2016). This research indicates the possible need for adjustment of this panel to facilitate equity in mutation detection between white and nonwhite or mixed-ethnicity CF patients, enabling an earlier diagnosis improving their quality of life.

Proprietary Testing

There are several cystic fibrosis genotyping assays approved by the FDA. eSensor Cystic Fibrosis Carrier Detection System detects 24 CFTR mutations (Coty, 2006), Tag-It™ Cystic Fibrosis Kit detects 39 mutations (FDA, 2006b), Cystic Fibrosis Genotyping Assay detects 30 mutations (FDA, 2007), InPlex™ CF Molecular Test detects 32 mutations (Hologic, 2008) , Verigene®CFTR and Verigene®CFTR PolyT Nucleic Acid Tests detect 26 mutations (FDA, 2009b), xTAG® Cystic Fibrosis 71 v2 Kit detects 71

mutations (Luminex, 2024) , and Illumina MiSeqDx™ Cystic Fibrosis 139-Variant Assay (Illumina, 2013).

Next generation sequencing (NGS) is also being integrated into CF diagnostic protocols. A diagnostic protocol in which a quick and relatively low-cost polymerase chain reaction (PCR)-based screening for the most common CF mutation, F508del, followed by full-gene next generation sequencing of *CFTR* was shown to improve genetic diagnosis and "holds promise to be a straightforward, convenient and rapid diagnostic protocol for CF patients" (Straniero et al., 2016). A study using NGS for NBS found that the NGS assay was 100% concordant with traditional methods. Retrospective analysis results indicate an IRT/NGS screening algorithm would enable high sensitivity, better specificity, and positive predictive value (Baker et al., 2016). Further, whole genome sequencing of the *CFTR* gene is also growing in popularity. This type of sequencing has revealed a high prevalence of the intronic variant c.3874-4522A>G in those affected by CF (Morris-Rosendahl et al., 2020).

Cystic fibrosis transmembrane conductance regulator (CFTR) modulators are a new class of medications targeting the underlying defect in CF by improving production, intracellular processing, and/or function of the defective CFTR protein. Ivacaftor (IVA), which improves chloride channel function, IVA combined with lumacaftor (LUM), which partially corrects the CFTR misfolding, and IVA combined with tezacaftor, which improves the intracellular processing and trafficking of CFTR, have been approved by the U.S. Food and Drug Administration for use in patients with CF. The indications and efficacy of these drugs depend upon the CFTR mutation in the individual patient. For example, ivacaftor has been FDA-approved and Clinical Pharmacogenetics Implementation Consortium (CPIC)-recommended only for CF patients with the *G551D* mutation. It is not recommended for any other CF mutation (Clancy et al., 2014).

Analytical Validity

Lyon et al. (2014) assessed clinical laboratories' proficiency at evaluating genetic alterations for CF. A total of 357 labs participated, performing approximately 120,000 tests monthly. Analytical sensitivity and specificity were 98.8% and 99.6%, respectively. Clinical interpretation matched intended response for zero, one, and two mutations. The authors concluded that laboratory testing for CF in the United States is of high quality (Lyon et al., 2014).

Sugunaraj et al. (2019) completed a cross-sectional study which included the analysis of *CFTR* variants in 50,778 exomes. Only 24 patients were identified to contain bi-allelic pathogenic *CFTR* variants; the authors identified 21 of these cases as true-positives and

three as potential false positives. Therefore, "genomic screening exhibited a positive predictive value of 87.5%, negative predictive value of 99.9%, sensitivity of 95.5%, and a specificity of 99.9%" (Sugunaraj et al., 2019). Overall, the presence and/or absence of *CFTR* variants was strongly related to a CF diagnosis.

Hendrix et al. (2020) assessed the performance of dried blood spot DNA extraction methods in NGS for cystic fibrosis. A total of 20 DNA samples were used in this study and sequenced using Illumina's MiSeqDx™ Cystic Fibrosis 139-Variant Assay and Swift Biosciences' Accel-Amplicon™ CFTR panel. The MiSeqDx CFTR Clinical Sequencing Assay identified 37 CF-causing variants in the 20 samples tested, for a total of 141 benign variants which were all confirmed with Sanger sequencing. The Swift Biosciences' Accel-Amplicon™ CFTR panel identified 39 CF-causing variants for a total of 123 benign variants which were all confirmed with Sanger sequencing. Illumina's MiSeqDx performed well with a very high average coverage per method (>3000×) despite most DNA extraction methods being low-concentration and relatively crude. Because of the higher input of samples on a single flow cell, the average coverage per target was lower in the Accel-Amplicon™ CFTR panel in comparison to the clinical sequencing assay, but still often had >1000× coverage. The authors conclude that "both these tests are appropriate DNA extraction methods that can be used with dried blood spots that will result in consistent, high-quality sequencing results" (Hendrix et al., 2020).

Sicko et al. (2021) validated the use of a custom NGS assay, Archer CF assay, for CF newborn screening. According to the New York State, CF NBS had high false positive rates. However, after the implementation of this three-tier IRT-DNA-SEQ approach using commercially available tests, the positive predictive value of CF NBS improved from 3.7% to 25.2%. The Archer CF assay involves full gene sequencing, analyzing a specific panel of 338 clinically relevant CFTR variants (second tier), followed by unblinding of all sequence variants and bioinformatic assessment of deletions/duplications (third tier). 227 infant samples were taken, and 190 of 227 carries one or more of the 338 panel variants. The second-tier panel variants were all called correctly, detecting 44 different variants with a 100% sensitivity and 100% specificity. All 227 samples also underwent third tier analysis which detected 62 additional unique SNV/indel variants (Sicko et al., 2021).

Clinical Utility and Validity

Sosnay et al. (2013) performed a comprehensive analysis of CF genotypes and phenotypes. The authors collected genotype and phenotype data from 39696 CF patients and found 159 variants with over 0.01% allele frequency, 127 of which met

both the clinical and functional criteria for CF. The phenotypic criterion used to define the pathogenic threshold was sweat chloride conductance of 10%. These 127 pathogenic genetic variants were estimated to represent 95.4% of CF alleles, leaving only 0.21% of CF patients without an identified pathological CFTR variant. The phenotypes of these variants vary (Sosnay et al., 2013).

Wainwright et al. (2015) evaluated the combination of IVA and LUM in CF patients homozygous for the *F508D* mutation (labeled Phe508del). A total of 1108 patients were examined. The mean baseline forced expiratory volume in one second "FEV1" was 61% of the predicted value. The absolute mean improvement in FEV1% was found to be 2.6-4%, which corresponded to a 4.3-6.7% relative treatment difference. The rate of pulmonary exacerbations was found to be 30-39% less in the treatment group compared to the placebo group. However, the rate of discontinuation due to an adverse event was 4.2% in the treatment group compared to the placebo group (Wainwright et al., 2015).

Sharma et al. (2018) performed a cost-effectiveness analysis of the FDA-approved IVA-LUM combination for the F508D mutation. The authors built a Markov-state model to assess the IVA-LUM for 12-year-old CF patients over several periods of time. "Markov states included mild CF (percentage of predicted forced expiratory volume in one second or FEV1 > 70%), moderate (FEV1 40–70%), severe (FEV1 < 40%) disease, post-transplant, and death. Pulmonary exacerbation and lung transplant were included as transition states." The IVA-LUM combination resulted in higher quality adjusted life years (QALY, 7.29 vs 6.84 for usual care) but at a cost of $1,778,920.88 per QALY compared to $116,155.76 for usual care. Both monetary amounts were over a 10-year period. The IVA-LUM combination was cost-effective at a threshold of $150,000 / QALY, which was estimated to occur at an annual drug cost of $4153 (Sharma et al., 2018).

Kessels et al. (2020) completed a systematic review of prenatal genetic testing for CF. Eight different databases were researched for this review. The authors found that after genetic testing "A change in clinical management was observed: termination of pregnancy (TOP) occurred in most cases where two pathogenic variants were identified in a fetus of carrier parents (158/167; 94.6%)" (Kessels et al., 2020). The authors conclude by stating that genetic testing for CF had good diagnostic performance and lead to fewer births affected by CF.

Avram et al. (2021) studied the cost-effectiveness of genotyping versus sequencing for prenatal CF carrier screening using three strategies: genotyping ("genotyping included the standard 23-variant panel recommended by ACMG/ACOG") both partners,

genotyping one partner then sequencing the second, and sequencing both partners. The authors used a decision-analytic model to generate a theoretical cohort of four million pregnant people and their partners. Sequencing both partners identified 1,099 carrier couples that would have been missed by genotyping of both partners, resulting in 273 fewer missed prenatal diagnoses, 152 more terminations, 152 fewer affected newborns, and screening with genotyping followed by sequencing identified 477 more carrier couples as compared to genotyping both partners, resulting in 119 fewer missed prenatal diagnoses, 66 more terminations, and 66 fewer affected newborns. The incremental cost-effectiveness ratio of genotyping followed by sequencing compared to genotyping both partners was $180,004/ quality-adjusted life year (QALY) and the incremental cost-effectiveness ratio of sequencing both partners compared to genotyping followed by sequencing was $17.6 million/QALY. "Compared to genotyping both partners, the cost per CF case averted for genotyping/sequencing was $1.2 million and sequencing both partners was $49.4 million. . . Sequencing both partners was cost-effective when the cost of sequencing fell below $339 per test. Genotyping/sequencing was cost-effective when the cost of sequencing was between $340 and 1837 per test. Lastly, genotyping both partners was cost-effective when sequencing cost above $1838 per test". The authors concluded that "before being routinely recommended, sequencing for CF carrier screening requires greater examination at a population-based level (Avram et al., 2021).

VI. Guidelines and Recommendations

American College of Medical Genetics and Genomics (ACMG)

The ACMG also recommends offering CF carrier screening to all individuals capable of becoming pregnant who are of reproductive age, regardless of ethnic background. Ideally, the testing would occur prior to pregnancy, but if the individual is pregnant, then they should be tested as early as possible during the pregnancy. ACMG also indicates that if the individual is found to be a CF carrier, then their reproductive partner should also be tested (Pletcher & Bocian, 2006). If the individual presents for testing late in the pregnancy, simultaneous testing of both reproductive partners of the fetus should be considered. When there is a paternal family history of CF, carrier testing of the father is warranted.

In 2020, the ACMG introduced technical guidelines for CFTR variant testing, particularly emphasizing considerations during pregnancy. These guidelines suggest that simultaneous testing might be necessary based on various factors like gestational age, family history, ethnicity, or patient preferences. Carrier testing is recommended for

individuals with a family history of CF, partners of those with such history, partners of males with CAVD, reproductive age women, and gamete donors. Additionally, CFTR variant testing can be conducted for prenatal diagnosis using cells obtained through procedures like amniocentesis or chorionic villus sampling (CVS). To ensure inclusivity, "the ACMG recommends either a classification-based reporting approach or a classification-based (targeted) testing approach (which has historically been used for CFTR carrier screening). For those laboratories who wish to continue using a targeted testing approach, the ACMG-23 variant panel remains as the minimum list of CFTR variants that should be included. Laboratories may want to consider adding additional variants to their panel depending on the ethnic composition of their expected test population. However, the minimum list of CFTR variants recommended for pan-ethnic carrier screening has not been increased at this time" (Deignan et al., 2020).

In 2023, the ACMG issued updated recommendations for CFTR carrier screening, introducing a new minimum CFTR variant set (Table 1) that replaces the previous set of 23 variants. These revised recommendations specifically apply to carrier screening and do not affect CFTR variant testing for diagnosis or newborn screening. However, all other aspects of the 2020 ACMG CFTR technical standards remain unchanged (Deignan et al., 2023).

Table 1 (adapted from Deignan et al. (2023)) : *CFTR* Carrier Screening Variant Set (n=100)

DNA Variant	Protein Variant	Legacy Name
c.4C>T	p.Gln2Ter	Q2X
c.178G>T	p.Glu60Ter	E60X
c.200C>T	p.Pro67Leu	P67L
c.223C>T	p.Arg75Ter	R75X
c.254G>A	p.Gly85Glu	G85E[a]
c.262_263del	p.Leu88IlefsTer22	394delTT
c.271G>A	p.Gly91Arg	G91R
c.274-1G>A	p.?	406-1G->A
c.292C>T	p.Gln98Ter	Q98X
c.293A>G	p.Gln98Arg	Q98R
c.313del	p.Ile105SerfsTer2	444delA
c.328G>C	p.Asp110His	D110H
c.349C>T	p.Arg117Cys	R117C

DNA Variant	Protein Variant	Legacy Name
c.350G>A	p.Arg117His	R117H[a]
c.489+1G>T	p.?	621+1G->T[a]
c.571T>G	p.Phe191Val	F191V
c.579+1G>T	p.?	711+1G->T[a]
c.579+3A>G	p.?	711+3A->G
c.617T>G	p.Leu206Trp	L206W
c.653T>A	p.Leu218Ter	L218X
c.695T>A	p.Val232Asp	V232D
c.803del	p.Asn268IlefsTer17	935delA
c.868C>T	p.Gln290Ter	Q290X
c.988G>T	p.Gly330Ter	G330X
c.1000C>T	p.Arg334Trp	R334W[a]
c.1013C>T	p.Thr338Ile	T338I
c.1021_1022dup	p.Phe342HisfsTer28	1154insTC
c.1029del	p.Cys343Ter	1161delC
c.1040G>A	p.Arg347His	R347H
c.1040G>C	p.Arg347Pro	R347P[a]
c.1055G>A	p.Arg352Gln	R352Q
c.1155_1156dup	p.Asn386IlefsTer3	1288insTA
c.1327_1330dup	p.Ile444ArgfsTer3	1461ins4
c.1364C>A	p.Ala455Glu	A455E[a]
c.1367T>C	p.Val456Ala	V456A
c.1373del	p.Gly458AspfsTer11	1504delG
c.1393-1G>A	p.?	1525-1G->A
c.1397C>G	p.Ser466Ter	S466X
c.1400T>C	p.Leu467Pro	L467P
c.1519_1521del	p.Ile507del	I507del[a]
c.1521_1523del	p.Phe508del	F508del[a]
c.1572C>A	p.Cys524Ter	C524X
c.1584+1G>A	p.?	1716+1G->A
c.1585-1G>A	p.?	1717-1G->A[a]
c.1624G>T	p.Gly542Ter	G542X[a]
c.1646G>A	p.Ser549Asn	S549N
c.1647T>G	p.Ser549Arg	S549R
c.1651G>A	p.Gly551Ser	G551S
c.1652G>A	p.Gly551Asp	G551D[a]

DNA Variant	Protein Variant	Legacy Name
c.1657C>T	p.Arg553Ter	R553X[a]
c.1673T>C	p.Leu558Ser	L558S
c.1675G>A	p.Ala559Thr	A559T
c.1679G>C	p.Arg560Thr	R560T[a]
c.1679+1G>A	p.?	1811+1G->A
c.1680-886A>G	p.?	1811+1634A->G
c.1680A>C	p.Arg560Ser	R560S
c.1682C>A	p.Ala561Glu	A561E
c.1692del	p.Asp565MetfsTer7	1824delA
c.1705T>G	p.Tyr569Asp	Y569D
c.1753G>T	p.Glu585Ter	E585X
c.1766+1G>A	p.?	1898+1G->A[a]
c.1766+5G>T	p.?	1898+5G->T
c.1837G>A	p.Ala613Thr	A613T
c.1882G>A	p.Gly628Arg	G628R
c.2052dup	p.Gln685ThrfsTer4	2184insA
c.2052del	p.Lys684AsnfsTer38	2184delA[a]
c.2125C>T	p.Arg709Ter	R709X
c.2175dup	p.Glu726ArgfsTer4	2307insA
c.2290C>T	p.Arg764Ter	R764X
c.2353C>T	p.Arg785Ter	R785X
c.2374C>T	p.Arg792Ter	R792X
c.2490+1G>A	p.?	2622+1G->A
c.2657+5G>A	p.?	2789+5G->A[a]
c.2668C>T	p.Gln890Ter	Q890X
c.2739T>A	p.Tyr913Ter	Y913X
c.2834C>T	p.Ser945Leu	S945L
c.2909G>A	p.Gly970Asp	G970D
c.2988G>A	p.Gln996=	3120G->A
c.2988+1G>A	p.?	3120+1G->A[a]
c.3067_3072del	p.Ile1023_Val1024del	3199del6

DNA Variant	Protein Variant	Legacy Name
c.3107C>A	p.Thr1036Asn	T1036N
c.3140-26A>G	p.?	3272-26A->G
c.3196C>T	p.Arg1066Cys	R1066C
c.3197G>A	p.Arg1066His	R1066H
c.3266G>A	p.Trp1089Ter	W1089X
c.3294G>C	p.Trp1098Cys	W1098C
c.3353C>T	p.Ser1118Phe	S1118F
c.3472C>T	p.Arg1158Ter	R1158X
c.3484C>T	p.Arg1162Ter	R1162X[a]
c.3528del	p.Lys1177SerfsTer15	3659delC[a]
c.3612G>A	p.Trp1204Ter	W1204X
c.3659del	p.Thr1220LysfsTer8	3791delC
c.3717+5G>A	p.?	3849+5G->A
c.3718-2477C>T	p.?	3849+10kbC->T[a]
c.3744del	p.Lys1250ArgfsTer9	3876delA
c.3764C>A	p.Ser1255Ter	S1255X
c.3808del	p.Asp1270MetfsTer8	3940delG
c.3846G>A	p.Trp1282Ter	W1282X[a]
c.3889dup	p.Ser1297PhefsTer5	4016insT
c.3909C>G	p.Asn1303Lys	N1303K[a]

[a]Variants that were part of the previously recommended minimum 23-variant set.

The ACMG also recommends that testing for the IVS8 polyT variant be performed only when carrier screening reveals a R117H mutation, as the polyT variant influences the clinical severity of the mutation but does not cause CF by itself (ACMG, 2011).

The ACMG provides guidance on indications for CFTR variant testing. Diagnostic testing can be used for the molecular confirmation of a clinical CF diagnosis, for infants with meconium ileus, for individuals with CAVD, individuals with idiopathic pancreatitis or bronchiectasis, and as a follow-up to newborn screening. Regarding carrier testing, ACMG recommends that carrier testing be offered to individuals with a positive family history of CF, to partners of individuals with a positive family history, to partners of individuals with CAVD, to reproductive age individuals capable of becoming pregnant, and to gamete donors (Deignan et al., 2020).

Prenatal testing can be performed using cells obtained for diagnostic cytogenetic testing (i.e., amniocentesis or chorionic villus sampling [CVS]). Testing can take place on cultured or uncultured amniocytes or villous trophoblasts. According to the guidelines, targeted sequencing for specific CFTR variants may be considered when:

a. "A pathogenic or likely pathogenic variant is confirmed in both partners.
b. A pathogenic or likely pathogenic variant is confirmed in one partner and a VUS or variant associated with variable expressivity is confirmed in the other partner
c. As part of preimplantation genetic testing when both biological parents are confirmed carriers of a pathogenic or likely pathogenic variant" (Deignan et al., 2020).

Comprehensive CFTR sequencing may be considered when:

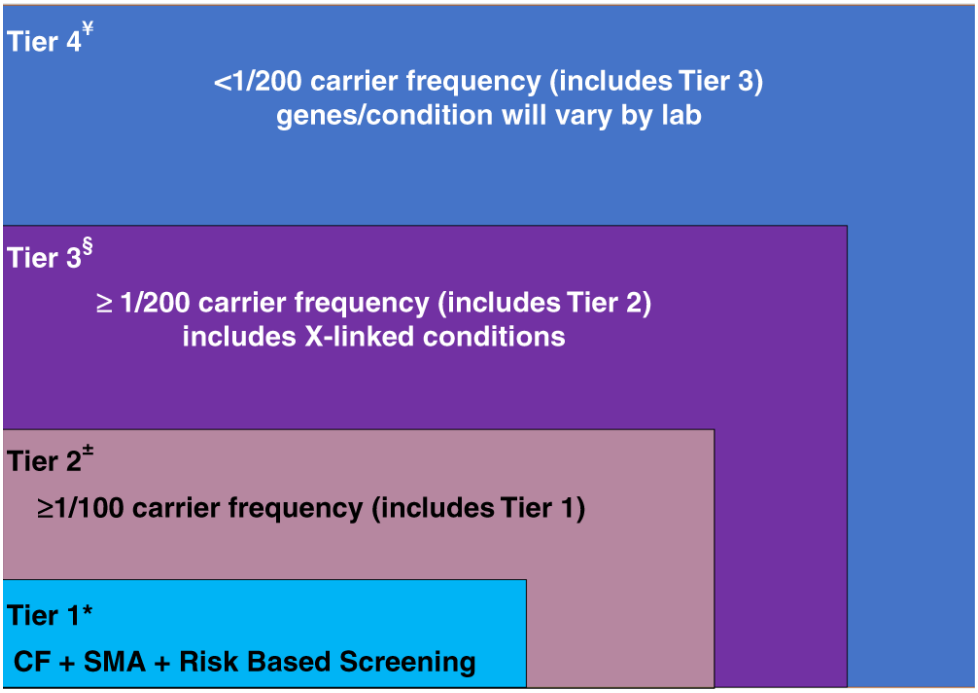
a. "One member of a couple is known to be a carrier of a pathogenic or likely pathogenic variant and any of the following is also true:
    - The partner is unavailable for screening
    - The partner has not been screened and any of the following is also true:
        - Screening that partner would be cost prohibitive
        - The results from the partner would not be available in time to allow for reproductive decision making
        - A diagnostic procedure (e.g., CVS, amniocentesis) is also being performed for other reasons (e.g., ultrasound abnormality).
b. An ultrasound finding (i.e., fetal echogenic bowel) suggests an affected fetus and CFTR variant information is not available from either biological parent" (Deignan et al., 2020).

The ACMG recommends the following regarding testing of intronic variants, deletions/duplications, reporting VUS and variable expressivity, and reporting of intron 9 polyT and TG regions:

- "ACMG recommends the reporting of the c.2657+5G>A and c.3718–2477C>T intronic variants. If these variants are not detectable with the laboratory's methodology (e.g., exome sequencing), then a separate assay should be performed to test for these variants.
- For all prenatal, postnatal, and adult diagnostic testing and carrier screening indications for CFTR testing, the ACMG does not recommend the testing of any specific exon-level or gene-level deletion or duplication variants.

- For all prenatal, postnatal, and adult diagnostic testing indications for CFTR where comprehensive methods are used, the ACMG recommends the reporting of VUS. For all adult carrier screening indications for CFTR where comprehensive methods are used, VUS should generally not be reported. However, laboratories may want to consider reporting VUS in the partner of an individual who had a pathogenic or likely pathogenic variant detected during screening.
- For all prenatal, postnatal, and adult diagnostic testing indications for CFTR where comprehensive methods are used, the ACMG recommends the reporting of any variants associated with variable expressivity.
- For all prenatal, postnatal, and adult diagnostic testing indications for CFTR, the ACMG recommends the reporting of R117H status as well as the results from at least the associated polyT tract. For all adult carrier screening indications for CFTR, polyT status should be reported when the R117H variant is detected; laboratories may also want to consider reporting the results from the associated polyT tract in the partner of an individual who had a pathogenic or likely pathogenic variant detected during screening" (Deignan et al., 2020).

The ACMG released recommendations for preconception and prenatal carrier screening. The following tier-based approach to carrier screening was produced and recommendations were made (Gregg et al., 2021):



CF: cystic fibrosis, SMA: spinal muscular atrophy.

- Preconception screening is recommended over prenatal screening.
- Concurrent partner testing should be offered if testing done during pregnancy.
- Tier 3 screening is recommended which includes 97 autosomal recessive genes and 16 X-linked genes, including DMD and Fragile X.
- The second biological parent may be offered Tier 3 carrier screening (for autosomal recessive conditions) when carrier screening is being performed at the same time in their reproductive partner who is capable of becoming pregnant.
- All pregnant patients and those planning a pregnancy should be offered Tier 3 carrier screening.
- Tier 4 screening should be considered when a pregnancy arises from a known or possible consanguineous relationship (second cousins or closer) or when a family or personal medical history warrants further risk assessment.
- ACMG does not recommend offering Tier 1 and/or Tier 2 screening, because these do not provide equitable evaluation of all racial/ethnic groups and routine offering of Tier 4 panels (Gregg et al., 2021).

Cystic Fibrosis Foundation

The Cystic Fibrosis Foundation (Farrell et al., 2017) convened a group of to develop clear and actionable consensus guidelines on the diagnosis of CF and to clarify diagnostic criteria and terminology for other disorders associated with *CFTR* mutations. The experts determined that "diagnoses associated with *CFTR* mutations in all individuals, from newborn to adult, be established by evaluation of CFTR function with a sweat chloride test. The latest mutation classifications annotated in the Clinical and Functional Translation of CFTR project (http://www.cftr2.org/index.php) should be used to aid in diagnosis."

The committee approved 27 consensus statements (Farrell et al., 2017):

1	Sweat chloride testing should be performed according to approved procedural guidelines published in established, international protocols such as the CLSI 2009 Guidelines.
2	Newborns with a positive CF newborn screen, to increase the likelihood of collecting an adequate sweat specimen, should have the test performed

	bilaterally and when the infant weighs >2 kg, and is at least 36 wk of corrected gestational age.
3	Newborns greater than 36 wk gestation and >2 kg body weight with a positive CF newborn screen, or positive prenatal genetic test, should have sweat chloride testing performed as soon as possible after 10 d of age, ideally by the end of the neonatal period (4 wk of age).
4	In infants with presumptive CF identified through NBS, CF treatment should not be delayed while efforts to establish a diagnosis of CF are initiated.
5	Sweat chloride analysis should be performed within a few hours of sweat collection and the results and interpretations should be reported to clinicians and parents or patients, as soon as possible and certainly on the same day.
6	In individuals presenting with a positive newborn screen, clinical features consistent with CF, or a positive family history, a diagnosis of CF can be made if the sweat chloride value is ≥60 mmol/L.
7	Individuals who are screen-positive and meet sweat chloride criteria for CF diagnosis should undergo *CFTR* genetic testing if the *CFTR* genotype was not available through the screening process or is incomplete.
8	In individuals with a positive newborn screen, a sweat chloride <30 mmol/L indicates that CF is unlikely.
9	Individuals with clinical features that may be consistent with CF who have a sweat chloride <30 mmol/L indicates that CF is less likely. It may, however, be considered if evolving clinical criteria and/or *CFTR* genotyping support CF and not an alternative diagnosis.
10	Individuals presenting with a positive newborn screen, symptoms of CF, or a positive family history, and sweat chloride values in the intermediate range (30-59 mmol/L) on two separate occasions may have CF. They should be considered for extended *CFTR* gene analysis and/or CFTR functional analysis.

11 The latest classifications identified in the CFTR2 project (http://www.cftr2.org/index.php) should be used to aid with CF diagnosis:

CF-causing mutation: individuals with 2 copies on separate alleles will likely have CF (clinical sweat confirmation needed)
Mutation of varying clinical consequence (MVCC): a mutation that in combination with a CF-causing mutation or another MVCC mutation may result in CF
Uncharacterized mutation/mutation of UNK: mutation that has not been evaluated by CFTR2 and may be disease causing or of variable clinical consequence or benign
Non-CF-causing mutation: individuals with 1 or more are unlikely to have CF (as a result of that allele)

12 In individuals presenting with a positive newborn screen, symptoms of CF, or a positive family history, the identification of 2 CF-causing mutations (defined by CFTR2) is consistent with a diagnosis of CF. Sweat chloride testing is necessary, though, to confirm the diagnosis.

13 The absence of detection of 2 CF-causing *CFTR* mutations does not exclude a diagnosis of CF.

14 If further CF functional testing is needed (NPD and ICM), it should be performed in a validated reference center with trained staff certified by the CF Foundation TDN or ECFS Clinical Trial Network.

15 In individuals with a positive newborn screen but variable or uncharacterized *CFTR* mutations (<2 CF-causing mutations), the diagnosis of CF can be made by demonstrating CFTR dysfunction (a sweat chloride ≥ 60 mmol/L or CF-typical NPD or ICM).

16 The term CRMS is used in the US for healthcare delivery purposes and CFSPID is used in other countries, but these both describe an inconclusive diagnosis following NBS.

17 The term CRMS/CFSPID is reserved for individuals who screen positive without clinical features consistent with a diagnosis of CF.

18 The definition of CRMS/CFSPID is an infant with a positive NBS test for CF and either:

A sweat chloride value <30 mmol/L and 2 *CFTR* mutations, at least 1 of which has unclear phenotypic consequences

OR

An intermediate sweat chloride value (30-59 mmol/L) and 1 or 0 CF-causing mutations

19 Children designated as CRMS/CFSPID should undergo at least one repeat sweat chloride test at CF centers with suitable expertise, such as an accredited CF center.

20 Children designated as CRMS/CFSPID should have clinical evaluation performed by CF providers to identify the minority that may develop clinical symptoms.

21 Children designated as CRMS/CFSPID can be considered for extended *CFTR* gene analysis (sequencing and or deletion duplication testing), as well as CFTR functional analysis (NPD/ICM) testing to further define their likelihood of developing CF.

22 The decision to reclassify children designated as CRMS/CFSPID as CF is an integrated decision that should take into account functional assessment of CFTR (sweat chloride, and possibly NPD/ICM), *CFTR* genetic analysis, and clinical assessment by the CF clinicians caring for the patient.

23 Genetic counseling should be offered to families of individuals followed for CRMS/CFSPID, including a discussion of the risk in future pregnancies.

24 Research Recommendation: Infants with a designation of CRMS/CFSPID (by definition) do not have clinical features consistent with a diagnosis of CF and

	further research is needed to determine the prognosis and best practices for frequency and duration of follow-up.
25	For individuals presenting with CF symptoms, the same diagnostic criteria recommended for the screened population for sweat chloride testing, *CFTR* genetic analysis, and CFTR functional testing should be used to confirm a CF diagnosis.
26	The diagnosis of CFTR-related disorder has been defined as a monosymptomatic clinical entity (CBAVD/pancreatitis/bronchiectasis) associated with CFTR dysfunction that does not fulfill the diagnostic criteria for CF.
27	Clinicians should avoid the use of terms like classic/nonclassic CF, typical/atypical CF, delayed CF, because these terms have no harmonized definition and could be confusing for families or caregivers.

The Cystic Fibrosis Foundation (Ren et al., 2018) also convened a multidisciplinary committee of CF caregivers to develop evidence-based guidelines for CFTR modulator therapy. The committee defined patients with CF as individuals who met the above CFF criteria for diagnosis of CF, combined with evidence of abnormal CFTR function, as demonstrated by elevated sweat chloride, detection of two CF-causing CFTR mutations, or abnormal nasal potential differences.

For adults and children aged six years and older with CF due to gating mutations other than G551D or R117H, the guideline panel made a conditional recommendation for treatment with IVA. For those with the R117H mutation, the guideline panel made a conditional recommendation for treatment with IVA for *1*) adults aged 18 years or older, and *2*) children aged 6–17 years with a forced expiratory volume in one second (FEV_1) less than 90% predicted. For those with the R117H mutation, the guideline panel made a conditional recommendation against treatment with IVA for *1*) children aged 12–17 years with an FEV_1 greater than 90% predicted, and *2*) children less than six years of age. Among those with two copies of F508del, the guideline panel made a strong recommendation for treatment with IVA/LUM for adults and children aged 12 years and older with an FEV_1 less than 90% predicted; and made a conditional recommendation for treatment with IVA/LUM for *1*) adults and children aged 12 years

or older with an FEV_1 greater than 90% predicted, and *2*) children aged 6–11 years (Ren et al., 2018).

American College of Obstetrics and Gynecology (ACOG)

The ACOG recommends offering CF carrier screening to all individuals who are considering becoming pregnant or who are currently pregnant. Expanded mutation panels which enhance the sensitivity for carrier screening can be considered for carrier detection, especially in non-Caucasian ethnic groups, but testing should not be repeated if performed previously (e.g., during previous pregnancy). If the patient is found to be a carrier, then her partner should be tested. They also indicate that newborn screening is not a replacement for carrier screening in a population.

Prenatal diagnosis is indicated after genetic counseling if both parents are carriers, or if the mother is a carrier and the father is unknown or unavailable for testing.

The ACOG recommends that complete gene sequencing of the CTFR gene is not appropriate for use in carrier screening, rather reserved for patients with cystic fibrosis, patients with negative carrier screening but a family history of cystic fibrosis, individuals with congenital bilateral absence of the vas deferens, or newborns with a positive newborn screening after the standard 23 gene screen has a negative result.

They also recommend referral to genetic counseling for couples in which both partners are CF carriers, prenatal diagnosis, and advanced reproductive technologies to decrease the risk of affected offspring should be discussed (ACOG, 2017).

These statements were reaffirmed in 2023.

c.

National Society of Genetic Counselors (NSGC)

The National Society of Genetic Counselors (NSGC) recommends that for all individuals capable of becoming pregnant and who are of reproductive age, carrier screening for CG should be offered "regardless of ancestry; preferably pre-conceptionally. CF carrier testing should also be offered to any individual with a family history of CF and to partners of mutation carriers and people with CF" (Langfelder-Schwind et al., 2014).

Regarding what mutations should be included in the carrier screening test, the NSGC states, "Carrier testing panels should include the mutations recommended by ACOG

and ACMG. For individuals of non- Northern European descent, pan-ethnic panels that include additional mutations more commonly identified in minority populations are appropriate to consider. Focus general population CF screening practices on identifying carriers of established disease-causing CFTR mutations" (Langfelder-Schwind et al., 2014).

The NSGC agrees with the ACMG regarding testing for IVS 8 5T/7T/9T as a reflex when mutation R117H is found in the CF carrier screen. They also assert that "in the absence of an R117H mutation, assessment of the intron 8 polyT or TG tracts is not recommended for routine CF carrier testing" (Langfelder-Schwind et al., 2014).

European Cystic Fibrosis Society (ECFS)

The ECFS mentioned CFTR modulators in their standards of care guideline. Recommendations state that "in patients with the G551D mutation ivacaftor should be part of standard of care"; at the time of writing, only one CFTR modulator had been shown to "demonstrate clinical efficacy" (Smyth et al., 2014).

In 2018, the ECFS published a revised version of their best practice guidelines. The ECFS has published the following requirement to when providing a CF diagnosis:

- "To be able to perform genetic testing for the most appropriate panel of *CFTR* mutations for the local population. Access to extended exon DNA analysis should be available when required" (Castellani et al., 2018).

The ECFS also states that genetic counseling should be offered when reporting a CF diagnosis to a patient.

National Institute for Health and Care Excellence (NICE)

In 2017, the NICE published guidelines on the diagnosis and management of CF. NICE includes the following recommendations on CF diagnoses:

- "Be aware that cystic fibrosis can be diagnosed based on:
  - positive test results in people with no symptoms, for example infant screening (blood spot immunoreactive trypsin test) followed by sweat and gene tests for confirmation or
  - clinical manifestations, supported by sweat or gene test results for confirmation or
  - clinical manifestations alone, in the rare case of people with symptoms who have normal sweat or gene test results.

- Assess for cystic fibrosis and, when clinically appropriate, perform a sweat test (for children and young people) or a cystic fibrosis gene test (for adults) in people with any of the following:
  - family history
  - congenital intestinal atresia
  - meconium ileus
  - symptoms and signs that suggest distal intestinal obstruction syndrome
  - faltering growth (in infants and young children)
  - undernutrition
  - recurrent and chronic pulmonary disease, such as:
    - recurrent lower respiratory tract infections
    - clinical or radiological evidence of lung disease (in particular bronchiectasis)
    - persistent chest X-ray changes
    - chronic wet or productive cough
  - chronic sinus disease
  - obstructive azoospermia (in young people and adults)
  - acute or chronic pancreatitis
  - malabsorption
  - rectal prolapse (in children)
  - pseudo-Bartter syndrome.
- Refer people with suspected cystic fibrosis to a specialist cystic fibrosis center if:
  - they have a positive or equivocal sweat test result
  - their assessment suggests they have cystic fibrosis but their test results are normal
  - gene testing reveals 1 or more cystic fibrosis mutations" (NICE, 2017).

Physicians Committee for Responsible Medicine (PCRM)

The PSRM has published guidelines on diagnosis of cystic fibrosis. Below are the recommended guidelines:

1. A sweat chloride test can be performed as early as 48 hours after birth, which is the mainstay of laboratory confirmation.
2. Newborn Screening is mandatory in all 50 states.

A patient with symptoms may require a sweat chloride test or genetic testing. "Patients who exhibit signs and symptoms but do not have a positive sweat test should have a genetic test. Prenatal testing and newborn screening may be used. Early detection renders a better clinical course. While not widely available, nasal potential difference

measurements can confirm the diagnosis if sweat testing and genetic testing results are inconclusive" (PCRM, 2023).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

On March 28, 2006, the FDA approved eSensor Cystic Fibrosis Carrier Detection System as a device for the detection of carrier status for cystic fibrosis for all adult couples contemplating pregnancy, regardless of ethnicity. It is a qualitative genotyping assay that simultaneously detects mutations currently recommended by the American College of Medical Genetics and American College of Obstetricians and Gynecologists (ACMG/ACOG). The eSensor® CFCD System is not indicated for prenatal screening or to establish a diagnosis for cystic fibrosis (FDA, 2006a).

On June 7, 2006, the FDA approved Tag-It™ Cystic Fibrosis Kit as a device used to simultaneously detect and identify a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene in human blood specimens. The panel includes mutations and variants currently recommended by the American College of Medical Genetics and American College of Obstetricians and Gynecologists (ACMG/ACOG), plus some of the world's most common and North American-prevalent mutations. The Tag-It™ Cystic Fibrosis Kit is a qualitative genotyping test which provides information intended to be used for carrier testing in adults of reproductive age, as an aid in newborn screening, and two in confirmatory diagnostic testing in newborns and children (FDA, 2006b).

On September 9, 2007, the FDA approved Cystic Fibrosis Genotyping Assay as a qualitative in vitro diagnostic device used to genotype a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene in genomic deoxyribonucleic acid (DNA) isolated from human whole blood specimens. The panel includes mutations and variants recommended by the American College of Medical Genetics (ACMG, 2004) and the American College of Obstetricians and

Gynecologists (ACOG, 2005) plus additional multiethnic mutations. The Cystic Fibrosis Genotyping Assay provides information intended to be used for carrier testing in adults of reproductive age, as an aid in newborn screening and in confirmatory diagnostic testing of newborns and children. This test is not indicated for use in fetal diagnostic or pre-implantation testing. This test is also not indicated for stand-alone diagnostic purposes (FDA, 2007).

On March 13, 2008, the FDA approved InPlex™ CF Molecular Test as an in vitro diagnostic device used to simultaneously detect and identify a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene in genomic DNA samples isolated from human peripheral whole blood specimens. The panel includes mutations and variants recommended by the 2004 American College of Medical Genetics (ACMG). The InPlex™ CF Molecular Test is a qualitative genotyping test that provides information intended to be used for cystic fibrosis carrier screening as recommended by ACMG and the 2005 American College of Obstetricians and Gynecologists (ACOG) for adults of reproductive age, as an aid in newborn screening for cystic fibrosis, and in confirmatory diagnostic testing for cystic fibrosis in newborns and children. The test is not indicated for use in fetal diagnostic or pre-implantation testing. This test is also not indicated for stand-alone diagnostic purposes and results should be used in conjunction with other available laboratory and clinical information (FDA, 2008).

On July 6, 2009, the FDA approved eSensor® CF Genotyping Test as an in vitro diagnostic device used to simultaneously detect and identify a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene in genomic DNA samples isolated from human peripheral whole blood specimens. The panel includes mutations and variants recommended by the 2004 American College of Medical Genetics (ACMG). The eSensor® CF Genotyping Test is a qualitative genotyping test that provides information intended to be used for cystic fibrosis carrier screening as recommended by ACMG and the 2005 American College of Obstetricians and Gynecologists (ACOG) for adults of reproductive age, as an aid in newborn screening for cystic fibrosis, and in confirmatory diagnostic testing for cystic fibrosis in newborns and children. The test is not indicated for use in fetal diagnostic or pre-implantation testing. This test is also not indicated for stand-alone diagnostic purposes and results should be used in conjunction with other available laboratory and clinical information (FDA, 2009a).

On July 24, 2009, the FDA approved Verigene®CFTR and Verigene®CFTR PolyT Nucleic Acid Tests as qualitative in vitro diagnostic devices used to genotype a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator

(CFTR) gene in genomic DNA isolated from human peripheral whole blood specimens. The panel includes mutations and variants recommended by the 2004 American College of Medical Genetics (ACMG) and the 2005 American College of Obstetricians and Gynecologists (ACOG). The Verigene®CFTR Nucleic Acid Test provides information intended to be used for carrier testing in adults of reproductive age and in confirmatory diagnostic testing of newborns and children. These tests are not indicated for use in fetal diagnostic or pre-implantation testing and not indicated for stand-alone diagnostic purposes and the results should be used in conjunction with other available laboratory and clinical information (FDA, 2009b).

On December 11, 2009, the FDA approved xTAG® Cystic Fibrosis 60 Kit v2 as a device used to simultaneously detect and identify a panel of mutations and variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene in human blood specimens. The panel includes mutations and variants currently recommended by the American College of Medical Genetics and American College of Obstetricians and Gynecologists (ACMG/ACOG), plus some of the world's most common and North American prevalent mutations. The xTAG® Cystic Fibrosis 60 Kit v2 is a qualitative genotyping test which provides information intended to be used for carrier testing in adults of reproductive age, as an aid in newborn screening, and in confirmatory diagnostic testing in newborns and children. The kit is not indicated for use in fetal diagnostic or pre-implantation testing. This kit is also not indicated for stand-alone diagnostic purposes (FDA, 2009c). On December 15, 2016, a modification to this test was approved for new software thresholds for 2183/2184 variants (FDA, 2016b).

On November 19, 2013, the FDA approved Illumina MiSeqDx™ Cystic Fibrosis 139-Variant Assay is a qualitative in vitro diagnostic system used to simultaneously detect 139 clinically relevant cystic fibrosis disease-causing mutations and variants of the cystic fibrosis transmembrane conductance regulator (CFTR) gene in genomic DNA isolated from human peripheral whole blood specimens. The variants include those recommended in 2004 by the American College of Medical Genetics (ACMG) and in 2011 by the American College of Obstetricians and Gynecologists (ACOG). The test is intended for carrier screening in adults of reproductive age, in confirmatory diagnostic testing of newborns and children, and as an initial test to aid in the diagnosis of individuals with suspected cystic fibrosis. The results of this test are intended to be interpreted by a board-certified clinical molecular geneticist or equivalent and should be used in conjunction with other available laboratory and clinical information. This test is not indicated for use for newborn screening, fetal diagnostic testing, preimplantation testing, or for stand-alone diagnostic purposes (FDA, 2013b).

On November 19, 2013, the FDA also approved Illumina MiSeqDxTM Cystic Fibrosis Clinical Sequencing Assay as a targeted sequencing in vitro diagnostic system that re-sequences the protein coding regions and intron/exon boundaries of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene in genomic DNA isolated from human peripheral whole blood specimens collected in K2EDTA. The test detects single nucleotide variants, and small InDels within the region sequenced, and additionally reports on two deep intronic mutations and two large deletions. The test is intended to be used on the Illumina MiSeqDx Instrument. The test is intended to be used as an aid in the diagnosis of individuals with suspected cystic fibrosis (CF). The test is most appropriate when the patient has an atypical or non-classic presentation of CF or when other mutation panels have failed to identify both causative mutations. The results of the test are intended to be interpreted by a board-certified clinical molecular geneticist or equivalent and should be used in conjunction with other available information including clinical symptoms, other diagnostic tests, and family history. This test is not indicated for use for stand-alone diagnostic purposes, fetal diagnostic testing, for pre-implantation testing, carrier screening, newborn screening, or population screening (FDA, 2013a).

On December 15, 2016, the FDA approved the XTAG Cystic Fibrosis 39 Kit V2 by Luminex Molecular Diagnostics, Inc. This test can identify the 23 CFTR mutations related to cystic fibrosis and could aid in carrier screening, newborn screening, and diagnostic testing (FDA, 2016a).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81220	CFTR (cystic fibrosis transmembrane conductance regulator) (e.g., cystic fibrosis) gene analysis; common variants (e.g., ACMG/ACOG guidelines)
81221	CFTR (cystic fibrosis transmembrane conductance regulator) (e.g., cystic fibrosis) gene analysis; known familial variants
81222	CFTR (cystic fibrosis transmembrane conductance regulator) (e.g., cystic fibrosis) gene analysis; duplication/deletion variants

CPT	Code Description
81223	CFTR (cystic fibrosis transmembrane conductance regulator) (e.g., cystic fibrosis) gene analysis; full gene sequence
81224	CFTR (cystic fibrosis transmembrane conductance regulator) (e.g., cystic fibrosis) gene analysis; intron 8 poly-T analysis (e.g., male infertility)
81479	Unlisted molecular pathology procedure
96040	Medical genetics and genetic counseling services, each 30 minutes face-to-face with patient/family
S0265	Genetic counseling, under physician supervision, each 15 minutes

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

ACMG. (2011). Technical Standards and Guidelines for CFTR Mutation Testing. *STANDARDS AND GUIDELINES FOR CLINICAL GENETICS LABORATORIES*. http://dx.doi.org/10.1038/gim.2014.93

ACOG. (2017). Committee Opinion No. 691: Carrier Screening for Genetic Conditions. *Obstet Gynecol*, *129*(3), e41-e55. https://doi.org/10.1097/aog.0000000000001952

Avram, C. M., Dyer, A. L., Shaffer, B. L., & Caughey, A. B. (2021). The cost-effectiveness of genotyping versus sequencing for prenatal cystic fibrosis carrier screening. *Prenatal Diagnosis*, *41*(11), 1449-1459. https://doi.org/10.1002/pd.6027

Baker, M. W., Atkins, A. E., Cordovado, S. K., Hendrix, M., Earley, M. C., & Farrell, P. M. (2016). Improving newborn screening for cystic fibrosis using next-generation sequencing technology: a technical feasibility study. *Genet Med*, *18*(3), 231-238. https://doi.org/10.1038/gim.2014.209

Barrett, P. M., Alagely, A., & Topol, E. J. (2012). Cystic fibrosis in an era of genomically guided therapy. *Hum Mol Genet*, *21*(R1), R66-71. https://doi.org/10.1093/hmg/dds345

Bell, S. C., De Boeck, K., & Amaral, M. D. (2015). New pharmacological approaches for cystic fibrosis: promises, progress, pitfalls. *Pharmacol Ther*, *145*, 19-34. https://doi.org/10.1016/j.pharmthera.2014.06.005

Castellani, C., Duff, A. J. A., Bell, S. C., Heijerman, H. G. M., Munck, A., Ratjen, F., Sermet-Gaudelus, I., Southern, K. W., Barben, J., Flume, P. A., Hodkova, P., Kashirskaya, N., Kirszenbaum, M. N., Madge, S., Oxley, H., Plant, B., Schwarzenberg, S. J., Smyth, A. R., Taccetti, G., . . . Drevinek, P. (2018). ECFS best practice guidelines: the 2018 revision. *J Cyst Fibros*, *17*(2), 153-178. https://doi.org/10.1016/j.jcf.2018.02.006

CF Foundation, U., Johns Hopkins University, The Hospital for Sick Children. (2023). *The Clinical and Functional TRanslation of CFTR (CFTR2)*. https://www.cftr2.org/resources

Clancy, J. P., & Jain, M. (2012). Personalized medicine in cystic fibrosis: dawning of a new era. *Am J Respir Crit Care Med*, *186*(7), 593-597. https://doi.org/10.1164/rccm.201204-0785PP

Clancy, J. P., Johnson, S. G., Yee, S. W., McDonagh, E. M., Caudle, K. E., Klein, T. E., Cannavo, M., & Giacomini, K. M. (2014). Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for ivacaftor therapy in the context of CFTR genotype. *Clin Pharmacol Ther*, *95*(6), 592-597. https://doi.org/10.1038/clpt.2014.54

Cohen-Cymberknoh, M., Shoseyov, D., & Kerem, E. (2011). Managing cystic fibrosis: strategies that increase life expectancy and improve quality of life. *Am J Respir Crit Care Med*, *183*(11), 1463-1471. https://doi.org/10.1164/rccm.201009-1478CI

Coty, W. A. (2006, February 28). *eSensor® Cystic Fibrosis Carrier Detection System 510(k) Summary*. https://pathsurveyor.com/api/pmn_pdf?knumber=K060543

Deignan, J. L., Astbury, C., Cutting, G. R., del Gaudio, D., Gregg, A. R., Grody, W. W., Monaghan, K. G., Richards, S., & Committee, A. L. Q. A. (2020). CFTR variant testing: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genetics In Medicine*, *22*(8), 1288-1295. https://doi.org/10.1038/s41436-020-0822-5

Deignan, J. L., Gregg, A. R., Grody, W. W., Guo, M. H., Kearney, H., Monaghan, K. G., Raraigh, K. S., Taylor, J., Zepeda-Mendoza, C. J., & Ziats, C. (2023). Updated recommendations for CFTR carrier screening: A position statement of the American College of Medical Genetics and Genomics (ACMG). *Genetics In Medicine*, *25*(8), 100867. https://doi.org/10.1016/j.gim.2023.100867

Farrell, P. M., Rosenstein, B. J., White, T. B., Accurso, F. J., Castellani, C., Cutting, G. R., Durie, P. R., Legrys, V. A., Massie, J., Parad, R. B., Rock, M. J., & Campbell, P. W., 3rd. (2008). Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. *J Pediatr*, *153*(2), S4-s14. https://doi.org/10.1016/j.jpeds.2008.05.005

Farrell, P. M., White, T. B., Ren, C. L., Hempstead, S. E., Accurso, F., Derichs, N., Howenstine, M., McColley, S. A., Rock, M., Rosenfeld, M., Sermet-Gaudelus, I., Southern, K. W., Marshall, B. C., & Sosnay, P. R. (2017). Diagnosis of Cystic Fibrosis: Consensus Guidelines from the Cystic Fibrosis Foundation. *J Pediatr*, *181s*, S4-S15.e11. https://doi.org/10.1016/j.jpeds.2016.09.064

FDA. (2006a). Decision Summary ESENSOR CYSTIC FIBROSIS CARRIER DETECTION TEST 510(k) Premarket Notification. http://www.accessdata.fda.gov/cdrh_docs/reviews/K051435.pdf

FDA. (2006b). *Decision Summary Tag It Cystic Fibrosis Carrier Detection System 510(k) Premarket Notification*. Retrieved from https://www.accessdata.fda.gov/cdrh_docs/reviews/K043011.pdf
FDA. (2007). *Decision Summary Cystic Fibrosis Genotyping Assay 510(k) Premarket Notification*. U.S. Food and Drug Administration Retrieved from https://www.accessdata.fda.gov/cdrh_docs/reviews/K062028.pdf
FDA. (2008). *Decision Summary Inplex CF Molecular Test 510(k) Premarket Notification*. U.S. Food and Drug Administration Retrieved from https://www.accessdata.fda.gov/cdrh_docs/reviews/K063787.pdf
FDA. (2009a). *Decision Summary eSensor® CF Genotyping Test 510(k) Premarket Notification*. U.S. Food and Drug Administration Retrieved from https://www.accessdata.fda.gov/cdrh_docs/reviews/K090901.pdf
FDA. (2009b). *Decision Summary Verigene® CFTR and Verigene® CFTR PolyT Nucleic Acid Tests 510(k) Premarket Notification*. U.S. Food and Drug Administration Retrieved from https://www.accessdata.fda.gov/cdrh_docs/reviews/K083294.pdf
FDA. (2009c). *Decision Summary xTAG® Cystic Fibrosis 60 Kit v2 510(k) Premarket Notification*. U.S. Food and Drug Administration Retrieved from https://www.accessdata.fda.gov/cdrh_docs/reviews/K083845.pdf
FDA. (2013a). *Decision Summary Illumina MiSeqDxTM Cystic Fibrosis Clinical Sequencing Assay 510(k) Premarket Notification*. U.S. Food and Drug Administration Retrieved from https://www.accessdata.fda.gov/cdrh_docs/reviews/K132750.pdf
FDA. (2013b). *Decision Summary Illumina MiSeqDx™ Cystic Fibrosis 139-Variant Assay 510(k) Premarket Notification*. U.S. Food and Drug Administration Retrieved from https://www.accessdata.fda.gov/cdrh_docs/reviews/K124006.pdf
FDA. (2016a). *XTAG Cystic Fibrosis 39 Kit V2*. https://www.accessdata.fda.gov/cdrh_docs/pdf16/K163347.pdf
FDA. (2016b). *XTAG Cystic Fibrosis 60 Kit V2, XTAG Data Analysis Software (TDAS) CFTR*. https://www.accessdata.fda.gov/cdrh_docs/pdf16/K163336.pdf
Gregg, A. R., Aarabi, M., Klugman, S., Leach, N. T., Bashford, M. T., Goldwaser, T., Chen, E., Sparks, T. N., Reddi, H. V., Rajkovic, A., Dungan, J. S., Practice, A. P., & Guidelines, C. (2021). Screening for autosomal recessive and X-linked conditions during pregnancy and preconception: a practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genetics In Medicine*, *23*(10), 1793-1806. https://doi.org/10.1038/s41436-021-01203-z
Grosse, S. D., Boyle, C. A., Botkin, J. R., Comeau, A. M., Kharrazi, M., Rosenfeld, M., & Wilfond, B. S. (2004). Newborn screening for cystic fibrosis: evaluation of benefits and risks and recommendations for state newborn screening programs. *MMWR Recomm Rep*, *53*(Rr-13), 1-36. https://pubmed.ncbi.nlm.nih.gov/15483524/

Hendrix, M. M., Cuthbert, C. D., & Cordovado, S. K. (2020). Assessing the Performance of Dried-Blood-Spot DNA Extraction Methods in Next Generation Sequencing. *International Journal of Neonatal Screening*, *6*(2), 36. https://doi.org/10.3390/ijns6020036

Hologic. (2008). InPlex Molecular Test. https://www.hologic.com/sites/default/files/package-insert/Hologic%20CF%20IVD%20Package%20Insert.pdf#:~:text=SUMMARY%20AND%20EXPLANATION%20A.%20Clinical%20Description%3A%20The%20InPlex%C2%AECF,establish%20the%20diagnosis%20of%20CF%20in%20an%20individual.

Illumina. (2013). Illumina MiSeqDx™ Cystic Fibrosis 139-Variant Assay. https://support.illumina.com/content/dam/illumina-marketing/documents/clinical/miseqdx-cystic-fibrosis-139-variant-assay-data-sheet-1000000006359.pdf

Kessels, S. J. M., Carter, D., Ellery, B., Newton, S., & Merlin, T. L. (2020). Prenatal genetic testing for cystic fibrosis: a systematic review of clinical effectiveness and an ethics review. *Genet Med*, *22*(2), 258-267. https://doi.org/10.1038/s41436-019-0641-8

Langfelder-Schwind, E., Karczeski, B., Strecker, M. N., Redman, J., Sugarman, E. A., Zaleski, C., Brown, T., Keiles, S., Powers, A., Ghate, S., & Darrah, R. (2014). Molecular testing for cystic fibrosis carrier status practice guidelines: recommendations of the National Society of Genetic Counselors. *J Genet Couns*, *23*(1), 5-15. https://doi.org/10.1007/s10897-013-9636-9

Lim, R. M., Silver, A. J., Silver, M. J., Borroto, C., Spurrier, B., Petrossian, T. C., Larson, J. L., & Silver, L. M. (2016). Targeted mutation screening panels expose systematic population bias in detection of cystic fibrosis risk. *Genet Med*, *18*(2), 174-179. https://doi.org/10.1038/gim.2015.52

Luminex. (2024). xTAG® Cystic Fibrosis 71 v2 Kit https://www.luminexcorp.com/cystic-fibrosis/#overview

Lyon, E., Schrijver, I., Weck, K. E., Ferreira-Gonzalez, A., Richards, C. S., & Palomaki, G. E. (2014). Molecular genetic testing for cystic fibrosis: laboratory performance on the College of American Pathologists external proficiency surveys [Original Research Article]. *Genetics In Medicine*, *17*, 219. https://doi.org/10.1038/gim.2014.93

Morris-Rosendahl, D. J., Edwards, M., McDonnell, M. J., John, S., Alton, E. W., Davies, J. C., & Simmonds, N. J. (2020). Whole Gene Sequencing of CFTR Reveals a High Prevalence of the Intronic Variant c.3874-4522A>G in Cystic Fibrosis. *Am J Respir Crit Care Med*. https://doi.org/10.1164/rccm.201908-1541LE

NICE. (2017). *Cystic fibrosis: diagnosis and management*. https://www.nice.org.uk/guidance/ng78/chapter/Recommendations#diagnosis-of-cystic-fibrosis

PCRM. (2023, February 16). *Nutrition Guide for Clinicians: Cystic Fibrosis*. Physicians Committee for Responsible Medicine. https://nutritionguide.pcrm.org/nutritionguide/view/Nutrition_Guide_for_Clinicians/1342064/all/Cystic_Fibrosis

Pletcher, B. A., & Bocian, M. (2006). Preconception and prenatal testing of biologic fathers for carrier status. American College of Medical Genetics. *Genet Med*, *8*(2), 134-135. https://doi.org/10.1097/01.gim.0000200948.58427.e2

Pritchard, L. L. (2016). Respiratory Conditions Update: Cystic Fibrosis. *FP Essent*, *448*, 35-43. https://www.ncbi.nlm.nih.gov/pubmed/27576234

Ren, C. L., Morgan, R. L., Oermann, C., Resnick, H. E., Brady, C., Campbell, A., DeNagel, R., Guill, M., Hoag, J., Lipton, A., Newton, T., Peters, S., Willey-Courand, D. B., & Naureckas, E. T. (2018). Cystic Fibrosis Foundation Pulmonary Guidelines. Use of Cystic Fibrosis Transmembrane Conductance Regulator Modulator Therapy in Patients with Cystic Fibrosis. *Ann Am Thorac Soc*, *15*(3), 271-280. https://doi.org/10.1513/AnnalsATS.201707-539OT

Schrijver, I., Pique, L., Graham, S., Pearl, M., Cherry, A., & Kharrazi, M. (2016). The Spectrum of CFTR Variants in Nonwhite Cystic Fibrosis Patients: Implications for Molecular Diagnostic Testing. *J Mol Diagn*, *18*(1), 39-50. https://doi.org/10.1016/j.jmoldx.2015.07.005

Sharma, D., Xing, S., Hung, Y. T., Caskey, R. N., Dowell, M. L., & Touchette, D. R. (2018). Cost-effectiveness analysis of lumacaftor and ivacaftor combination for the treatment of patients with cystic fibrosis in the United States. *Orphanet J Rare Dis*, *13*(1), 172. https://doi.org/10.1186/s13023-018-0914-3

Sicko, R. J., Stevens, C. F., Hughes, E. E., Leisner, M., Ling, H., Saavedra-Matiz, C. A., Caggana, M., & Kay, D. M. (2021). Validation of a Custom Next-Generation Sequencing Assay for Cystic Fibrosis Newborn Screening. *Int J Neonatal Screen*, *7*(4). https://doi.org/10.3390/ijns7040073

Smyth, A. R., Bell, S. C., Bojcin, S., Bryon, M., Duff, A., Flume, P., Kashirskaya, N., Munck, A., Ratjen, F., Schwarzenberg, S. J., Sermet-Gaudelus, I., Southern, K. W., Taccetti, G., Ullrich, G., & Wolfe, S. (2014). European Cystic Fibrosis Society Standards of Care: Best Practice guidelines. *Journal of Cystic Fibrosis*, *13*, S23-S42. https://doi.org/10.1016/j.jcf.2014.03.010

Sosnay, P. R., Siklosi, K. R., Van Goor, F., Kaniecki, K., Yu, H., Sharma, N., Ramalho, A. S., Amaral, M. D., Dorfman, R., Zielenski, J., Masica, D. L., Karchin, R., Millen, L., Thomas, P. J., Patrinos, G. P., Corey, M., Lewis, M. H., Rommens, J. M., Castellani, C., . . . Cutting, G. R. (2013). Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. *Nat Genet*, *45*(10), 1160-1167. https://doi.org/10.1038/ng.2745

Stafler, P., Mei-Zahav, M., Wilschanski, M., Mussaffi, H., Efrati, O., Lavie, M., Shoseyov, D., Cohen-Cymberknoh, M., Gur, M., Bentur, L., Livnat, G., Aviram, M., Alkrinawi, S., Picard, E., Prais, D., Steuer, G., Inbar, O., Kerem, E., & Blau, H. (2016). The impact of a national population carrier screening program on cystic fibrosis birth rate and age at diagnosis: Implications for newborn screening. *J Cyst Fibros*, *15*(4), 460-466. https://doi.org/10.1016/j.jcf.2015.08.007

Straniero, L., Solda, G., Costantino, L., Seia, M., Melotti, P., Colombo, C., Asselta, R., & Duga, S. (2016). Whole-gene CFTR sequencing combined with digital RT-PCR improves genetic diagnosis of cystic fibrosis. *J Hum Genet*. https://doi.org/10.1038/jhg.2016.101

Sugunaraj, J. P., Brosius, H. M., Murray, M. F., Manickam, K., Stamm, J. A., Carey, D. J., & Mirshahi, U. L. (2019). Predictive value of genomic screening: cross-sectional study of cystic fibrosis in 50,788 electronic health records. *NPJ Genom Med*, *4*, 21. https://doi.org/10.1038/s41525-019-0095-6

Wainwright, C. E., Elborn, J. S., Ramsey, B. W., Marigowda, G., Huang, X., Cipolli, M., Colombo, C., Davies, J. C., De Boeck, K., Flume, P. A., Konstan, M. W., McColley, S. A., McCoy, K., McKone, E. F., Munck, A., Ratjen, F., Rowe, S. M., Waltz, D., & Boyle, M. P. (2015). Lumacaftor-Ivacaftor in Patients with Cystic Fibrosis Homozygous for Phe508del CFTR. *N Engl J Med*, *373*(3), 220-231. https://doi.org/10.1056/NEJMoa1409547

Wilschanski, M., Zielenski, J., Markiewicz, D., Tsui, L. C., Corey, M., Levison, H., & Durie, P. R. (1995). Correlation of sweat chloride concentration with classes of the cystic fibrosis transmembrane conductance regulator gene mutations. *J Pediatr*, *127*(5), 705-710. https://doi.org/10.1016/s0022-3476(95)70157-5

Genetic Testing for Diagnosis of Inherited Peripheral Neuropathies

Policy Number: AHS – M2072 – Genetic Testing for Diagnosis of Inherited Peripheral Neuropathies	Prior Policy Name and Number, as applicable: • AHS – M2124 - Charcot-Marie-Tooth Disease
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

The inherited peripheral neuropathies are a heterogeneous group of diseases that may be inherited in an autosomal dominant, autosomal recessive, or X-linked dominant manner. The inherited peripheral neuropathies can be divided into hereditary motor and sensory neuropathies (such as Charcot-Marie-Tooth disease), hereditary neuropathy with liability to pressure palsies, hereditary sensory and autonomic neuropathies, and other miscellaneous types (e.g., hereditary brachial plexopathy, giant axonal neuropathy). In addition to clinical presentation, nerve conduction studies, and family history, genetic testing can be used to diagnose specific inherited peripheral neuropathies (Kang, 2024c).

When pursuing genetic testing for inherited peripheral neuropathies, genetic counseling is strongly recommended.

II. Related Policies

Policy Number	Policy Title
AHS-M2112	Nerve Fiber Density Testing
AHS-M2145	General Genetic Testing, Germline Disorders
AHS-M2146	General Genetic Testing, Somatic Disorders
AHS-M2179	Prenatal Screening (Prevention Genetics)

AHS-G2043	Celiac Disease Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with clinical features of Charcot-Marie-Tooth (CMTA) disease, but for whom a definitive diagnosis cannot be made without genetic testing, genetic testing for *PMP22* deletions/duplication, *GJB1* mutations, **and/or** *MFN2* mutations **MEETS COVERAGE CRITERIA**.

2) For individuals with clinical features of CMT disease who have tested negative for common deleterious variants (*PMP22* deletions/duplication; *GJB1* or *MFN2* mutations), single gene or multi-gene panel testing for CMT disease risk genes **MEETS COVERAGE CRITERIA**.

3) For asymptomatic individuals who have a close blood relative (see Note 1) with a known deleterious mutation in a CMT gene, genetic testing for the known familial mutation **MEETS COVERAGE CRITERIA**.

4) For individuals who are clinically suspected of having hereditary neuropathy with liability to pressure palsies (HNPP), but for whom a definitive diagnosis cannot be made without genetic testing, genetic testing for *PMP22* deletions and duplications **MEETS COVERAGE CRITERIA**.

5) For individuals who are clinically suspected of having hereditary motor neuropathy (HMN), but for whom a definitive diagnosis cannot be made without genetic testing, genetic testing for *BSCL2* mutations **MEETS COVERAGE CRITERIA**.

NOTES:

Note 1: Close blood relatives include 1st-degree relatives (e.g., parents, siblings, and children), 2nd-degree relatives (e.g., grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings), and 3rd-degree relatives (great-grandparents, great-aunts, great-uncles, great-grandchildren, and first cousins).

Note 2: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
AAN	American Academy of Neurology
AANEM	American Academy of Neuromuscular and Electrodiagnostic
AAPM&R	American Academy of Physical Medicine and Rehabilitation
AARS	Alanine-tRNA ligase, cytoplasmic
AIFM1	*Apoptosis-inducing factor 1*
BSCL2	*Seipin lipid droplet biogenesis associated*
DYNC1H1	*Dynein cytoplasmic 1 heavy chain 1*
EFNS	European Federation of Neurological Societies
EGR2	*Early growth response protein 2*
GARS	Glycyl-t-ribonucleic acid synthetase
GDAP1	*Ganglioside-induced differentiation-associated protein-1*
GJB1	Gap junction beta-1 protein (connexin 32)
HMN	Hereditary motor neuropathy
HNPP	Hereditary neuropathy with predisposition to pressure palsy
HSANs	Hereditary sensory and autonomic neuropathies
HSPB1	*Heat-shock protein beta-1*
HSPB8	*Heat-shock protein beta-8*
KIF1B	*Kinesin family member 1B*
LITAF	Lipopolysaccharide induced tumor necrosis factor
LMNA	Lamin A/C
MED25	*Mediator complex subunit 25*
MPZ	Myelin protein P_0
NEFL	Neurofilament light polypeptide
NINDS	National Institute of Neurological Disorders and Stroke
PDK3	*Pyruvate dehydrogenase kinase isoform 3*
PMP22	*Peripheral myelin protein 22*
PRPS1	*Ribose-phosphate pyrophosphokinase 1*
RAB7A	*Member RAS oncogene family*
Sep T9	*Septin 9*
SH3TC2	*SH3 domain and tetratricopeptide*
TRPV4	*Transient receptor potential cation channel subfamily V*
WES/WGS	Whole exome or genome sequencing

V. Scientific Background

Peripheral neuropathies encompass the set of disorders that primarily lead to peripheral nerve dysfunction. Symptoms typically include weakness of muscles at extremities, spine curvature, and loss of sensation at extremities (Kang, 2024c; UpToDate, 2024). Neuropathies may be caused by a variety of different factors, such as metabolic issues (including Fabry disease, Niemann-Pick disease, et al.) or present as a secondary symptom to another condition (such as Tangier disease) (Kang, 2024c).

Charcot-Marie-Tooth (CMTA) disease, also known as hereditary motor sensory neuropathy, is a group of progressive disorders that affect the peripheral nerves. CMT is caused by a mutation in one of several myelin genes that result in defects in myelin structure, maintenance, or function within peripheral nerves. Charcot-Marie-Tooth disease is one of the most common inherited neurological disorders, affecting approximately 1 in 2,500 people in the United States (Kang, 2024a).

Symptoms

The neuropathy of CMT affects both motor and sensory nerves. Symptoms usually start in childhood and have a gradual progression. The severity of symptoms varies greatly among individuals and even among family members with the disease and gene mutation (Bird, 2023; NINDS, 2007, 2023). Typical symptoms include the following:

- "Weakness or paralysis of the foot and lower leg muscles, which can cause difficulty lifting the foot (foot drop)
- A high-stepped gait with frequent tripping or falling
- Balance problems
- Foot deformities, such as high arches and curled toes (hammertoes)
- Lower legs may take on an "inverted champagne bottle" shape due to the loss of muscle bulk
- Reduced ability to feel heat, cold, and touch
- Weakness and atrophy may occur in the hands, causing difficulty with fine motor skills
- Decreased sense of vibration and position (proprioception)
- Curvature of the spine (scoliosis)
- Hip displacement
- Contractures (chronic shortening of muscles or tendons around joints)
- Muscle cramping
- Nerve pain" (NINDS, 2023)

Pain can range from mild to severe, and some people may need to rely on foot or leg braces or other orthopedic devices to maintain mobility. Some people living with CMT

experience tremor, and vision and hearing can also be affected. In rare cases, breathing difficulties may occur if the nerves that control the muscles of the diaphragm are affected (NINDS, 2023).

Causes

CMT is caused by mutations in genes that produce proteins involved in the structure and function of either the peripheral nerve axon or the myelin sheath. Although different proteins are abnormal in different forms of CMT disease, all mutations affect the normal function of the peripheral nerves. There is little correlation between the genotype and phenotype of CMT; it is common to see differing mutations result in various clinical phenotypes all within the same gene (Kang, 2024a).

Pattern of Inheritance

The pattern of inheritance varies with the type of CMT disease. CMT1, most cases of CMT2, and most intermediate forms are inherited in an autosomal dominant pattern. CMT4, a few CMT2 subtypes, and some intermediate forms are inherited in an autosomal recessive pattern. CMTX is inherited in an X-linked pattern. Some cases of CMT disease result from a new mutation and occur in people with no history of the disorder in their family. In rare cases the gene mutation causing CMT disease is a new mutation which occurs spontaneously in the individual's genetic material and has not been passed down through the family (Kang, 2024a).

CMT1

Charcot-Marie-Tooth disease type 1 (CMT1) is a demyelinating peripheral neuropathy characterized by distal muscle weakness and atrophy, sensory loss, and are usually slowly progressive and often associated with *pes cavus* foot deformity and bilateral foot drop (Bird, 2023). The six subtypes of CMT1 shown in Table 1 are clinically indistinguishable and are designated solely on molecular findings (Saporta et al., 2011)

Table 1: Molecular Genetics of CMT1 (Saporta et al., 2011)

Locus Name	Proportion of CMT1 (excluding CMTX)	Gene	Protein Product
CMT1A	70%-80%	*PMP22*	Peripheral myelin protein 22
CMT1B	10%-12%	*MPZ*	Myelin protein P_0
CMT1C	~1%	*LITAF*	Lipopolysaccharide-induced tumor necrosis factor-alpha factor

CMT1D	Unknown	*EGR2*	Early growth response protein 2
CMT1E	~1%	*PMP22*	Peripheral myelin protein 22 (sequence changes)
CMT1F/2E	Unknown	*NEFL*	Neurofilament light polypeptide

Charcot-Marie-Tooth disease type 1A (CMT1A) is an autosomal dominant disease that results from a duplication of the gene on chromosome 17 that carries the instructions for producing the peripheral myelin protein-22 (PMP-22). Overexpression of this gene causes the structure and function of the myelin sheath to be abnormal. A different neuropathy distinct from CMT1A called hereditary neuropathy with predisposition to pressure palsy (HNPP) is caused by a deletion of one of the PMP-22 genes. In this case, abnormally low levels of the PMP-22 gene result in episodic, recurrent demyelinating neuropathy (NINDS, 2007, 2023).

Charcot-Marie-Tooth disease type 1B (CMT1B) is an autosomal dominant disease caused by mutations in the gene that carries the instructions for manufacturing the myelin protein zero (P0), which is another critical component of the myelin sheath. Most of these mutations are point mutations. As a result of abnormalities in P0, CMT1B produces symptoms similar to those found in CMT1A (NINDS, 2007, 2023).

Other less common causes genetic causes of CMT1 result from mutations within *LITAF, EGR2, PMP22*, and *NEFL* genes, respectively (NINDS, 2023).

CMT2

Charcot-Marie-Tooth disease type 2 (CMT2) is an axonal (non-demyelinating) peripheral neuropathy characterized by distal muscle weakness and atrophy. Axonal peripheral neuropathy shows extensive clinical overlap with CMT1 (Bird, 2023). In general, individuals with CMT2 tend to be less disabled and have less sensory loss than individuals with CMT1 (Bird, 2023). It is less common than CMT1. CMT2A, the most common axonal form of CMT, is caused by mutations in Mitofusin 2, a protein associated with mitochondrial fusion. Symptoms are similar to those seen in CMT1, but people with CMT2 often have less disability and sensory loss than individuals with CMT1. Additionally, symptoms for CMT2 may have vocal cord or phrenic nerve involvement, causing speech or respiratory problems (NINDS, 2023).

Table 2: Molecular Genetics of CMT2 (Anthony Antonellis, 2018; Bird, 2023; Peter De Jonghe, 2011; Schindler, 2014; Züchner, 2013)

Locus	Proportion of CMT	Gene / Chromosome Locus	Protein Product
CMT2A1	Unknown	*KIF1B*	Kinesin-like protein KIF1B
CMT2A2[1]	20%	*MFN2*	Mitofusin-2
CMT2B	Unknown	*RAB7*A	Ras-related protein Rab-7
CMT2B1	Unknown	*LMNA*	Lamin A/C
CMT2B2	Unknown	*MED25*	Mediator of RNA polymerase II transcription subunit 25
CMT2C[2]	Unknown	*TRPV4*	Transient receptor potential cation channel subfamily V member 4
CMT2D[3]	3%	*GARS*	Glycyl-tRNA synthetase
CMT2E/1F[4]	4%	*NEFL*	Neurofilament light polypeptide
CMT2F	Unknown	*HSPB1*	Heat-shock protein beta-1
CMT2G	Unknown	12q12-q13	Unknown
CMT2H/2K	5%	*GDAP1*	Ganglioside-induced differentiation-associated protein-1
CMT2I/2J	Unknown	*MPZ*	Myelin protein P_0
CMT2L	Unknown	*HSPB8*	Heat-shock protein beta-8
CMT2N	Unknown	*AARS*	Alanine--tRNA ligase, cytoplasmic
CMT2O	Unknown	*DYNC1H1*	Cytoplasmic dynein 1 heavy chain 1
CMT2P	Unknown	*LRSAM1*	E3 ubiquitin-protein ligase LRSAM1
CMT2S	Unknown	*IGHMBP2*	DNA-binding protein SMUBP-2
CMT2T	Unknown	*DNAJB2*	DnaJ homolog subfamily B member 2
CMT2U	Unknown	*MARS*	Methionine--tRNA ligase, cytoplasmic

CMT3

Dejerine-Sottas disease (CMT3), is a severe demyelinating neuropathy that begins in infancy. Infants have severe muscle atrophy, weakness, and sensory problems. This rare disorder can be caused by mutations in multiple genes, including *PMP22, MPZ,* and *EGR2*, and can be inherited either dominantly or recessively (NINDS, 2023).

CMT4

Charcot-Marie-Tooth disease type 4 (CMT4) comprises several different subtypes of autosomal recessive demyelinating motor and sensory axonal neuropathies. Each neuropathy subtype is caused by a different genetic mutation, may affect a particular

ethnic population, and produces distinct physiologic or clinical characteristics. Affected individuals have the typical CMT phenotype of distal muscle weakness and atrophy associated with sensory loss and, frequently, pes cavus foot deformity. Several genes have been identified as causing CMT4, including *GDAP1* (CMT4A), *MTMR13* (CMT4B1), *MTMR2* (CMT4B2), *SH3TC2* (CMT4C), *NDG1* (CMT4D), *EGR2* (CMT4E), *PRX* (CMT4F), *FDG4* (CMT4H), and *FIG4* (CMT4J) (Kang, 2024a; NINDS, 2007, 2023).

Table 3: Molecular Genetics of CMT4 (Bird, 2017; Delague, 2013; Hamid Azzedine, 2015; Li, 2013)

Locus Name	Proportion of CMT4	Gene	Protein Product
CMT4A[1]	Unknown	*GDAP1*	Ganglioside-induced differentiation-associated protein 1
CMT4B1		*MTMR2*	Myotubularin-related protein 2
CMT4B2		*SBF2*	Myotubularin-related protein 13
CMT4C[2]		*SH3TC2*	SH3 domain and tetratricopeptide repeats-containing protein 2
CMT4D		*NDRG1*	Protein NDRG1
CMT4E		*EGR2*	Early growth response protein 2
CMT4F		*PRX*	Periaxin
CMT4H[3]		*FGD4*	FYVE, RhoGEF and PH domain-containing protein 4
CMT4J[4]		*FIG4*	Phosphatidylinositol 3, 5 biphosphate

CMTX

CMTX is caused by a point mutation in the connexin-32 gene on the X chromosome. The connexin-32 protein is expressed in Schwann cells, which wrap around nerve axons and make up a single segment of the myelin sheath (NINDS, 2007, 2023). CMTX type 1 is characterized by a moderate to severe motor and sensory neuropathy. Hearing loss and central nervous system symptoms may also occur in certain affected families (Abrams, 2020).

Table 4: Molecular Genetics of CMTX (Bird, 2023; Kim, 2013)

Disease Name	Proportion of X-Linked CMT	Gene / Chromosome Locus	Protein Product
CMTX1[1]	90%	*GJB1*	Gap junction beta-1 protein (connexin 32)

CMTX2[2]	Unknown	Xp22.2	
CMTX3[1]			Not applicable
CMTX4[1]		*AIFM1*	Apoptosis-inducing factor 1
CMTX5[2]		*PRPS1*	Ribose-phosphate pyrophosphokinase 1
CMTX6[1]		*PDK3*	Pyruvate dehydrogenase kinase isoform 3

Hereditary Brachial Plexopathy (Hereditary Neuralgic Amyotrophy)

This condition is primarily characterized by painful injuries to the brachial plexus nerves as well as episodic weakness of the shoulder and arm. Other symptoms such as winging of the scapula, short stature, neck folds, small face, and hypotelorism may be present. Nerve conduction velocity is typically normal, and the histopathology of this condition is non-specific. The *septin 9 gene* (*SEPT9*) on chromosome 17 has been associated with this condition (Bromberg, 2023).

Giant Axonal Neuropathy

This condition is characterized by disorganization of cytoskeletal intermediate filaments stemming from a mutated form of gigaxonin. Patients with this disorder often have a signature physical appearance; red and kinked hair, high foreheads, long eyelashes, and pale complexions are all hallmarks of this condition. The central nervous system may be affected as well with cerebellar dysfunction, spasticity, and potentially intellectual disability as possible symptoms. Nerve biopsy may show axonal loss or another axonal dysfunction. This diagnosis is confirmed by testing of the *GAN* gene (Kang, 2024c).

Hereditary Sensory and Autonomic Neuropathies (HSANs)

This subsection of disorders primarily encompasses non-motor neuropathies and are characterized by major loss of myelinated and unmyelinated fibers. These conditions are not as common as hereditary motor neuropathies and primarily present with sensory dysfunction, although motor functions may be affected. There are five main types of HSAN, each caused by different genes. Genes are associated as shown below (Eichler, 2024):

Disease Name (subtype)	Gene(s) or Locus	Examples of symptoms
HSAN1 (A)	*SPTLC1*	Distal sensory loss, distal muscle wasting
HSAN1 (B)	3p24-p22	Axonal neuropathy with distal sensory impairment

HSAN1 (C)	*SPTLC2*	Distal sensory loss, distal muscle wasting
HSAN1 (D)	*ATL1*	Distal sensory loss, distal muscle wasting
HSAN1 (E)	*DNMT1*	Hearing loss, progressive dementia
HSAN1 (F)	*ATL3*	Distal sensory impairment
HSAN2 (A)	*HSN2*	Loss of pain, pressure, touch, and temperature sensation
HSAN2 (B)	*FAM134 B*	Loss of pain, pressure, touch, and temperature sensation
HSAN2 (C)	*KIF1A*	Loss of pain, pressure, touch, and temperature sensation
HSAN2 (D)	*SCN9A*	Loss of pain and temperature sensation, hearing loss
HSAN3/Familial Dysautonomia	9q31	Dysautonomic crises, orthostatic hypotension
HSAN4/Congenital Insensitivity to Pain with Anhidrosis	*NTRK1*	Loss of pain sensation, thermoregulatory dysfunction
HSAN5	*NGFB*	Loss of pain and temperature sensation
HSAN6	*DST*	Lack of psychomotor development, respiratory difficulties
HSAN7	*SCN11A*	Inability to experience pain

Other unclassified HSANs exist, such as spastic paraplegia with ulcerations of the hands and feet (associated with *CCT5*) and sensory neuropathy with ichthyosis and anterior chamber syndrome (Eichler, 2024).

Genetic Testing

Charcot-Marie-Tooth disease is usually diagnosed by an extensive history and physical examination. The clinical diagnosis is then confirmed by electrodiagnostic tests like electromyography and nerve conduction velocity tests, and sometimes by nerve biopsy. Genetic testing is available for most types of CMT, and results are usually enough to confirm a diagnosis. Genetic testing can simplify the diagnosis of CMT by avoiding invasive procedures, such as nerve biopsy. In addition, early diagnosis can facilitate early interventions, including physical therapy. However, most therapies are only supportive (occupational, physical) and generally do not rely on the results of specific genetic testing (Kang, 2024a, 2024b). A positive genetic test can confirm diagnosis in most people with CMT. But a negative result does not exclude the disease, as an unidentified gene may be missed by DNA sampling (Charcot-Marie-Tooth News, 2023).

Genetic testing for CMT is complicated by the extensive underlying genetic heterogeneity. The CMT spectrum of disorders can be inherited in an autosomal dominant, autosomal recessive, or X-linked manner. The most commonly identified CMT subtypes are CMT1A (*PMP22* duplication), CMTX1 (*GJB1* mutation), hereditary neuropathy with liability to pressure palsies (*PMP22* deletion), CMT1B (*MPZ* mutation), and CMT2A (*MFN2* mutation). Together, these five subtypes account for 92 percent of genetically defined CMT cases. All other CMT subtypes and associated mutations each account for <1 percent of genetically defined CMT (CMTA, 2023; Kang, 2024a). Genetic screening for relatives of a patient diagnosed with CMT is an option, but risk assessment depends on several factors, including accuracy of the diagnosis, determination of the mode of inheritance for the individual family, and results of molecular genetic testing (Kang, 2024a).

Proprietary Testing

Numerous genetic panels are available for the assessment of peripheral neuropathies, such as GeneDx's panel (64 genes) and Invitae's panel (83 genes) (GeneDx, 2024; Invitae, 2024). Other pane's include ones by Athena Diagnostics (23 genes) (Athena Diagnostics, 2023), Claritas Genomics (*PMP22* gene) (Claritas Genomics, 2023), MNG Laboratories (139 genes) (MNG Laboratories, 2022), Prevention Genetics (44 genes) (Prevention Genetics, 2023), and Variantyx's Genomic Unity panel (25 genes).

Clinical Utility and Validity

DiVincenzo et al. (2014) performed an analysis of the genetic landscape of CMT. 14 genes associated with CMT (*PMP22, GJB1, MPZ, MFN2, SH3TC2, GDAP1, NEFL, LITAF, GARS, HSPB1, FIG4, EGR2, PRX*, and *RAB7A*) were evaluated out of 3312 individuals. Deletions and duplications in the PMP22 gene consisted of about 78% of positive findings, followed by mutations in the *GJB1* (6.7%), *MPZ* (5.3%), and *MFN2* (4.3%) genes. 71% of the pathogenic mutations found were missense mutations. Overall, 95% of the positive results involved one of four genes (*PMP22, GJB1, MPZ, MFN2*). The authors conclude that these four genes should be screened first before proceeding with further genetic testing (DiVincenzo et al., 2014).

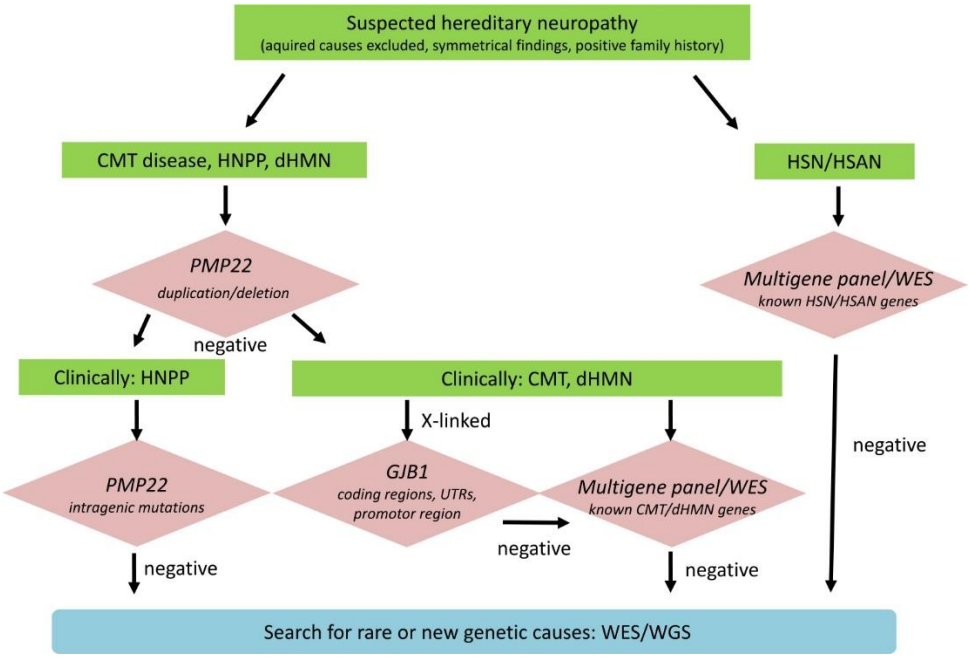
Pareyson et al. (2017) reviewed the current literature on CMT diagnosis stating that data justifies a step-wise algorithm considering a variety of factors, such as phenotype, nerve conduction velocities, and ethnicity. The authors note that NGS is steadily replacing older methods of sequencing in this algorithm. The authors propose evaluating the first few common genes (*PMP22*, *MPZ*, et al) and then considering larger sequencing methods such as NGS. However, due to the growing number of genes associated with CMT, these larger sequencing methods may be considered first-line.

Finally, the authors state that due to the growing number of associated genes, newer classifications need to be discussed and validated further (Pareyson et al., 2017).

Rudnik-Schoneborn et al. (2016) evaluated the clinical features and genetic results of 1206 CMT patients and 124 affected relatives. Genetic detection rates were 56% in demyelinating CMT and 17% in axonal CMT. "Three genetic defects (*PMP22* duplication/deletion, *GJB1*/*Cx32* or *MPZ*/P0 mutation) were responsible for 89.3% of demyelinating CMT index patients in whom a genetic diagnosis was achieved, and the diagnostic yield of the three main genetic defects in axonal CMT (*GJB1*/*Cx32*, *MFN2*, *MPZ*/P0 mutations) was 84.2%". The authors concluded that "diagnostic algorithms are still useful for cost-efficient mutation detection and for the interpretation of large-scale genetic data made available by next generation sequencing strategies" (Rudnik-Schoneborn et al., 2016).

Vaeth et al. (2019) evaluated the effect of implementing a targeted next-generation sequencing (NGS) approach for identifying CMT. The authors stated that from 1992-2012, a total of 1442 CMT analyses were performed (through Sanger sequencing and other quantitative analyses) and a pathogenic variant was discovered in 21.6% of these cases. From this cohort, 195 samples that did not reach a definitive diagnosis were sequenced by a custom 63-gene panel. The authors identified a 5.6% increase in diagnostic yield using this targeted NGS approach (Vaeth et al., 2019).

Cortese et al. (2020) investigated the effectiveness of NGS panels in CMT. 220 patients were enrolled in the study and a targeted CMT NGS panel was performed. After NGS sequencing, a molecular diagnosis based on a pathogenic variant was found in 30% of the cases and variants of unknown significance were found in 33% of the cases. 39% of the cases held mutations in *GJB1, MFN2*, and *MPZ* while the others held mutations in *SH3TC2, GDAP1, IGHMBP2, LRSAM1, FDG4,* and *GARS*. Copy number changes were detected in *PMP22, MPZ, MFN2, SH3TC2,* and *FDG4*. The authors conclude that "NGS panels are effective tools in the diagnosis of CMT, leading to genetic confirmation in one-third of cases negative for *PMP22* duplication/deletion, thus highlighting how rarer and previously undiagnosed subtypes represent a relevant part of the genetic landscape of CMT" (Cortese et al., 2020).



Rudnik-Schöneborn et al. (2020) suggested a diagnostic algorithm for genetic testing of suspected hereditary neuropathy. Advanced genetic sequencing allows for comprehensive evaluation of the pathogenic relevance of identified variants. As shown in the chart above, "If *PMP22* copy number analysis is negative, then clinical distinction of HNPP and CMT/dHMN will sort out patients for *PMP22* mutation analysis only and those for broader multigene testing. If a pedigree is compatible with X-linked inheritance, it is recommended to analyze coding and non-coding regions of *GBJ1*. Patients who are tested negative for known neuropathy genes may be included in further whole exome or genome sequencing (WES/WGS) to detect mutations in rare and new genes" (Rudnik-Schöneborn et al., 2020).

Yalcintepe et al. (2021) studied the importance of multiple gene analysis for diagnosis of Charcot Marie Tooth Disease. Fifty-five patients with suspected CMT phenotype were examined using a customized multigene panel which was compared to the Multiplex Ligand Probe Amplification (MLPA) method. The custom panel identified 13 cases (7.15%) with a pathogenic/likely pathogenic variant. "The affected genes were *MARS1, NDRG1, GJB1, GDAP1, MFN2, PRX, SH3TC2*, and *FGD4*. Six cases (10.9%) had pathogenic variants in *GJB1* and *FGD4* genes, variants of unknown significance (VUS) in *HSPB3, CHRNA1, ARHGEF10,* and *KIF5A* genes. 21 cases (11.55%) had VUS with the genes *HSPB3, KIF1B, SCN11A, CHRNA1, HSPB1, FIG4, ARHGEF10, DHTKD1, SBF1, EGR2, SBF2, IGHMBP2, KIF5A*, and *DNAJB2*." The authors concluded that the NGS customized panel was beneficial, time-saving, and cost-effective in the diagnosis of CMT (Yalcintepe et al., 2021).

VI. Guidelines and Recommendations

American Academy of Neurology (AAN), the American Academy of Neuromuscular and Electrodiagnostic Medicine (AANEM), and the American Academy of Physical Medicine and Rehabilitation (AAPM&R)

The Polyneuropathy Task Force that included 19 physicians with representatives from the American Academy of Neurology (AAN), the American Academy of Neuromuscular and Electrodiagnostic Medicine (AANEM), and the American Academy of Physical Medicine and Rehabilitation (AAPM&R) concluded that "genetic testing is established as useful for the accurate diagnosis and classification of hereditary polyneuropathies (Class I)" (England et al., 2009).

The Task Force stated that "for patients with a cryptogenic polyneuropathy who exhibit a classic hereditary neuropathy phenotype, routine genetic screening may be useful for CMT1A duplication/deletion and *Cx32* mutations in the appropriate phenotype (Class III). Further genetic testing may be considered guided by the clinical question." The Task Force recommended that "genetic testing should be conducted for the accurate diagnosis and classification of hereditary neuropathies (Level A)". The Task force further recommended that "Genetic testing may be considered in patients with a cryptogenic polyneuropathy and classic hereditary neuropathy phenotype (Level C). Initial genetic testing should be guided by the clinical phenotype, inheritance pattern, and electrodiagnostic features and should focus on the most common abnormalities which are CMT1A [*PMP22]* duplication/HNPP deletion, Cx32 (*GJB1*), and *MFN2* mutation screening. There is insufficient evidence to support or refute the usefulness of routine genetic testing in cryptogenic polyneuropathy patients without a classic hereditary phenotype (Level U)" (England et al., 2009).

These guidelines were reaffirmed on January 22, 2022.

European Federation of Neurological Societies (EFNS)

The EFNS released recommendations on genetic testing for various types of peripheral neuropathies. Regarding CMT, they noted that "Given the rarity of AR CMT in the European population routine diagnostic screening of the many known genes is currently not feasible" but acknowledged that "Currently, molecular genetic testing can be offered for several of the more prevalent CMT genes." EFNS stated that PMP22 duplication should be tested first in patients presenting with CMT1, followed by sequencing of *GJB1*, *MPZ*, and *PMP22*. If a patient presents with CMT2, *MFN2* should be screened first, followed by *MPZ*. If a patient presents with intermediate CMT, *GJB1* and *MPZ* should be screened. EFNS notes that in patients with hereditary neuropathy

with liability to pressure palsies will be investigated for a *PMP22* deletion at the same time as a screening for a *PMP22* duplication (Burgunder et al., 2011).

However, routine diagnostic screenings for hereditary motor neuropathy (HMN) and hereditary sensory-autonomic neuropathy (HSAN) are not feasible due to low mutation frequencies. If screening is performed for these conditions, EFNS recommends *BSCL2* as the first candidate for screening in HMN. NTRK1 may also be screened for in congenital insensitivity to pain with anhidrosis patients (CIPA, a sub-phenotype of HSAN) and *RAB7* may be screened in CMT2B patients. Finally, *SEPT9* may be screened in the context of hereditary neuralgic amyotrophy (HNA) (Burgunder et al., 2011).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81324	PMP22 (peripheral myelin protein 22) (eg, Charcot-Marie-Tooth, hereditary neuropathy with liability to pressure palsies) gene analysis; duplication/deletion analysis
81325	PMP22 (peripheral myelin protein 22) (eg, Charcot-Marie-Tooth, hereditary neuropathy with liability to pressure palsies) gene analysis; full sequence analysis

81326	PMP22 (peripheral myelin protein 22) (eg, Charcot-Marie-Tooth, hereditary neuropathy with liability to pressure palsies) gene analysis; known familial variant
81403	Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)
81404	Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons)
81448	Hereditary peripheral neuropathies (eg, Charcot-Marie-Tooth, spastic paraplegia), genomic sequence analysis panel, must include sequencing of at least 5 peripheral neuropathy-related genes (eg, BSCL2, GJB1, MFN2, MPZ, REEP1, SPAST, SPG11, SPTLC1)

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Abrams, C. (2020). GJB1 Disorders: Charcot Marie Tooth Neuropathy (CMT1X) and Central Nervous System Phenotypes. https://www.ncbi.nlm.nih.gov/books/NBK1374/

Anthony Antonellis, P., Lev G Goldfarb, MD, and Kumaraswamy Sivakumar, MD. (2018). GARS-Associated Axonal Neuropathy. https://www.ncbi.nlm.nih.gov/books/NBK1242/

Athena Diagnostics. (2023). CMT Advanced Evaluation - Comprehensive. https://www.athenadiagnostics.com/view-full-catalog/c/cmt-advanced-evaluation-comprehensive

Bird, T. (2017). GDAP1-Related Hereditary Motor and Sensory Neuropathy. https://www.ncbi.nlm.nih.gov/books/NBK1539/

Bird, T. (2023). Charcot-Marie-Tooth (CMT) Hereditary Neuropathy Overview. https://www.ncbi.nlm.nih.gov/books/NBK1358/

Bromberg, M. B. (2023, September 19). *Brachial plexus syndromes*. https://www.uptodate.com/contents/brachial-plexus-syndromes

Burgunder, J. M., Schols, L., Baets, J., Andersen, P., Gasser, T., Szolnoki, Z., Fontaine, B., Van Broeckhoven, C., Di Donato, S., De Jonghe, P., Lynch, T., Mariotti, C., Spinazzola, A., Tabrizi, S. J., Tallaksen, C., Zeviani, M., Harbo, H. F., & Finsterer, J. (2011). EFNS guidelines for the molecular diagnosis of neurogenetic disorders: motoneuron, peripheral nerve and muscle disorders. *Eur J Neurol*, *18*(2), 207-217. https://doi.org/10.1111/j.1468-1331.2010.03069.x

Charcot-Marie-Tooth News. (2023). *Genetic Tests and Genetic Counseling*. https://charcot-marie-toothnews.com/genetic-tests/

Claritas Genomics. (2023). PMP22 Deletion/Duplication. http://www.claritasgenomics.com/test/pmp22-deletionduplication/index.html

CMTA. (2023). Genetic Testing. https://www.cmtausa.org/living-with-cmt/find-help/genetic-testing/

Cortese, A., Wilcox, J. E., Polke, J. M., Poh, R., Skorupinska, M., Rossor, A. M., Laura, M., Tomaselli, P. J., Houlden, H., Shy, M. E., & Reilly, M. M. (2020). Targeted next-generation sequencing panels in the diagnosis of Charcot-Marie-Tooth disease. *Neurology*, *94*(1), e51-e61. https://doi.org/10.1212/WNL.0000000000008672

Delague, V. (2013). Charcot-Marie-Tooth Neuropathy Type 4H. https://www.ncbi.nlm.nih.gov/books/NBK153601/

DiVincenzo, C., Elzinga, C. D., Medeiros, A. C., Karbassi, I., Jones, J. R., Evans, M. C., Braastad, C. D., Bishop, C. M., Jaremko, M., Wang, Z., Liaquat, K., Hoffman, C. A., York, M. D., Batish, S. D., Lupski, J. R., & Higgins, J. J. (2014). The allelic spectrum of Charcot-Marie-Tooth disease in over 17,000 individuals with neuropathy. *Mol Genet Genomic Med*, *2*(6), 522-529. https://doi.org/10.1002/mgg3.106

Eichler, F. (2024, 05/02/2023). *Hereditary sensory and autonomic neuropathies*. https://www.uptodate.com/contents/hereditary-sensory-and-autonomic-neuropathies

England, J. D., Gronseth, G. S., Franklin, G., Carter, G. T., Kinsella, L. J., Cohen, J. A., Asbury, A. K., Szigeti, K., Lupski, J. R., Latov, N., Lewis, R. A., Low, P. A., Fisher, M. A., Herrmann, D. N., Howard, J. F., Lauria, G., Miller, R. G., Polydefkis, M., & Sumner, A. J. (2009). Practice Parameter: Evaluation of distal symmetric polyneuropathy: Role of laboratory and genetic testing (an evidence-based review). *Neurology*, *72*(2), 185. https://doi.org/10.1212/01.wnl.0000336370.51010.a1

GeneDx. (2024). Hereditary Neuropathy Panel. https://www.genedx.com/tests/detail/hereditary-neuropathy-panel-800

Hamid Azzedine, E. L., and Mustafa A Salih. (2015). Charcot-Marie-Tooth Neuropathy Type 4C. https://www.ncbi.nlm.nih.gov/books/NBK1340/

Invitae. (2024). *Invitae Comprehensive Neuropathies Panel*. https://www.invitae.com/us/providers/test-catalog/test-03200

Kang, P. (2024a, 05/30/2024). *Charcot-Marie-Tooth disease: Genetics, clinical features, and diagnosis*. https://www.uptodate.com/contents/charcot-marie-tooth-disease-genetics-clinical-features-and-diagnosis

Kang, P. (2024b, September 22). *Charcot-Marie-Tooth disease: Management and prognosis*. https://www.uptodate.com/contents/charcot-marie-tooth-disease-management-and-prognosis

Kang, P. (2024c, 04/15/2024). *Overview of hereditary neuropathies*. https://www.uptodate.com/contents/overview-of-hereditary-neuropathies

Kim, J.-W., Kim, Hee-Jin. (2013). Charcot-Marie-Tooth Neuropathy X Type 5. https://www.ncbi.nlm.nih.gov/books/NBK1876/

Li, J. (2013). Charcot-Marie-Tooth Neuropathy Type 4J. https://www.ncbi.nlm.nih.gov/books/NBK169431/

MNG Laboratories. (2022). Charcot-Marie-Tooth Disease, Axonal (NGS Panel and Copy Number Analysis + mtDNA). https://mnglabs.com/tests/NGS345A/charcot-marie-tooth-disease-axonal-ngs-panel-and-copy-number-analysis-mtdna

NINDS. (2007). Charcot-Marie-Tooth Disease Fact Sheet. https://www.ninds.nih.gov/Disorders/Patient-Caregiver-Education/Fact-Sheets/Charcot-Marie-Tooth-Disease-Fact-Sheet#3092_5

NINDS. (2023). *Charcot-Marie-Tooth Disease*. https://www.ninds.nih.gov/health-information/disorders/charcot-marie-tooth-disease

Pareyson, D., Saveri, P., & Pisciotta, C. (2017). New developments in Charcot-Marie-Tooth neuropathy and related diseases. *Curr Opin Neurol*, *30*(5), 471-480. https://doi.org/10.1097/wco.0000000000000474

Peter De Jonghe, M., PhD and Albena K Jordanova, PhD. (2011). Charcot-Marie-Tooth Neuropathy Type 2E/1F. https://www.ncbi.nlm.nih.gov/books/NBK1187/

Prevention Genetics. (2023). Charcot-Marie-Tooth (CMT) - Comprehensive Panel. https://www.preventiongenetics.com/testInfo?val=Charcot-Marie-Tooth+%28CMT%29+-+Comprehensive+Panel

Rudnik-Schöneborn, S., Auer-Grumbach, M., & Senderek, J. (2020). Charcot-Marie-Tooth disease and hereditary motor neuropathies – Update 2020. *Medizinische Genetik*, *32*(3), 207-219. https://doi.org/doi:10.1515/medgen-2020-2038

Rudnik-Schoneborn, S., Tolle, D., Senderek, J., Eggermann, K., Elbracht, M., Kornak, U., von der Hagen, M., Kirschner, J., Leube, B., Muller-Felber, W., Schara, U., von Au, K., Wieczorek, D., Bussmann, C., & Zerres, K. (2016). Diagnostic algorithms in Charcot-Marie-Tooth neuropathies: experiences from a German genetic laboratory on the basis of 1206 index patients. *Clin Genet*, *89*(1), 34-43. https://doi.org/10.1111/cge.12594

Saporta, A. S., Sottile, S. L., Miller, L. J., Feely, S. M., Siskind, C. E., & Shy, M. E. (2011). Charcot-Marie-Tooth disease subtypes and genetic testing strategies. *Ann Neurol*, *69*(1), 22-33. https://doi.org/10.1002/ana.22166

Schindler, A. (2014). TRPV4-Associated Disorders. https://www.ncbi.nlm.nih.gov/books/NBK201366/

UpToDate. (2024). *Patient education: Charcot-Marie-Tooth disease (The Basics)*. https://www.uptodate.com/contents/charcot-marie-tooth-disease-the-basics

Vaeth, S., Christensen, R., Duno, M., Lildballe, D. L., Thorsen, K., Vissing, J., Svenstrup, K., Hertz, J. M., Andersen, H., & Jensen, U. B. (2019). Genetic analysis of Charcot-Marie-Tooth disease in Denmark and the implementation of a next generation sequencing platform. *Eur J Med Genet*, *62*(1), 1-8. https://doi.org/10.1016/j.ejmg.2018.04.003

Yalcintepe, S., Gurkan, H., Dogan, I. G., Demir, S., Sag, S. O., Kabayegit, Z. M., Atli, E. I., Atli, E., Eker, D., & Temel, S. G. (2021). The Importance of Multiple Gene Analysis for Diagnosis and Differential Diagnosis in Charcot Marie Tooth Disease. *Turk Neurosurg*, *31*(6), 888-895. https://doi.org/10.5137/1019-5149.jtn.33661-21.3

Züchner, S. (2013). Charcot-Marie-Tooth Neuropathy Type 2A. https://www.ncbi.nlm.nih.gov/books/NBK1511/

Genetic Testing for Muscular Dystrophies

Policy Number: AHS – M2074 – Genetic Testing for Dystrophies	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Muscular dystrophies, genetic conditions characterized by progressive muscle atrophy, can be caused by several genetic mutations. Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are due to mutations in the dystrophin gene on the X chromosome (Darras, 2024a). Facioscapulohumeral muscular dystrophy (FSHD) occurs due to a contraction of the polymorphic macrosatellite repeat D4Z4 on chromosome 4q35 (Darras, 2024c). The limb-girdle muscular dystrophies (LGMDs) are a group of approximately 30 rare hereditary progressive neuromuscular disorders (Murphy & Straub, 2015) LGMDs result from mutations in genes required for normal muscle function and vary in severity, phenotype, pathology, and age of onset (Darras, 2024d).

Pretest genetic counseling and genetic counseling at time of result disclosure is strongly recommended for individuals pursuing genetic testing for muscular dystrophies.

Terms such as male and female are used when necessary to refer to sex assigned at birth.

For guidance on prenatal or preconception screening for muscular dystrophies, please see AHS-M2179-Prenatal Screening (Genetic).

II. Related Policies

Policy Number	Policy Title
AHS-M2179	Prenatal Screening (Genetic)

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with clinical signs of a dystrophinopathy, genetic testing for likely pathogenic or pathogenic variants of *dystrophin (DMD)* **MEETS COVERAGE CRITERIA**.

2) For individuals with clinical signs of facioscapulohumeral muscular dystrophy (FSHD), genetic testing to confirm a diagnosis of FSHD **MEETS COVERAGE CRITERIA**.

3) For individuals who are clinically suspected of having limb-girdle muscular dystrophy (LGMD), genetic testing for likely pathogenic or pathogenic variants associated with LGMD **MEETS COVERAGE CRITERIA**.

4) For individuals with clinical signs of congenital muscular dystrophy (CMD), genetic testing to confirm a diagnosis of CMD **MEETS COVERAGE CRITERIA**.

5) For individuals for whom genetic testing was negative for dystrophinopathies, FSHD, LGMD, or CMD, but for whom the clinical suspicion of a muscular dystrophy remains, genetic testing for rare muscular dystrophies (e.g., myotonic dystrophy, Emery-Dreifuss muscular dystrophy, oculopharyngeal muscular dystrophy, distal muscular dystrophy) **MEETS COVERAGE CRITERIA**.

6) For first- and second-degree relatives (see Note 1) of individuals with a muscular dystrophy, the following genetic testing **MEETS COVERAGE CRITERIA**:
   a) Testing restricted to the known familial likely pathogenic or pathogenic variant.
   b) Comprehensive disorder specific genetic testing when the specific familial likely pathogenic or pathogenic variant is unknown.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

7) For all other situations not discussed above, genetic testing for muscular dystrophies **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: First-degree relatives include parents, full siblings, and children of the individual. Second-degree relatives include grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings of the individual.

Note 2: For two or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
AAN	American Academy of Neurology
AANEM	American Association of Neuromuscular and Electrodiagnostic Medicine
ACMG	American College of Medical Genetics
ALT	Alanine aminotransferase
AMP	Association for Molecular Pathology
ANO5	*Anoctamin 5*
AST	Aspartate aminotransferase
BMD	Becker Muscular Dystrophy
CAPN3	*Calpain 3*
cDNA	Copy/complementary DNA
CGH	Comparative genomic hybridization
CK	Creatine phosphokinase
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMA	Chromosomal microarray
CMD	Congenital muscular dystrophy
CMS	Centers for Medicare and Medicaid
CNBP	*CCHC-type zinc finger nucleic acid binding protein*
CNVs	Copy number variations
COL6A1/2/3	*Collagen type VI alpha [1, 2, or 3] chain*
CRPPA	CDP-L-ribitol pyrophosphorylase A
CVS	Chorionic villus sampling
DAG1	*Dystroglycan 1*
DCM	Dilated cardiomyopathy
DM1/DM2	Myotonic dystrophy
DMD	Duchenne muscular dystrophy
DMD	*Dystrophin*

DMPK	*Dystrophia myotonica protein kinase*
DNA	Deoxyribonucleic acid
DNAJB6	*DnaJ heat shock protein family (Hsp40) member B6*
DNMT3B	*DNA-methyltransferase 3 beta*
DPPI	Duchenne Parent Project Italy
DUX4	Double homeobox protein 4
DYSF	*Dysferlin*
EDMD	Emery-Dreifuss Muscular Dystrophy
EMQN	European Molecular Genetics Quality Network
EPNS	European Paediatric Neurology Society
FCMD	Fukuyama congenital muscular dystrophy
FDA	Food and Drug Administration
FKRP	*Fukutin-related protein*
FKTN	*Fukutin*
FSHD	Facioscapulohumeral Muscular Dystrophy
GMPPB	*GDP-Mannose pyrophosphorylase B*
HNRNPDL	*Heterogeneous nuclear ribonucleoprotein D like protein*
Hsp40	Heat shock protein 40 kD
LAMA2	*Laminin subunit alpha 2*
LDTs	Laboratory-developed tests
LGMDs	Limb-girdle muscular dystrophies
LRIF1	*Ligand-dependent nuclear receptor-interacting factor 1*
MDA	Muscular Dystrophy Association
MLPA	Multiplex ligation-dependent probe amplification
mPCR	Multiplex polymerase chain reaction
mRNA	Messenger ribonucleic acid
NGS	Next-generation sequencing
NMDs	Neuromuscular disorders
OMIM	Online Mendelian Inheritance in Man
OPMD	Oculopharyngeal muscular dystrophy
PABPN1	*Polyadenosine RNA binding protein, polyadenylate-binding nuclear protein 1*
PCR	Polymerase chain reaction
PLEC1	*Plectin*
POGLUT1	*Protein O-glucosyltransferase 1*
POMGNT1	*Protein O-linked mannose N-acetylglucosaminyltransferase 1*
POMGNT2	*Protein O-linked mannose N-acetylglucosaminyltransferase 2*
POMT1	*Protein O-mannosyltransferase 1*
POMT2	*Protein O-mannosyltransferase 2*

PPMD	Parent Project Muscular Dystrophy
RHDO	Relative haplotype dosage analysis
RNA	Ribonucleic acid
SEPN1	*Selenon*
SGCA	*Sarcoglycan alpha*
SGCB	*Sarcoglycan beta*
SGCD	*Sarcoglycan delta*
SGCG	*Sarcoglycan gamma*
SMCHD1	*Structural maintenance of chromosomes flexible hinge domain containing 1*
SNPs	Single nucleotide polymorphisms
SNVs	Single nucleotide variants
TCAP	*Telethonin*
TNPO3	*Transportin 3*
TRAPPC11	*Trafficking protein particle complex 11*
TRIM32	*Tripartite m-containing 32*
TTN	*Titin*
USPSTF	U.S. Preventive Services Task Force
WES	Whole-exome sequencing
ZAK	Former gene name of *MAP3K20 (mitogen-activated protein kinase kinase kinase 20)*

V. Scientific Background

Neuromuscular disorders (NMDs) encompass a diverse set of conditions, many of which arise from specific genetic mutations that impair muscle function and nervous system communication. NMDs are an inherited group of progressive disorders that involve muscle weakness as the primary symptom. Among the most prominent are DMD, BMD, facioscapulohumeral muscular dystrophy (FSHD) and LGMD. Each disorder has distinct genetic causes and clinical presentations.

Duchenne Muscular Dystrophy is caused by mutations in the dystrophin gene on the X chromosome, leading to a lack of functional dystrophin, a protein vital for stabilizing muscle fibers. This disorder typically presents in early childhood with muscle weakness, progressive loss of mobility and cardiac or respiratory complications (Darras, 2024a).

Becker muscular dystrophy, similar to DMD, results from mutation in the dystrophin gene but allows for the production of partially functional dystrophin. As a result, symptoms may appear later in life and progress more slowly, often enabling individuals to remain ambulatory into adulthood (Darras, 2024a).

Facioscapulohumeral Muscular Dystrophy occurs due to the contraction of the D4Z4 repeat region on chromosome 4q35, which leads to the abnormal expression of the *DUX4* gene with a protein toxic to muscle cells. Symptoms include weakness in the facial, shoulder and upper arm muscles, with variability in disease severity and progression among individuals (Darras, 2024c).

Limb-Girdle Muscular Dystrophy represents a group of over 30 hereditary disorders characterized by weakness in the muscles around the hips and shoulders. Different genetic mutations affect the proteins required for normal muscle structure and function, resulting in a wide range of severity, onset stages, and disease progression patterns (Darras, 2024d).

In addition to the more well-known forms of muscular dystrophy, several lesser-known types of muscular dystrophy may present clinically. Congenital muscular dystrophies (CMDs) are a heterogeneous group of disorders that present at birth or in early infancy (within the first two years), caused by mutations in genes involved in muscle and connective tissue development. Examples include Walker-Warburg syndrome and Ullrich CMD (Darras, 2024e). Myotonic Dystrophy (DM1 and DM2) occurs as an adult-onset muscular dystrophy characterized by muscle stiffness, weakness, and systemic involvement (such as cardiac issues). This disorder results from mutations in the *DMPK* or *CNBP* genes. Emery-Dreifuss Muscular Dystrophy (EDMD) is caused by mutations in genes encoding nuclear envelope proteins, and leads to early contractures, progressive muscle wasting and cardiomyopathy (Darras, 2024b). Oculopharyngeal Muscular Dystrophy (OPMD) is a rare adult-onset dystrophy (mainly autosomal dominant inheritance) associated with mutation in the *PABPN1* gene, causing ptosis, difficulty swallowing and proximal muscle weakness (Darras, 2024e). Distal muscular dystrophies are a heterogenous group (over 12 different disorders of myopathies) that affect distal muscles (hands, feet, and forearms) (Darras, 2024e).

Duchenne Muscular Dystrophy (DMD) and Becker Muscular Dystrophy (BMD)

Dystrophinopathies are due to mutations in the *DMD* (Dystrophin) gene located on the X chromosome inherited in a recessive pattern. All hemizygous males will exhibit the characteristic phenotype whereas heterozygous females for a pathogenic mutation may exhibit a range of clinical manifestations. If skeletal muscles are affected, dystrophinopathies are classified as either DMD or BMD; however, if cardiac muscle is primarily affected, it is characterized as *DMD*-associated dilated cardiomyopathy (DCM) (Darras, 2024c; Darras et al., 2000).

Duchenne muscular dystrophy, the more severe phenotype of the skeletal muscle dystrophinopathies, typically presents in males before the age of five with progressive, symmetric muscle weakness and calf hypertrophy. Affected males are typically

wheelchair-dependent before their teens, and the individual rarely survives beyond their thirties due to respiratory complications and heart failure. BMD is often less severe and manifests later in affected individuals at 30 years of age. Most of these individuals remain ambulatory into adulthood, with some reported to remain ambulatory as late as their sixties. Although skeletal muscle deterioration progresses more slowly in BMD, cardiomyopathy is the most common cause of death in these patients, shortening the life expectancy to the mid-forties. Many BMD patients receive heart transplants within five years after diagnosis of cardiomyopathy (Darras et al., 2000). The prevalence of DMD in the United States has been estimated between 1.3 and 2.1 cases per 10,000 live male births, though the number differed by race/ethnicity in a recent study (Romitti et al., 2015).

Duchenne muscular dystrophy or BMD should be suspected in males with elevated serum creatine phosphokinase (CK) levels (>ten-times of normal and >five-times of normal, respectively) and clinical symptoms of dystrophinopathy. As permeability of the sarcolemma increases due to muscle damage, creatine phosphokinase is released beyond the normal range, which can be used as a diagnostic value. In DMD, serum CK peaks by age two years, with levels reaching ten to 20 times the upper limit of normal (Darras, 2024a). As the disease progresses, dystrophic muscle fibers begin to decay and the rate of CK release decreases (Kim et al., 2017). Hemizygous female carriers can also exhibit elevated serum CK levels, which are two to ten times that of the normal range. (Darras et al., 2000). A normal CK level, however, makes DMD and BMD unlikely and alternative diagnoses should be considered in these cases.

A variety of genetic testing methodologies are available to assist in the diagnosis of DMD/BMD. In general, it is advisable to try larger deletion/duplication genetic testing first and, if negative, move on to next-generation exome or genome sequence analysis, which can detect "small" mutations [like single nucleotide polymorphisms (SNPs)] as well as micro deletion and duplications (Darras, 2024a). Multiplex ligation-dependent probe amplification (MLPA) has been used successfully to detect duplication or deletion mutations that cause DMD/BMD. It has also been reported that up to 98% of disease-causing deletions could be detected with multiplex polymerase chain reaction (PCR) (Chamberlain et al., 1992). Quantitative PCR, long-range PCR, and chromosomal microarray (CMA) may also be considered; however, CMA is not recommended as a primary confirmatory assay for dystrophinopathies as the sensitivity of the assay may not be sufficient to detect all exon level DMD deletions and duplications (Darras et al., 2000).

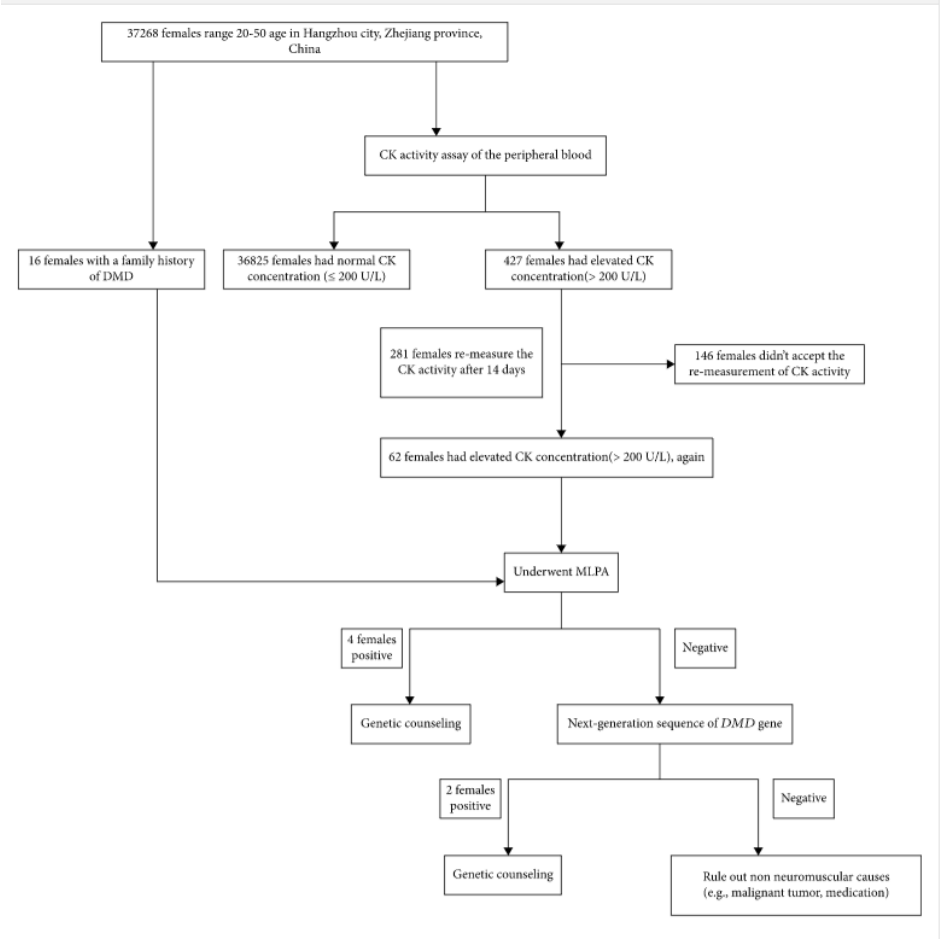
Clinical Utility and Validity of DMD/BMD Genetic Testing

A recent Chinese study of 146 at-risk pregnancies in 131 DMD families report a 99% mutation detection rate using "a prenatal diagnosis algorithm for dystrophinopathies that combines multiplex ligation-dependent probe amplification (MLPA), quantitative PCR, sequencing and linkage analyses" (Wang et al., 2017). This data also shows that 51.1% of the probands had de novo exon deletions. Recombination of the *DMD* gene occurred in nine of the 146 pregnancies. The authors conclude that "the present results demonstrate the importance of considering maternal germline mosaicism in the genetic assessment. Prenatal diagnosis should be suggested to the parent with a DMD proband whether carrier testing found the causative mutation in the mother's blood or not" (Wang et al., 2017). The reported accuracy rate of this multiplex/quantitative PCR-based method is considerably higher that the reported accuracy rate (>70%) of a real-time PCR assay of the *DMD* gene (Zhang et al., 2013).

The MLPA-based genetic testing of dystrophinopathies has been reported in many studies with varying degrees of sensitivity. A large study of 1053 Chinese DMD/BMD patients using MLPA testing reported identifying 70.56% of the probands (Yang et al., 2013) whereas a smaller study of 121 individuals (both male and female) reports confirmation of only 63% of patients and symptomatic females (Luce et al., 2016). A third study of using an algorithm of mPCR and MLPA on 150 male patients reported a 75% confirmation rate (Murugan et al., 2010). Another study using MLPA concluded that "the reading-frame rule held in 90% to 94% of children, which is consistent with reports from other parts of the world. However, testing by MLPA is a limitation, and advanced sequencing methods including analysis of the structure of mutant dystrophin is needed for more-accurate assessments of the genotype-phenotype correlation" (Vengalil et al., 2017).

In a study by Han et al. (2020), 37,268 females of reproductive age were recruited in Hangzhou, China, to identify definite DMD carriers prior to or early in pregnancy. Participants were screened for high CK activity (>200 U/L), a typical finding in 50-70% of asymptomatic heterozygous female DMD carriers (Brandsema & Darras, 2015), and DMD family history. The screening process narrowed down the number of participants for DMD genetic sequencing performed with MLPA to detect gross deletions and duplications and next-generation sequencing (NGS) based on multiplex PCR to detect the small point mutations, insertions, and deletions. From the 37,268 participants, 427 females were identified to have CK levels above 200 U/L and 16 females with a previous family history of DMD. A total of 427 females with high CK levels were asked to repeat CK testing, to which 281 females agreed, and 62 females showed to have sustained elevated serum CK levels. The 16 participants with DMD family history and 62 females with sustained CK levels >200 U/L were asked to undergo *DMD* genetic

testing. "MLPA and NGS of the *DMD* gene identified six definite DMD carriers with clear pathogenic variants (3 of the 16 subjects with positive family history and three of the 62 subjects with negative family history) … Four cases had deletions and/or duplications of *DMD* gene, mostly located in a hotspot mutation region (exons 44-55), one case carried a previous reported pathogenic missense variant, and a novel deleterious frameshift pathogenic variant was found in one case" (Han et al., 2020). Results of the screening for DMD carriers are summarized in the figure below (Han et al., 2020).



Confirmed carriers of DMD pathogenic variant were provided professional genetic counseling and several reproductive choices such as preimplantation genetic diagnosis, prenatal genetic testing through chorionic villus, amniotic fluid, or umbilical cord blood sampling during different gestation weeks as needed. The authors state that current carrier screening for DMD, which is provided for females with a family history of DMD, misses the many spontaneous cases resulting from unsuspected variants. Therefore, the authors suggest that "Carrier screening before or early in pregnancy will allow carrier females to receive genetic counseling and be informed of fertility choices and recurrent risk. Moreover, carrier screening will help carriers prepare

for the possibility of manifesting DMD-related symptoms later on in life… Furthermore, female relatives of positive carriers should be recommended for genetic testing in order to evaluate their carrier status" (Han et al., 2020).

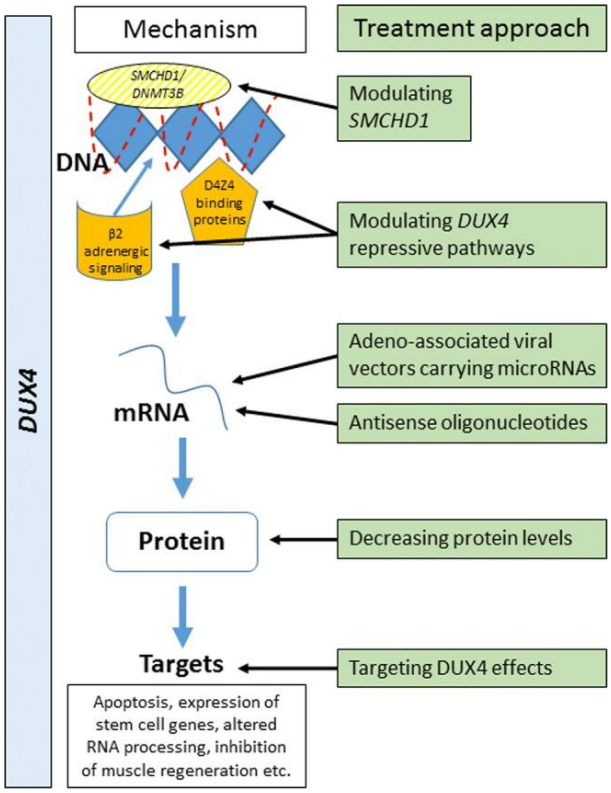
Facioscapulohumeral Muscular Dystrophy (FSHD)

Facioscapulohumeral muscular dystrophy is the third most common type of muscular dystrophy , with an estimated prevalence of four to 12 per 100,000 population (Darras et al., 2000). It is an autosomal dominant genetic disorder that is caused by a deletion of the macrosatellite repeat regions D4Z4 of the *DUX4* gene in the subtelomeric region of chromosome 4q. In healthy individuals, the *DUX4* gene is epigenetically silenced in somatic tissues; however, contraction of the D4Z4 repeats allows for inefficient chromatin silencing due to abnormal chromatin structure, resulting in inappropriate somatic expression. Unaffected individuals have a variable number of D4Z4 repeats, ranging from 11 to more than 100, whereas FSHD1 patients have only one to ten repeats on one of the copies of chromosome four. The DUX4 protein is usually only expressed in the germline as a DNA-binding protein with presumed transcription factor activity. Its toxicity in somatic cells is unknown. Two forms of FSHD have been classified—FSHD1, the major form due to a major contraction of the D4Z4 macrosatellite repeat sequences, and FSHD2, a minor form with a normal number of D4Z4 repeats but abnormal D4Z4 chromatin structure. FSHD2 patients have a disease status that cannot be confirmed by using the standard molecular diagnostic testing used in FSHD1 patients (van der Maarel et al., 2011). Further, 85% of patients with FSHD2 have mutations in the *SMCHD1* gene on chromosome 18, which encodes for a chromatin modifier believed to be involved in maintaining the D4Z4 chromatin structure (R. J. Lemmers et al., 2012). Pathogenic variants of *DNMT3B* and *LRIF1* genes are less common causes of FSHD2 (Hamanaka et al., 2020; van den Boogaard et al., 2016). Despite the different genetic causes, the phenotypes of FSHD1 and FSHD2 patients are often clinically indistinguishable (de Greef et al., 2010).

Facioscapulohumeral muscular dystrophy patients exhibit a progressive muscular dystrophy with variability of affected muscles between patients. Generally, muscles of the face, arms, legs, shoulders, and abdomen can be affected. Serum-based diagnostic testing for FSHD has been elusive. A cross-sectional study by Petek and colleagues, using high-throughput proteomics, shows that the levels of creatine kinase MM and MB isoforms, carbonic anhydrase III, and troponin I type two were elevated at least 1.5-fold in affected individuals and correlated with the severity and state of disease (Petek et al., 2016). Because of the variability of FSHD, genetic testing is still "the preferred diagnostic choice" (R. J. L. F. Lemmers et al., 2012).

Methylation of the D4Z4 regions also plays a role in disease expression and progression (Haynes et al., 2018; Mul et al., 2018; van der Maarel et al., 2011). A study by Mul and colleagues researched the clinical variability of FSHD1 patients for a possible linkage between the severity of disease, the repeat array size of D4Z4, and D4Z4 methylation. Unsurprisingly, unaffected gene carriers had both a higher number of array repeats and higher methylation levels. One interesting result is that the location of the affected body region did show a correlation between disease severity and DNA modification. "The D4Z4 repeat array size and D4Z4 methylation contribute to variability in disease severity and penetrance, but other disease modifying factors must be involved as well, such as polyadenylation of the *DUX4* transcript. Polyadenylated *DUX4* transcripts are not degraded and lead to the development of FSHD by a toxic gain of function mechanism (Darras, 2024c). The larger effect of the D4Z4 repeat array on facial muscle involvement suggests that these muscles are more sensitive to the influence of the FSHD1 locus itself, whereas leg muscle involvement seems highly dependent on modifying factors" (Mul et al., 2018).

Inappropriate expression of *DUX4* in somatic tissue is ultimately responsible for the pathogenesis of FSHD; hence, recent efforts to treat the disorder have targeted the expression of this gene and/or its protein product, as summarized in the table below (Hamel & Tawil, 2018).



Clinical Utility and Validity of FSHD Genetic Testing

The data on the clinical validity and utility of FSHD genetic testing is limited. The American Academy of Neurology and the American Association of Neuromuscular & Electrodiagnostic Medicine released joint guidelines stating the following: "our systematic review identified nine Class III studies from specialty clinics that, together, demonstrate that the finding of a D4Z4 contraction on chromosome 4q35 likely has a sensitivity of 93% and a specificity of 98% for diagnosis of clinically defined FSHD" (Tawil et al., 2015). A 2010 study of more than 800 individuals, however, question the criteria for the molecular diagnosis of FSHD. In this study, three percent of asymptomatic, healthy individuals reported a reduced number of D4Z4 repeats, varying four to eight units on chromosome four; further, almost one-half of probands had a normal copy number of D4Z4 repeats. These "results suggest that the genetic basis of FSHD, which is remarkably heterogeneous, should be revisited because this has important implications for genetic counseling and prenatal diagnosis of at-risk families" (Scionti et al., 2012).

The most common method of molecular diagnosis for FSHD is pulsed-field gel electrophoresis with Southern Blotting to discriminate between chromosome four and chromosome ten D4Z4 arrays. A study by Dai and colleagues describes a novel method to characterize the D4Z4 repeat numbers in FSHD by using a single molecule optical mapping platform that helps detect structural variants. With Bionano genome mapping, the number of D4Z4 repeats with 4qA allelic configuration and the levels of postzygotic mosaicism were determined. In the study, the primary cohort consisting of five patients had a confirmed positive diagnosis of FSHD based on Southern blot analysis. The second cohort consisted of eight patients that were suspected to have FSHD, but the patients did not have a prior molecular diagnosis. Optical mapping was performed, and Southern blot was used as a validation assay. In both cohorts, the results obtained from optical mapping have 100% concordance with the results from Southern blot analysis. Although optical mapping is a novel method that can improve accuracy and reliability of FSHD molecular testing, the authors also discuss the disadvantages of the optical mapping platform. Optical mapping is an expensive and time consuming tool compared to Southern blot, which works reliably for diagnosing patients with one to ten D4Z4 repeats in a cost-effective manner. Despite the disadvantages, the authors believe that costs for optical mapping will decrease and the tool will be incorporated into the clinical setting (Dai et al., 2020).

The genetic diagnosis of FSHD can be confounded by false positive or false negative results. A common test for FSHD uses a p13E-11 DNA probe to confirm a specific deletion that is indicative of the disorder. However, a false negative may occur if the patient has a deletion that affects the diagnostic probe's region of recognition (Lemmers et al., 2003). A "false" negative result may also occur with this test for patients with FSHD2, since they may not possess the characteristic deletion that is

present in typical FSHD1 cases. If clinical suspicion of FSHD exists in patients with a negative test result, then further genetic testing that includes the *SMCHD1*, *DNMT3B,* and/or *LRIF1* genes may be warranted to confirm FSHD2. Genetic testing should also include haplotype analysis, since a false positive result may occur "if the contracted D4Z4 array is located on the non-permissive 4qB haplotype" (Darras, 2024c).

Limb-Girdle Muscular Dystrophies (LGMDs)

Together, the group of disorders that constitute LGMD occur with an estimated minimum prevalence between ten and 23 per 100,000 (Wicklund, 2019).The LGMDs vary widely in their genetics and clinical features, ranging from mild forms allowing patients to maintain a fairly normal life to severe deterioration of proximal limb muscles with significant physical weakness and shortened life-span (Monies et al., 2016). A newer definition of LGMD has been proposed as "a genetically inherited condition that primarily affects skeletal muscle leading to progressive, predominantly proximal muscle weakness at presentation caused by a loss of muscle fibres. To be considered a form of limb girdle muscular dystrophy the condition must be described in at least two unrelated families with affected individuals achieving independent walking, must have an elevated serum creatine kinase activity, must demonstrate degenerative changes on muscle imaging over the course of the disease, and have dystrophic changes on muscle histology, ultimately leading to end-stage pathology for the most affected muscles" (Straub et al., 2018).

Approximately 30 LGMDs are recognized currently, most of which are inherited in an autosomal recessive pattern. The table below displays the recognized LGMDs according to the updated classification system (Wicklund, 2019):

Nomenclature, genes, and protein products of limb-girdle muscular dystrophies

New nomenclature	Old nomenclature	Gene	Protein product
Autosomal dominant			
LGMD D1	LGMD1D	*DNAJB6*	DnaJ heat shock protein family (Hsp40) member B6
LGMD D2	LGMD1F	*TNPO3*	Transportin 3
LGMD D3	LGMD1G	*HNRNPDL*	Heterogeneous nuclear ribonucleoprotein D like protein
LGMD D4	LGMD1I	*CAPN3*	Calpain 3
LGMD D5		*COL6A1*	Collagen type VI alpha 1 chain
Autosomal recessive			

New nomenclature	Old nomenclature	Gene	Protein product
LGMD R1	LGMD2A	*CAPN3*	Calpain 3
LGMD R2	LGMD2B	*DYSF*	Dysferlin
LGMD R3	LGMD2D	*SGCA*	Sarcoglycan alpha
LGMD R4	LGMD2E	*SGCB*	Sarcoglycan beta
LGMD R5	LGMD2C	*SGCG*	Sarcoglycan gamma
LGMD R6	LGMD2F	*SGCD*	Sarcoglycan delta
LGMD R7	LGMD2G	*TCAP*	Telethonin
LGMD R8	LGMD2H	*TRIM32*	Tripartite motif-containing 32
LGMD R9	LGMD2I	*FKRP*	Fukutin-related protein
LGMD	LGMD2J	*TTN*	Titin
LGMD	LGMD2K	*POMT1*	Protein O-
LGMD	LGMD2L	*ANO5*	Anoctamin 5
LGMD	LGMD2M	*FCMD*	Fukutin
LGMD	LGMD2N	*POMT2*	Protein O-
LGMD R15	LGMD2O	*POMGNT1*	Protein O-linked mannose *N*-acetylglucosaminyltransferase 1 (beta 1,2–)
LGMD	LGMD2P	*DAG1*	Dystroglycan 1
LGMD	LGMD2Q	*PLEC1*	Plectin
LGMD	LGMD2S	*TRAPPC11*	Trafficking protein particle
LGMD	LGMD2T	*GMPPB*	GDP-mannose
LGMD	LGMD2U	*CRPPA*	CDP-L-ribitol
LGMD	LGMD2Z	*POGLUT1*	Protein O-glucosyltransferase
LGMD		*COL6A1/2/3*	Collagen type VI alpha 1, 2, or
LGMD		*LAMA2*	Laminin subunit alpha 2
LGMD R24		*POMGNT2*	Protein O-linked mannose *N*-acetylglucosaminyltransferase 2 (beta 1,4–)

The autosomal recessive LGMD R1 is considered the most common form of LGMD worldwide, making up an estimated 15 to 40 percent of all cases of LGMD (Nallamilli et al., 2018; Wicklund & Kissel, 2014).

Dilated cardiomyopathy (DCM) is the most common cardiac phenotype associated with LGMD, and its prevalence varies in the different subgroups of patients. The risk of developing DCM is particularly high in the LGMD1B patient subgroup, such that clinicians might consider preventive interventions due to the high risk of sudden death (Arbustini et al., 2018).

Clinical Utility and Validity of LGMD Genetic Testing

Based on published literature, the clinical validity of genetic testing for LGMD is difficult to ascertain; however, broad genetic testing is becoming the standard for LGMD diagnosis for patients suspected of having LGMD (Wicklund, 2019). Testing should be performed by way of an LGMD or neuromuscular gene panel that looks for alterations in genes known to be involved in LGMD or other dystrophies and myopathies. If such a panel is inconclusive, broader testing by way of whole-exome sequencing or whole genome sequence with NGS may be used (Ghaoui et al., 2015; Ozyilmaz et al., 2019). Unless clinical findings suggest a particular subtype of LGMD, whole-exome or whole genome testing may increase the likelihood of an accurate diagnosis as compared to single gene sequencing (Narayanaswami et al., 2014).

The yield of genetic testing in patients with signs and symptoms of LGMD varies depending on the mutation and population characteristics. Some studies conclude that the clinical validity is reasonably high (Fanin et al., 2009; Norwood et al., 2007). According to Norwood et al. (2007), "DNA analysis directed to provide confirmation of mutation in the affected gene(s) is the gold standard of diagnosis, and necessary to be able to offer carrier or presymptomatic testing to other family members." Other researchers have attempted to utilize whole-exome sequencing to diagnose LGMD in 100 individuals with familial LGMD; however, a diagnostic success rate of only 45% was achieved (Ghaoui et al., 2015).

Monies et al. (2016) screened 50 random genetically unstudied families with LGMD via a gene panel incorporating 759 OMIM (Online Mendelian Inheritance in Man) genes associated with neurological disorders. OMIM is a catalogue of human genes and genetic disorders that provides comprehensive information based on the periodical biomedical literature (Amberger et al., 2014). The panel "identified the mutation in 76% of families (38/50; 11 novel). A total of 34 families had mutations in LGMD-related genes with four others having variants not typically associated with LGMD. The majority of cases had recessive inheritance with homoallelic pathogenic variants (97.4%, 37/38), as expected considering the high rate of consanguinity in the study population." The authors concluded that the "neurological panel achieved a high clinical sensitivity (76%) and is an effective first-line laboratory test in patients with LGMD and other myopathies. This sensitive, cost-effective, and rapid assay significantly assists clinical practice especially in these phenotypically and genetically heterogeneous disorders. Moreover, the application of the American College of Medical Genetics (ACMG) and Association for Molecular Pathology (AMP) guidelines applied in the classification of variant pathogenicity provides a clear interpretation for physicians on the relevance of such findings" (Monies et al., 2016).

Harris et al. (2017) performed whole-exome sequencing (WES) on 104 patients with LGMD in which standard gene testing had not yet yielded a diagnosis, and 91 patients using sequential gene by gene testing. They found that "patients selected for WES had undergone more extensive prior testing than those undergoing standard genetic testing, and on average had had eight genes screened already. In this extensively investigated cohort WES identified the genetic diagnosis in 28 families (28/75, 37%), including the identification of the novel gene ZAK and two unpublished genes. WES of a single affected individual with sporadic disease yielded a diagnosis in 13/38 (34%) of cases. In comparison, conventional gene by gene testing provided a genetic diagnosis in 28/84 (33%) families." The authors concluded that "WES was able to overcome many limitations of standard testing and achieved a higher rate of diagnosis than standard testing even in this cohort of extensively investigated patients. Earlier application of WES is therefore likely to yield an even higher diagnostic rate. We obtained a high diagnosis rate in simplex cases and therefore such individuals should be included in exome or genome sequencing projects. Disease due to somatic mosaicism may be increasingly recognized due to the increased sensitivity of next generation sequencing techniques to detect low level mosaicism" (Harris et al., 2017). A similar study by Reddy and colleagues reported 40% of the LGMD families tested "had novel and previously reported pathogenic mutations, primarily in LGMD genes, and also in genes for Duchenne muscular dystrophy, facioscapulohumeral muscular dystrophy, congenital myopathy, myofibrillar myopathy, inclusion body myopathy and Pompe disease" (Reddy et al., 2017).

Congenital Muscular Dystrophy (CMD)

Congenital Muscular Dystrophy is a group of genetically diverse disorders that typically manifest at birth or within the first two years of life, marked by muscle weakness, hypotonia, and delayed motor milestones. Initially, CMD was recognized in infants who presented with generalized weakness and abnormal muscle biopsies, revealing fibrosis, fatty tissue infiltration and degeneration of muscle fibers. Arthrogryposis, or joint contractures present at birth, is also common in newborns with CMD (Darras, 2024e).

CMD encompasses both "classic" forms, like merosin-deficient CMD, and more complex "syndromic" forms such as Walker-Warburg syndrome, Fukuyama muscular dystrophy, and muscle-eye-brain disease. Syndromic CMDs often involve structural abnormalities in the brain detected by neuroimaging; these can present with cognitive impairments, developmental delays, and cardiac involvement. Genes associated with CMD in targeted gene panels encompass genes such as *LAMA2, COL6A1-3, FKTN*, and *POMT1-2*, among others. Dysfunction can occur across multiple organ symptoms in merosin-deficient CMD (associated with mutations in the *LAMA2* gene) (Darras, 2024e).

Clinical Utility and Validity of CMD Genetic Testing

O'Grady et al. (2016) compared targeted next-generation (NGS) panels with traditional candidate gene sequencing in diagnosing CMD. The study included 123 participants with evidence of muscle weakness and hypotonia in the first two years of life. These participants were identified over a 35-year period. Genetic testing targeted key genes, including *LAMA2, FKRP, COL6A1-3*, and *SEPN1*. A genetic diagnosis was confirmed for 39 participants (32%), with two additional probable cases. Further NGS using a 45-gene panel, a 336-gene panel, or WES identified causative variants in 11 more individuals, bringing the total diagnostic yield to 59 out of 123 (48%). The study concluded that NGS should serve as the first-line diagnostic tool for CMD, with muscle biopsy reserved for secondary evaluation (O'Grady et al., 2016).

Clinical Utility and Validity of Multi-gene Panel Testing for NMDs

Ebert et al. (2024) drove a retrospective review to analyze the clinical utility and detection rate of genetic testing for individuals with suspected neuromuscular disease. Genetic testing results were gathered for individuals who had received multi-gene panel testing for neuromuscular disorders; the authors analyzed individual records to determine whether the results confirmed or denied a neuromuscular disease diagnosis. A pathogenic mutation or a variant of unknown significance (or both) was found in 77.1% of the study individuals, allowing the confirmation of diagnosis in 35.9%. The most common presenting symptom and indication for testing was suspected neuropathy (53.3%). Myopathy was found in 48.7% of cases. Overall, the authors concluded that the existing evidence constitutes additional confirmatory evidence towards the use of multi-gene panel genetic testing to help the diagnosis and management of neuromuscular disorders. The genetic panel testing was done through Invitae (Ebert et al., 2024).

Rosenberg et al. (2023) investigated the use of multi-gene panel genetic testing to confirm a diagnosis of neuromuscular diseases among 155 patients. They note that clinical evaluation for NMDs has increasingly incorporated molecular genetics as a first or second tier diagnosis practice, especially over the past ten years and that “curated multigene panels of several hundreds of genes remain the most used methodology for the diagnosis of many NMDs. WES is an increasingly popular testing choice when disease presentation is complex and nonclassical.” The authors gathered patient data between 2018 and 2020 using 26 separate genetic tests for a total of 262 individual tests (average of 1.7 tests per patient). Of the patients who had tests performed, over half had a comprehensive NMD panel ordered with between 110 and 211 genes known to be associated with NMDs, inherited myopathies and congenital myasthenic syndrome. The comprehensive NMD panel that was ordered for over half of patients

had a diagnostic yield of 14% with 21% of all patients receiving a confirmative diagnosis, which the authors note was low due to the heterogeneity of NMDs and the specific challenge of conditions such as myotonic dystrophy type 1 and facioscapulohumeral muscular dystrophy, which require specialized molecular testing (Rosenberg et al., 2023).

VI. Guidelines and Recommendations

Best Practice Guidelines on Molecular Diagnostics in Duchenne/Becker Muscular Dystrophies Workshop Report

The international workshop comprised of scientists from Europe, the US, India, and Australia was organized and sponsored by the European Neuro-Muscular Centre, the European Molecular Genetics Quality Network, TREAT-NMD, and Euro-Gentest. The flow chart for the recommended diagnostic work-up of a dystrophinopathy is shown below in Figure 1, located below (Abbs et al., 2010).

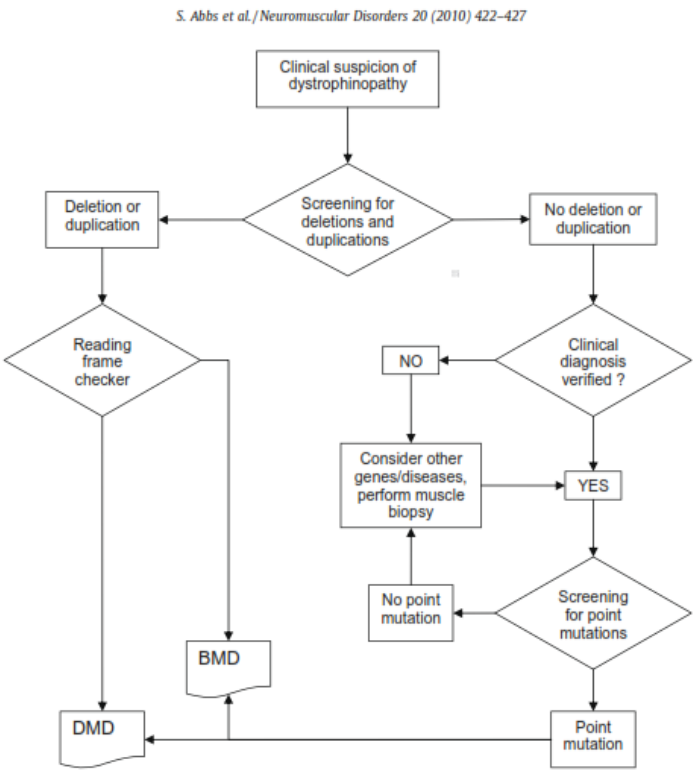


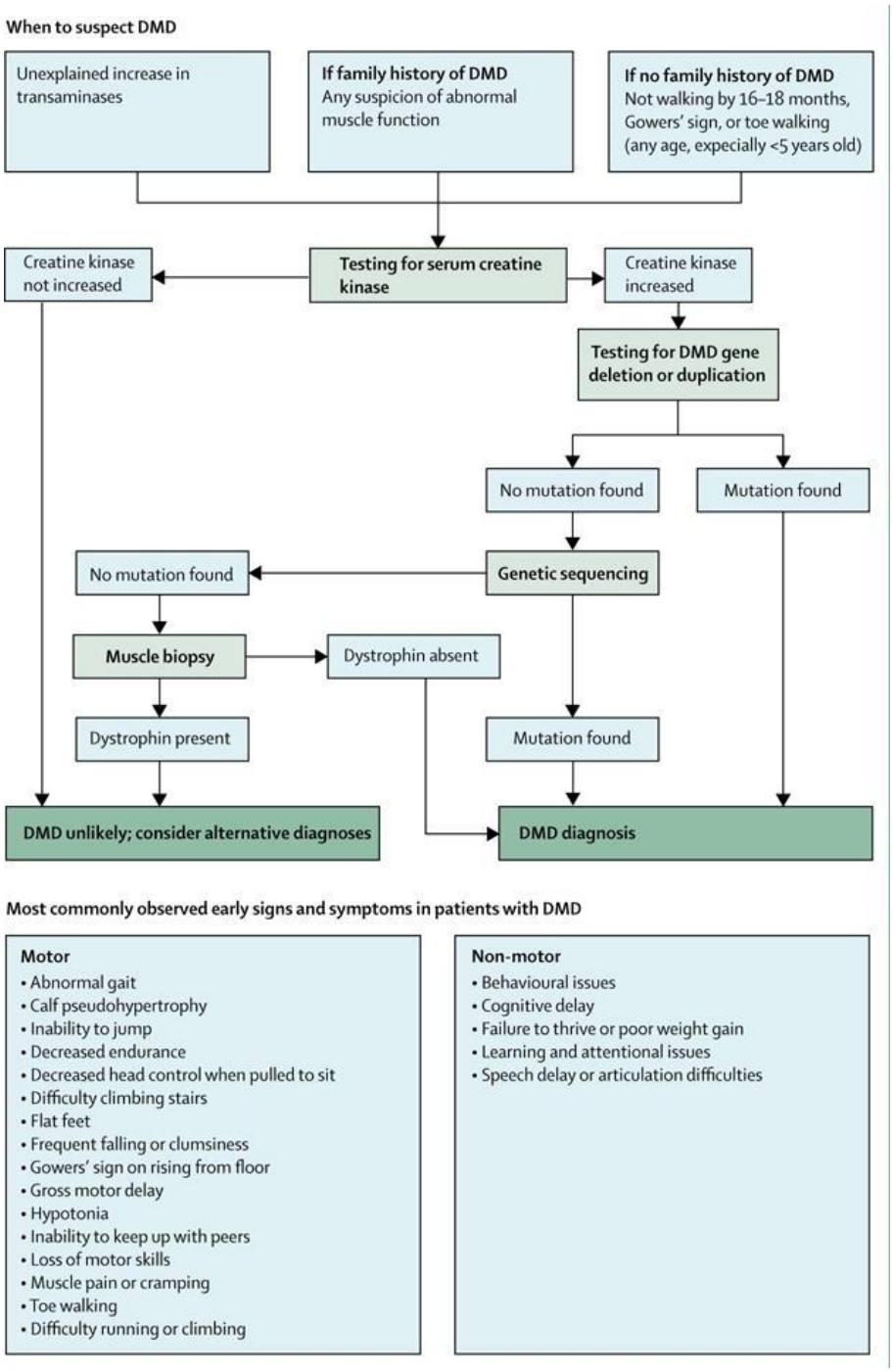
Fig. 1. Flow chart for the diagnostic work-up of a dystrophinopathy.

Recommendations for testing are as follows: if there is a clinical suspicion of a dystrophinopathy, first screen for deletions and duplications. Then, if no deletion or duplication is detected, but the clinical diagnosis is verified, screening for point mutations should be performed (Abbs et al., 2010).

The DMD Care Considerations Working Group

The CDC selected 84 clinicians to comprise the DMD Care Considerations Working Group to develop recommendations regarding all aspects of DMD care, including the diagnosis and genetic testing of muscular dystrophy. They state the following: "Testing for a DMD mutation in a blood sample is always necessary even if DMD is first confirmed by the absence of dystrophin protein expression on muscle biopsy. The results of genetic testing provide the clinical information required for genetic counselling, prenatal diagnosis, and consideration for future mutation-specific therapies... If analysis by one or more of these techniques leads to the identification and full characterisation of a dystrophin mutation, then no further testing is required. If deletion/ duplication testing is negative, then dystrophin gene sequencing should be done to look for point mutations or small deletions/insertions. Full characterisation of the mutation (deletion endpoints or exact position of any point mutation) is required to allow correlation of the predicted effect of the mutation on the reading frame of the gene, which is the major determinant of the phenotypic variability seen in dystrophinopathy, as well as to determine eligibility for the mutation-specific treatments currently in trials" (Bushby et al., 2010).

An update of the 2010 DMD Care considerations was published to further improve patient care. A diagnostic flow chart for DMD was provided and can be found below (Birnkrant et al., 2018).



The DMD Care Considerations Working Group has stated that "Because approximately 70% of individuals with DMD have a single-exon or multi-exon deletion or duplication in the dystrophin gene, dystrophin gene deletion and duplication testing is usually the first confirmatory test. Testing is best done by multiplex ligation-dependent probe amplification (MLPA) or comparative genomic hybridisation array, since use of multiplex PCR can only identify deletions…If deletion or duplication testing is negative, genetic sequencing should be done to screen for the remaining types of mutations

that are attributed to DMD (approximately 25–30%)…Finally, if genetic testing does not confirm a clinical diagnosis of DMD, then a muscle biopsy sample should be tested for the presence of dystrophin protein by immunohistochemistry of tissue cryosections or by western blot of a muscle protein extract" (Birnkrant et al., 2018). Also stated is that "Family members of an individual with DMD should receive genetic counselling to establish who is at risk of being a carrier. Carrier testing is recommended for female relatives of a boy or man who has been genetically confirmed to have DMD" (Birnkrant et al., 2018).

American Academy of Neurology (AAN) and the American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM)

The Guideline Development Subcommittee of the AAN and the Practice Issues Review Panel of the AANEM published recommendations that "Targeted genetic testing often identifies causative mutations in the classic CMD subtypes... Genetic diagnoses are beneficial to the patient, as they often enable physicians to provide more accurate prognoses and facilitate genetic counseling and family planning discussions, and may enable patients to become more aware of future clinical trials for which they may be eligible… when available and feasible, physicians might order targeted genetic testing for specific CMD subtypes that have well-characterized molecular causes" and "In individuals with CMD who either do not have a mutation identified in one of the commonly associated genes or have a phenotype whose genetic origins have not been well characterized, physicians might order whole-exome or whole-genome sequencing when those technologies become more accessible and affordable for routine clinical use" (Kang et al., 2015).

In 2014, the AAN and the Practices Issues Review Panel of the AANEM issued evidenced-based guidelines for the diagnosis and treatment of limb-girdle and distal dystrophies, who made the following recommendations (Narayanaswami et al., 2014) that were reaffirmed in 2022:

For the diagnosis of LGMD:

- For patients with suspected muscular dystrophy, clinicians should use a clinical approach to guide genetic diagnosis based on the clinical phenotype, including the pattern of muscle involvement, inheritance pattern, age at onset and associated manifestations (e.g., early contractures, cardiac or respiratory involvement) (Level B recommendation).
- In patients with suspected muscular dystrophy in whom initial clinically directed genetic testing does not provide a diagnosis, clinicians may obtain genetic consultation or perform parallel sequencing of targeted exomes, whole-exome

sequencing, whole genome screening, or next-generation sequencing to identify the genetic abnormality (Level C recommendation).

The AAN Guidelines state: "Diagnosis assists in defining the long-term prognosis, since some dystrophies are more rapidly progressive, involve the cardiorespiratory systems more frequently, or are associated with other disorders. The identification of these dystrophies through genetic testing will not only inform long-term prognosis but will also assist in directing care more efficiently (e.g., more frequent cardiorespiratory monitoring and prophylactic treatments such as pacer/defibrillator placement for those disorders known to be associated with cardiac involvement). Precise identification of the disorder also eliminates the need for repeated testing for an acquired, treatable disorder such as an inflammatory myopathy, because some dystrophies have inflammation on muscle biopsy, making diagnosis difficult on the basis of routine biopsy findings. In addition, the temptation to try immunosuppressive agents repeatedly, looking for a therapeutic response, is not unusual when there is no diagnosis and the patient is worsening. This exposes patients to potentially serious side effects of immunosuppressive medications. Patients on immunosuppressants need regular monitoring, adding logistical difficulties to a population that may have significantly impaired mobility. Health care costs are increased by repeated investigations, immunosuppressive treatments, and laboratory monitoring. Although establishing a genetic diagnosis is expensive on the front end, the costs of continued investigation for other causes and the risks and expenses associated with empiric trials of immunosuppressants make a strong case for establishing a genetic diagnosis, which often provides patients a sense of closure. Establishing a genetic diagnosis is crucial for genetic counseling to inform decision-making about having children and for screening of offspring. Treatment of cardiomyopathy, arrhythmias, and ventilatory failure prolongs life and improves quality of life in patients with other neuromuscular diseases" (Narayanaswami et al., 2014). These guidelines were reaffirmed in 2022.

For the diagnosis of CMD:

"CMDs are often autosomal recessive, but some cases have been found to follow autosomal dominant patterns, by direct inheritance, spontaneous mutations, or mosaicism. The genetic origins of many cases of CMD have been discovered. However, many affected individuals remain without a genetic diagnosis, an indicator that novel disease genes have yet to be identified. Clinical genetic testing is available for virtually all genes known to be associated with CMD."

Two recommendations regarding CMD diagnosis via genetic testing (reaffirmed 2021):

"1. When available and feasible, physicians might order targeted genetic testing for specific CMD subtypes that have well-characterized molecular causes (Level C). Our

systematic review indicates that many patients with CMD do not have mutations in one of the currently known genes. The cost of next-generation sequencing (whole-exome and whole-genome sequencing) is dropping rapidly, to the point where these technologies are now readily available to many researchers who seek novel causative disease genes.

2. In individuals with CMD who either do not have a mutation identified in one of the commonly associated genes or have a phenotype whose genetic origins have not been well characterized, physicians might order whole-exome or whole genome sequencing when those technologies become more accessible and affordable for routine clinical use (Level C)" (Kang et al., 2015).

American Academy of Neurology (AAN)

The American Academy of Neurology published evidence-based guidelines which found that "the finding of a D4Z4 contraction on chromosome 4q35 likely has a sensitivity of 93% and a specificity of 98% for diagnosis of clinically defined FSHD." They recommend that "Clinicians should obtain genetic confirmation of FSHD1 in patients with atypical presentations and no first-degree relatives with genetic confirmation of the disease [Level B]." Concerning the use of genetics as a predictor of severity in FSHD, they recommend, "Large D4Z4 deletion sizes (contracted D4Z4 allele of 10–20 kb) should alert the clinician that the patient is more likely to develop more significant disability and at an earlier age. Patients with large deletions are also more likely to develop symptomatic extramuscular manifestations [Level B]" (AAN, 2018; Tawil et al., 2015). These guidelines were reaffirmed in 2021.

International Standard of Care Committee for Congenital Muscular Dystrophy

As a part of the guidelines concerning newly diagnosed patients, the International Standard of Care Committee for Congenital Muscular Dystrophy recommends that "if a genetic diagnosis is known, the recurrence risk and impact on future family planning should be discussed. Even if the exact genetic defect is not known, recurrence risk can sometimes be discussed using a common genetic model that is often associated with the diagnosis" (Wang et al., 2010).

171st European Neuromuscular Centre International Workshop on Standards of Care and Management of Facioscapulohumeral Muscular Dystrophy (FSHD)

In a report from the 171st European Neuromuscular Centre International Workshop Standards of Care and Management of FSHD held in January 2010, it is stated that "when a physician concludes facioscapulohumeral syndrome based on clinical findings,

the odds are in favor of FSHD, and genetic testing is the preferred diagnostic choice" (R. J. L. F. Lemmers et al., 2012).

Evidence-Based Consensus and Systematic Review on Reducing the Time to Diagnosis of Duchenne Muscular Dystrophy Joint Report of the EPNS, MDA, PPMD, TREAT-NMD, and DPPI

According to this evidence-based report endorsed by the European Paediatric Neurology Society (EPNS), the Muscular Dystrophy Association (MDA), Duchenne Parent Project Italy (DPPI), Parent Project Muscular Dystrophy (PPMD), and TREAT-NMD, "Genetic testing is crucial for obtaining a complete diagnosis of DMD, and should be considered the gold standard" (Aartsma-Rus et al., 2019); accepted DMD symptoms are listed and include "calf hypertrophy (pseudohypertrophy); delayed walking; difficulty climbing/descending stairs; difficulty rising from the floor; difficulty running/walking; elevated serum CK levels (including elevated ALT and AST); a family history of DMD; frequent falls; Gowers' sign; male sex; and muscle weakness;" additional recommendations include that "patients with signs and symptoms of DMD and elevated serum CK levels should be referred for genetic testing to either a clinical geneticist or a neuromuscular specialist" (Aartsma-Rus et al., 2019). Regarding testing of other family members, Aartsma-Rus et al. (2019) recommends that "After a patient receives a complete genetic diagnosis of DMD, it is mandatory that carrier testing of the mother and other at-risk female family members be offered with appropriate pre- and postgenetic counseling."

U.S. Preventive Services Task Force (USPSTF)

No U.S. Preventive Services Task Force recommendations for genetic testing for muscular dystrophy have been identified. A search for "muscular dystrophy" on the USPSTF website turned up zero relevant results in October of 2024.

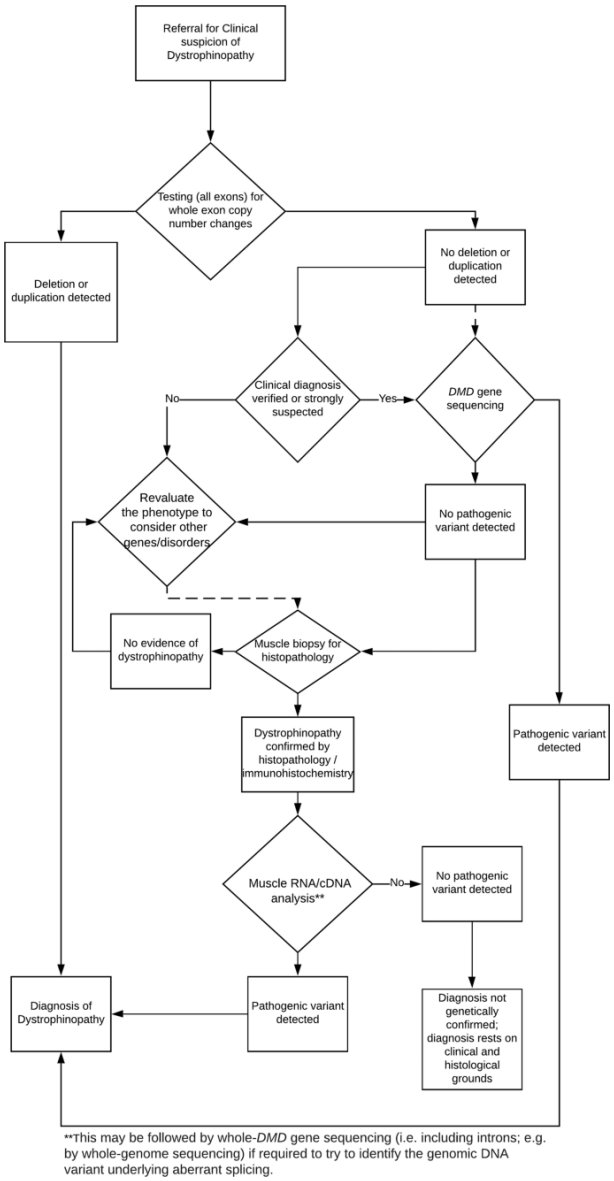
European Molecular Genetics Quality Network (EMQN) Best Practice Guidelines on molecular diagnostics in the Duchenne/Becker muscular dystrophies

The EMQN summarizes current diagnostic methods to analyze the *DMD* gene and categorizes these methods according to specificity of the tests into Level one (copy number variations detection), Level two (small variants detection), and Level three (RNA Analysis). Seventy-eight percent of the pathogenic *DMD* variants is due to whole-exon deletions and duplications; therefore, detection of the relative copy number of all exons within the *DMD* gene (copy number variations) is the first level of diagnostic tests offered. Several quantitative tests can be used to detect these copy number variations (CNVs). The most reliable method is MLPA, which detects the number of deletions or duplications to the exon level. MLPA is more reliable when there are

multiple exons involved, but less if there is a single-exon deletion. Therefore, real-time PCR, multiplex PCR, Sanger sequencing, or microsatellite marker analysis must follow to confirm single-exon deletions. Comparative genomic hybridization (CGH) is another method which provides a slightly higher detection rate than MLPA by use of oligonucleotide probes to interrogate copy number across the entire 2.2 Mb genomic region of the *DMD* gene. The last method is NGS, which can detect single nucleotide variants (SNVs) and CNVs; however, NGS is not routinely used to detect CNVs because it is not sensitive enough to pick up on all types of CNVs. Level two testing detects missense, nonsense, small insertions and deletions, and indel and splicing variants through either Sanger sequencing or NGS. NGS is less time consuming as it allows for many targets to be sequenced with deep sequence coverage in multiple patients at a time and enhances detection of low level somatic mosaicism in patients' or probands' mothers; however, Sanger sequencing is still the standard method used for known familial variant testing. Level three testing, such as muscle cDNA analysis, is used when a patient presents with clinical symptoms of a dystrophinopathy, but no CNVs or small variants were detected from Level one and Level two diagnostic techniques.

A flowchart for the recommended molecular diagnostic algorithm for dystrophinopathy is shown in the figure above (Fratter et al., 2020). When dystrophinopathy is suspected due to clinical symptoms, high serum creatine kinase levels, and a possible family history, molecular testing to detect CNVs is recommended. If a pathogenic deletion and duplication is not detected, the next step is to sequence the coding region of the entire *DMD* gene. If a pathogenic variant is not detected from these two tests, a muscle biopsy, dystrophin gene analysis via immunohistochemistry, or muscle cDNA sequencing may be performed. If the patient presents with a positive muscle biopsy test and positive symptoms of a dystrophinopathy, but no pathogenic variant is detected through genetic testing, the patient should still be diagnosed with a dystrophinopathy (Fratter et al., 2020).

The EMQN also provides guidelines on genetic testing for females affected by dystrophinopathies. Although dystrophinopathies predominantly affect males, females



could have a pathogenic *DMD* variant and present with a milder phenotype most likely due to a skewed X-inactivation. In this case, the diagnostic method would be identical to the one presented in the flowchart: evaluation of clinical symptoms, CNV analysis and *DMD* gene sequencing followed by muscle biopsy, dystrophin gene analysis, and muscle cDNA sequencing as needed. If a female presents with the complete DMD phenotype, karyotyping is recommended due to possible chromosomal aberrations or autosomal translocations that could cause 100% skewed X-inactivation.

Females who are carriers of dystrophinopathies are usually clinically asymptomatic but may develop some symptoms during their lifetime or pass it down to their children.

Therefore, carrier testing is important for family planning. If the familial pathogenic variant is known, then two complementary tests should be performed (MLPA and Sanger Sequencing or MLPA and microsatellite analysis). High density array CGH can also be used, which is a single test that interrogates multiple loci within the deletion or duplication. If the familial pathogenic variant is not known and an affected male in the family is not available, then female carriers who are at risk of being carriers should be offered CNV analysis and *DMD* gene sequencing followed by muscle biopsy, dystrophin gene analysis, and muscle cDNA sequencing as needed. Measuring serum creatine kinase levels may also be helpful (Fratter et al., 2020).

The EMQN also comments on prenatal diagnosis of dystrophinopathies. Since it is not possible to predict whether a female will present symptoms of a dystrophinopathy, prenatal diagnosis should only be performed on pregnancies with a male fetus unless there is documented familial reoccurrence of complete skewed X-inactivation. Prenatal testing is performed on placental biopsy through chorionic villus sampling (CVS) within 11-12 weeks of gestation or amniocentesis within 15-17 weeks of gestation. CVS is the preferred method as it provides higher DNA quality and greater safety for the pregnancy. The obtained DNA from placental biopsy can be analyzed through NGS or CNV detection. For non-invasive prenatal diagnostic options, relative haplotype dosage analysis (RHDO) may be performed. "RHDO analysis examines SNPs in the cell-free DNA from a maternal blood sample and shows whether the male foetus has inherited the high risk or low risk haplotype across the *DMD* gene" (Fratter et al., 2020).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81161	DMD (dystrophin) (eg, duchenne/becker muscular dystrophy) deletion analysis, and duplication analysis, if performed
81187	CNBP (CCHC-type zinc finger nucleic acid binding protein) (eg, myotonic dystrophy type 2) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81234	DMPK (DM1 protein kinase) (eg, myotonic dystrophy type 1) gene analysis; evaluation to detect abnormal (expanded) alleles
81239	DMPK (DM1 protein kinase) (eg, myotonic dystrophy type 1) gene analysis; characterization of alleles (eg, expanded size)
81312	PABPN1 (poly[A] binding protein nuclear 1) (eg, oculopharyngeal muscular dystrophy) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81400	Molecular pathology procedure, Level 1 (eg, identification of single germline variant [eg, SNP] by techniques such as restriction enzyme digestion or melt curve analysis)
81404	Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons)
81408	Molecular pathology procedure, Level 9 (eg, analysis of >50 exons in a single gene by DNA sequence analysis)
81479	Unlisted molecular pathology procedure
0218U	Neurology (muscular dystrophy), DMD gene sequence analysis, including small sequence changes, deletions, duplications, and variants in non-uniquely mappable regions, blood or saliva, identification and characterization of genetic variants Proprietary test: Genomic Unity® DMD Analysis Lab/Manufacturer: Variantyx Inc
S3853	Genetic testing for myotonic muscular dystrophy

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

AAN. (2018). Evidence-based guideline summary: evaluation, diagnosis, and management of facioscapulohumeral muscular dystrophy., *2018*. https://doi.org/10.1212/WNL.0000000000001783

Aartsma-Rus, A., Hegde, M., Ben-Omran, T., Buccella, F., Ferlini, A., Gallano, P., Howell, R. R., Leturcq, F., Martin, A. S., Potulska-Chromik, A., Saute, J. A., Schmidt, W. M., Sejersen, T., Tuffery-Giraud, S., Uyguner, Z. O., Witcomb, L. A., Yau, S., & Nelson, S. F. (2019). Evidence-Based Consensus and Systematic Review on Reducing the Time to Diagnosis of Duchenne Muscular Dystrophy. *J Pediatr*, *204*, 305-313.e314. https://doi.org/10.1016/j.jpeds.2018.10.043

Abbs, S., Tuffery-Giraud, S., Bakker, E., Ferlini, A., Sejersen, T., & Mueller, C. R. (2010). Best practice guidelines on molecular diagnostics in Duchenne/Becker muscular dystrophies. *Neuromuscul Disord*, *20*(6), 422-427. https://doi.org/10.1016/j.nmd.2010.04.005

Amberger, J. S., Bocchini, C. A., Schiettecatte, F., Scott, A. F., & Hamosh, A. (2014). OMIM.org: Online Mendelian Inheritance in Man (OMIM®), an online catalog of human genes and genetic disorders. *Nucleic Acids Research*, *43*(D1), D789-D798. https://doi.org/10.1093/nar/gku1205

Arbustini, E., Di Toro, A., Giuliani, L., Favalli, V., Narula, N., & Grasso, M. (2018). Cardiac Phenotypes in Hereditary Muscle Disorders: JACC State-of-the-Art Review. *J Am Coll Cardiol*, *72*(20), 2485-2506. https://doi.org/10.1016/j.jacc.2018.08.2182

Birnkrant, D. J., Bushby, K., Bann, C. M., Apkon, S. D., Blackwell, A., Brumbaugh, D., Case, L. E., Clemens, P. R., Hadjiyannakis, S., Pandya, S., Street, N., Tomezsko, J., Wagner, K. R., Ward, L. M., & Weber, D. R. (2018). Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. *Lancet Neurol*, *17*(3), 251-267. https://doi.org/10.1016/s1474-4422(18)30024-3

Brandsema, J. F., & Darras, B. T. (2015). Dystrophinopathies. *Semin Neurol*, *35*(04), 369-384. https://doi.org/10.1055/s-0035-1558982

Bushby, K., Finkel, R., Birnkrant, D. J., Case, L. E., Clemens, P. R., Cripe, L., Kaul, A., Kinnett, K., McDonald, C., Pandya, S., Poysky, J., Shapiro, F., Tomezsko, J., & Constantin, C. (2010). Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and pharmacological and psychosocial management. *Lancet Neurol*, *9*(1), 77-93. https://doi.org/10.1016/s1474-4422(09)70271-6

Chamberlain, J. S., Chamberlain, J. R., Fenwick, R. G., Ward, P. A., Caskey, C. T., Dimnik, L. S., Bech-Hansen, N. T., Hoar, D. I., Richards, S., Covone, A. E., Govanni, R., Abbs, S., Bentley, D. R., Bobrow, M., Rysiecki, G., Ray, P. N., Boileau, C., Junien, C., Boehm, C., . .

. Rysiecki, G. (1992). Diagnosis of Duchenne and Becker muscular dystrophies by polymerase chain reaction. A multicenter study. *JAMA*, *267*(19), 2609-2615. https://doi.org/10.1001/jama.1992.03480190051030

Dai, Y., Li, P., Wang, Z., Liang, F., Yang, F., Fang, L., Huang, Y., Huang, S., Zhou, J., Wang, D., Cui, L., & Wang, K. (2020). Single-molecule optical mapping enables quantitative measurement of D4Z4 repeats in facioscapulohumeral muscular dystrophy (FSHD). *Journal of Medical Genetics*, *57*(2), 109. https://doi.org/10.1136/jmedgenet-2019-106078

Darras, B. T. (2024a, 09/30/2024). *Duchenne and Becker muscular dystrophy: Clinical features and diagnosis*. https://www.uptodate.com/contents/duchenne-and-becker-muscular-dystrophy-clinical-features-and-diagnosis

Darras, B. T. (2024b, March 20, 2024). *Emery-Dreifuss muscular dystrophy*. https://www.uptodate.com/contents/emery-dreifuss-muscular-dystrophy

Darras, B. T. (2024c, 01/19/2024). *Facioscapulohumeral muscular dystrophy*. https://www.uptodate.com/contents/facioscapulohumeral-muscular-dystrophy

Darras, B. T. (2024d, 02/01/2024). *Limb-girdle muscular dystrophy*. https://www.uptodate.com/contents/limb-girdle-muscular-dystrophy

Darras, B. T. (2024e, July 12, 2024). *Oculopharyngeal, distal and congenital muscular dystrophies*. https://www.uptodate.com/contents/oculopharyngeal-distal-and-congenital-muscular-dystrophies

Darras, B. T., Urion, D. K., & Ghosh, P. S. (2000, 01/20/2022). *Dystrophinopathies*. University of Washington, Seattle. https://www.ncbi.nlm.nih.gov/books/NBK1119/

de Greef, J. C., Lemmers, R. J., Camano, P., Day, J. W., Sacconi, S., Dunand, M., van Engelen, B. G., Kiuru-Enari, S., Padberg, G. W., Rosa, A. L., Desnuelle, C., Spuler, S., Tarnopolsky, M., Venance, S. L., Frants, R. R., van der Maarel, S. M., & Tawil, R. (2010). Clinical features of facioscapulohumeral muscular dystrophy 2. *Neurology*, *75*(17), 1548-1554. https://doi.org/10.1212/WNL.0b013e3181f96175

Ebert, S. E., Meiling, J. B., Caress, J. B., Gandhi Mehta, R. K., Baute Penry, V., Puwanant, A., & Cartwright, M. S. (2024). Clinical Utility and Diagnostic Yield of Genetic Testing for Inherited Neuromuscular Disorders in a Single, Large Neuromuscular Center. *Neurology Clinical Practice*, *14*(2), e200268. https://doi.org/doi:10.1212/CPJ.0000000000200268

Fanin, M., Nascimbeni, A. C., Aurino, S., Tasca, E., Pegoraro, E., Nigro, V., & Angelini, C. (2009). Frequency of LGMD gene mutations in Italian patients with distinct clinical phenotypes. *Neurology*, *72*(16), 1432-1435. https://doi.org/10.1212/WNL.0b013e3181a1885e

Fratter, C., Dalgleish, R., Allen, S. K., Santos, R., Abbs, S., Tuffery-Giraud, S., & Ferlini, A. (2020). EMQN best practice guidelines for genetic testing in dystrophinopathies. *European Journal of Human Genetics*, *28*(9), 1141-1159. https://doi.org/10.1038/s41431-020-0643-7

Ghaoui, R., Cooper, S. T., Lek, M., Jones, K., Corbett, A., Reddel, S. W., Needham, M., Liang, C., Waddell, L. B., Nicholson, G., O'Grady, G., Kaur, S., Ong, R., Davis, M., Sue, C. M., Laing, N. G., North, K. N., MacArthur, D. G., & Clarke, N. F. (2015). Use of Whole-Exome Sequencing for Diagnosis of Limb-Girdle Muscular Dystrophy: Outcomes and Lessons Learned. *JAMA Neurol*, *72*(12), 1424-1432. https://doi.org/10.1001/jamaneurol.2015.2274

Hamanaka, K., Sikrova, D., Mitsuhashi, S., Masuda, H., Sekiguchi, Y., Sugiyama, A., Shibuya, K., Lemmers, R., Goossens, R., Ogawa, M., Nagao, K., Obuse, C., Noguchi, S., Hayashi, Y. K., Kuwabara, S., Balog, J., Nishino, I., & van der Maarel, S. M. (2020). Homozygous nonsense variant in LRIF1 associated with facioscapulohumeral muscular dystrophy. *Neurology*, *94*(23), e2441-e2447. https://doi.org/10.1212/WNL.0000000000009617

Hamel, J., & Tawil, R. (2018). Facioscapulohumeral Muscular Dystrophy: Update on Pathogenesis and Future Treatments. *Neurotherapeutics*, *15*(4), 863-871. https://doi.org/10.1007/s13311-018-00675-3

Han, S., Xu, H., Zheng, J., Sun, J., Feng, X., Wang, Y., Ye, W., Ke, Q., Ren, Y., Yao, S., Zhang, S., Chen, J., Griggs, R. C., Zhao, Z., Qi, M., & Gatheridge, M. A. (2020). Population-Wide Duchenne Muscular Dystrophy Carrier Detection by CK and Molecular Testing. *BioMed Research International*, *2020*, 8396429. https://doi.org/10.1155/2020/8396429

Harris, E., Topf, A., Barresi, R., Hudson, J., Powell, H., Tellez, J., Hicks, D., Porter, A., Bertoli, M., Evangelista, T., Marini-Betollo, C., Magnusson, O., Lek, M., MacArthur, D., Bushby, K., Lochmuller, H., & Straub, V. (2017). Exome sequences versus sequential gene testing in the UK highly specialised Service for Limb Girdle Muscular Dystrophy. *Orphanet J Rare Dis*, *12*(1), 151. https://doi.org/10.1186/s13023-017-0699-9

Haynes, P., Bomsztyk, K., & Miller, D. G. (2018). Sporadic DUX4 expression in FSHD myocytes is associated with incomplete repression by the PRC2 complex and gain of H3K9 acetylation on the contracted D4Z4 allele. *Epigenetics Chromatin*, *11*(1), 47. https://doi.org/10.1186/s13072-018-0215-z

Kang, P. B., Morrison, L., Iannaccone, S. T., Graham, R. J., Bonnemann, C. G., Rutkowski, A., Hornyak, J., Wang, C. H., North, K., Oskoui, M., Getchius, T. S., Cox, J. A., Hagen, E. E., Gronseth, G., & Griggs, R. C. (2015). Evidence-based guideline summary: evaluation, diagnosis, and management of congenital muscular dystrophy: Report of the Guideline Development Subcommittee of the American Academy of Neurology and the Practice Issues Review Panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. *Neurology*, *84*(13), 1369-1378. https://doi.org/10.1212/wnl.0000000000001416

Kim, E. Y., Lee, J. W., Suh, M. R., Choi, W. A., Kang, S. W., & Oh, H. J. (2017). Correlation of Serum Creatine Kinase Level With Pulmonary Function in Duchenne Muscular

Dystrophy. *Ann Rehabil Med*, *41*(2), 306-312. https://doi.org/10.5535/arm.2017.41.2.306

Lemmers, R. J., Osborn, M., Haaf, T., Rogers, M., Frants, R. R., Padberg, G. W., Cooper, D. N., van der Maarel, S. M., & Upadhyaya, M. (2003). D4F104S1 deletion in facioscapulohumeral muscular dystrophy: phenotype, size, and detection. *Neurology*, *61*(2), 178-183. https://doi.org/10.1212/01.wnl.0000078889.51444.81

Lemmers, R. J., Tawil, R., Petek, L. M., Balog, J., Block, G. J., Santen, G. W., Amell, A. M., van der Vliet, P. J., Almomani, R., Straasheijm, K. R., Krom, Y. D., Klooster, R., Sun, Y., den Dunnen, J. T., Helmer, Q., Donlin-Smith, C. M., Padberg, G. W., van Engelen, B. G., de Greef, J. C., . . . van der Maarel, S. M. (2012). Digenic inheritance of an SMCHD1 mutation and an FSHD-permissive D4Z4 allele causes facioscapulohumeral muscular dystrophy type 2. *Nat Genet*, *44*(12), 1370-1374. https://doi.org/10.1038/ng.2454

Lemmers, R. J. L. F., O'Shea, S., Padberg, G. W., Lunt, P. W., & van der Maarel, S. M. (2012). Best practice guidelines on genetic diagnostics of Facioscapulohumeral muscular dystrophy: Workshop 9th June 2010, LUMC, Leiden, The Netherlands. *Neuromuscular Disorders*, *22*(5), 463-470. https://doi.org/10.1016/j.nmd.2011.09.004

Luce, L. N., Dalamon, V., Ferrer, M., Parma, D., Szijan, I., & Giliberto, F. (2016). MLPA analysis of an Argentine cohort of patients with dystrophinopathy: Association of intron breakpoints hot spots with STR abundance in DMD gene. *J Neurol Sci*, *365*, 22-30. https://doi.org/10.1016/j.jns.2016.03.047

Monies, D., Alhindi, H. N., Almuhaizea, M. A., Abouelhoda, M., Alazami, A. M., Goljan, E., Alyounes, B., Jaroudi, D., Allssa, A., Alabdulrahman, K., Subhani, S., El-Kalioby, M., Faquih, T., Wakil, S. M., Altassan, N. A., Meyer, B. F., & Bohlega, S. (2016). A first-line diagnostic assay for limb-girdle muscular dystrophy and other myopathies. *Hum Genomics*, *10*(1), 32. https://doi.org/10.1186/s40246-016-0089-8

Mul, K., Voermans, N. C., Lemmers, R., Jonker, M. A., van der Vliet, P. J., Padberg, G. W., van Engelen, B. G. M., van der Maarel, S. M., & Horlings, C. G. C. (2018). Phenotype-genotype relations in facioscapulohumeral muscular dystrophy type 1. *Clin Genet*. https://doi.org/10.1111/cge.13446

Murphy, A. P., & Straub, V. (2015). The Classification, Natural History and Treatment of the Limb Girdle Muscular Dystrophies. *J Neuromuscul Dis*, *2*(s2), S7-s19. https://doi.org/10.3233/jnd-150105

Murugan, S., Chandramohan, A., & Lakshmi, B. R. (2010). Use of multiplex ligation-dependent probe amplification (MLPA) for Duchenne muscular dystrophy (DMD) gene mutation analysis. *Indian J Med Res*, *132*, 303-311. https://www.researchgate.net/publication/46288551_Use_of_multiplex_ligation-dependent_probe_amplification_MLPA_for_Duchenne_muscular_dystrophy_DMD_gene_mutation_analysis

Nallamilli, B. R. R., Chakravorty, S., Kesari, A., Tanner, A., Ankala, A., Schneider, T., da Silva, C., Beadling, R., Alexander, J. J., Askree, S. H., Whitt, Z., Bean, L., Collins, C., Khadilkar,

S., Gaitonde, P., Dastur, R., Wicklund, M., Mozaffar, T., Harms, M., . . . Hegde, M. (2018). Genetic landscape and novel disease mechanisms from a large LGMD cohort of 4656 patients. *Ann Clin Transl Neurol*, *5*(12), 1574-1587. https://doi.org/10.1002/acn3.649

Narayanaswami, P., Weiss, M., Selcen, D., David, W., Raynor, E., Carter, G., Wicklund, M., Barohn, R. J., Ensrud, E., Griggs, R. C., Gronseth, G., & Amato, A. A. (2014). Evidence-based guideline summary: diagnosis and treatment of limb-girdle and distal dystrophies: report of the guideline development subcommittee of the American Academy of Neurology and the practice issues review panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. *Neurology*, *83*(16), 1453-1463. https://doi.org/10.1212/wnl.0000000000000892

Norwood, F., de Visser, M., Eymard, B., Lochmuller, H., & Bushby, K. (2007). EFNS guideline on diagnosis and management of limb girdle muscular dystrophies. *Eur J Neurol*, *14*(12), 1305-1312. https://doi.org/10.1111/j.1468-1331.2007.01979.x

O'Grady, G. L., Lek, M., Lamande, S. R., Waddell, L., Oates, E. C., Punetha, J., Ghaoui, R., Sandaradura, S. A., Best, H., Kaur, S., Davis, M., Laing, N. G., Muntoni, F., Hoffman, E., MacArthur, D. G., Clarke, N. F., Cooper, S., & North, K. (2016). Diagnosis and etiology of congenital muscular dystrophy: We are halfway there. *Annals of Neurology*, *80*(1), 101-111. https://onlinelibrary.wiley.com/doi/abs/10.1002/ana.24687

Ozyilmaz, B., Kirbiyik, O., Ozdemir, T. R., Kaya Ozer, O., Kutbay, Y. B., Erdogan, K. M., Guvenc, M. S., Kale, M. Y., Gazeteci, H., Kilic, B., Sertpoyraz, F., Diniz, G., Baydan, F., Gencpinar, P., Dundar, N. O., & Yis, U. (2019). Impact of next-generation sequencing panels in the evaluation of limb-girdle muscular dystrophies. *Ann Hum Genet*, *83*(5), 331-347. https://doi.org/10.1111/ahg.12319

Petek, L. M., Rickard, A. M., Budech, C., Poliachik, S. L., Shaw, D., Ferguson, M. R., Tawil, R., Friedman, S. D., & Miller, D. G. (2016). A cross sectional study of two independent cohorts identifies serum biomarkers for facioscapulohumeral muscular dystrophy (FSHD). *Neuromuscul Disord*, *26*(7), 405-413. https://doi.org/10.1016/j.nmd.2016.04.012

Reddy, H. M., Cho, K. A., Lek, M., Estrella, E., Valkanas, E., Jones, M. D., Mitsuhashi, S., Darras, B. T., Amato, A. A., Lidov, H. G., Brownstein, C. A., Margulies, D. M., Yu, T. W., Salih, M. A., Kunkel, L. M., MacArthur, D. G., & Kang, P. B. (2017). The sensitivity of exome sequencing in identifying pathogenic mutations for LGMD in the United States. *J Hum Genet*, *62*(2), 243-252. https://doi.org/10.1038/jhg.2016.116

Romitti, P. A., Zhu, Y., Puzhankara, S., James, K. A., Nabukera, S. K., Zamba, G. K., Ciafaloni, E., Cunniff, C., Druschel, C. M., Mathews, K. D., Matthews, D. J., Meaney, F. J., Andrews, J. G., Conway, K. M., Fox, D. J., Street, N., Adams, M. M., Bolen, J., & STARnet, M. D. (2015). Prevalence of Duchenne and Becker muscular dystrophies in the United States. *Pediatrics*, *135*(3), 513-521. https://doi.org/10.1542/peds.2014-2044

Rosenberg, A., Tian, C., He, H., Ulm, E., Collins Ruff, K., & B. Nagaraj, C. (2023). An evaluation of clinical presentation and genetic testing approaches for patients with neuromuscular disorders. *American Journal of Medical Genetics Part A*, *191*(11), 2679-2692. https://onlinelibrary.wiley.com/doi/abs/10.1002/ajmg.a.63356

Scionti, I., Greco, F., Ricci, G., Govi, M., Arashiro, P., Vercelli, L., Berardinelli, A., Angelini, C., Antonini, G., Cao, M., Di Muzio, A., Moggio, M., Morandi, L., Ricci, E., Rodolico, C., Ruggiero, L., Santoro, L., Siciliano, G., Tomelleri, G., . . . Tupler, R. (2012). Large-scale population analysis challenges the current criteria for the molecular diagnosis of fascioscapulohumeral muscular dystrophy. *Am J Hum Genet*, *90*(4), 628-635. https://doi.org/10.1016/j.ajhg.2012.02.019

Straub, V., Murphy, A., Udd, B., & group, L. w. s. (2018). 229th ENMC international workshop: Limb girdle muscular dystrophies - Nomenclature and reformed classification Naarden, the Netherlands, 17-19 March 2017. *Neuromuscul Disord*, *28*(8), 702-710. https://doi.org/10.1016/j.nmd.2018.05.007

Tawil, R., Kissel, J. T., Heatwole, C., Pandya, S., Gronseth, G., & Benatar, M. (2015). Evidence-based guideline summary: Evaluation, diagnosis, and management of facioscapulohumeral muscular dystrophy: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology and the Practice Issues Review Panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. *Neurology*, *85*(4), 357-364. https://doi.org/10.1212/wnl.0000000000001783

van den Boogaard, M. L., Lemmers, R., Balog, J., Wohlgemuth, M., Auranen, M., Mitsuhashi, S., van der Vliet, P. J., Straasheijm, K. R., van den Akker, R. F. P., Kriek, M., Laurense-Bik, M. E. Y., Raz, V., van Ostaijen-Ten Dam, M. M., Hansson, K. B. M., van der Kooi, E. L., Kiuru-Enari, S., Udd, B., van Tol, M. J. D., Nishino, I., . . . van der Maarel, S. M. (2016). Mutations in DNMT3B Modify Epigenetic Repression of the D4Z4 Repeat and the Penetrance of Facioscapulohumeral Dystrophy. *Am J Hum Genet*, *98*(5), 1020-1029. https://doi.org/10.1016/j.ajhg.2016.03.013

van der Maarel, S. M., Tawil, R., & Tapscott, S. J. (2011). Facioscapulohumeral muscular dystrophy and DUX4: breaking the silence. *Trends Mol Med*, *17*(5), 252-258. https://doi.org/10.1016/j.molmed.2011.01.001

Vengalil, S., Preethish-Kumar, V., Polavarapu, K., Mahadevappa, M., Sekar, D., Purushottam, M., Thomas, P. T., Nashi, S., & Nalini, A. (2017). Duchenne Muscular Dystrophy and Becker Muscular Dystrophy Confirmed by Multiplex Ligation-Dependent Probe Amplification: Genotype-Phenotype Correlation in a Large Cohort. *J Clin Neurol*, *13*(1), 91-97. https://doi.org/10.3988/jcn.2017.13.1.91

Wang, C. H., Bonnemann, C. G., Rutkowski, A., Sejersen, T., Bellini, J., Battista, V., Florence, J. M., Schara, U., Schuler, P. M., Wahbi, K., Aloysius, A., Bash, R. O., Beroud, C., Bertini, E., Bushby, K., Cohn, R. D., Connolly, A. M., Deconinck, N., Desguerre, I., . . . Zeller, R. (2010). Consensus statement on standard of care for congenital muscular

dystrophies. *J Child Neurol*, *25*(12), 1559-1581. https://doi.org/10.1177/0883073810381924

Wang, H., Xu, Y., Liu, X., Wang, L., Jiang, W., Xiao, B., Wei, W., Chen, Y., Ye, W., & Ji, X. (2017). Prenatal diagnosis of Duchenne muscular dystrophy in 131 Chinese families with dystrophinopathy. *Prenat Diagn*, *37*(4), 356-364. https://doi.org/10.1002/pd.5019

Wicklund, M. P. (2019). The Limb-Girdle Muscular Dystrophies. *Continuum (Minneap Minn)*, *25*(6), 1599-1618. https://doi.org/10.1212/CON.0000000000000809

Wicklund, M. P., & Kissel, J. T. (2014). The limb-girdle muscular dystrophies. *Neurol Clin*, *32*(3), 729-749, ix. https://doi.org/10.1016/j.ncl.2014.04.005

Yang, J., Li, S. Y., Li, Y. Q., Cao, J. Q., Feng, S. W., Wang, Y. Y., Zhan, Y. X., Yu, C. S., Chen, F., Li, J., Sun, X. F., & Zhang, C. (2013). MLPA-based genotype-phenotype analysis in 1053 Chinese patients with DMD/BMD. *BMC Med Genet*, *14*, 29. https://doi.org/10.1186/1471-2350-14-29

Zhang, T., Liu, S., Wei, T., Yong, J., Mao, Y., Lu, X., Xie, J., Ke, Q., Jin, F., & Qi, M. (2013). Development of a comprehensive real-time PCR assay for dystrophin gene analysis and prenatal diagnosis of Chinese families. *Clin Chim Acta*, *424*, 33-38. https://doi.org/10.1016/j.cca.2013.05.006

Genetic Testing for Epilepsy

Policy Number: AHS – M2075 – Genetic Testing for Epilepsy	Prior Policy Name and Number, as applicable:
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Epilepsy is a group of disorders characterized by recurrent, unprovoked seizures due to abnormal, synchronized neuronal firing in the brain that can be distinguished by seizure type, age of onset, developmental status, co-morbid features and etiology (Berg et al., 2010; Myers & Mefford, 2015).

For guidance on the use of chromosomal microarray or low-pass whole genome sequencing for the diagnosis of epilepsy, please reference policy AHS-M2033-Chromosomal Microarray and Low-pass Whole Genome Sequencing.

II. Related Policies

Policy Number	Policy Title
AHS-M2033	Chromosomal Microarray and Low-pass Whole Genome Sequencing
AHS-M2085	Genetic Testing of Mitochondrial Disorders
AHS-M2176	Testing for Autism Spectrum Disorder and Developmental Delay

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with infantile- and early-childhood–onset epilepsy syndromes (in which epilepsy is the core clinical symptom), genetic testing for mutations associated with infantile- and early-childhood–onset epilepsy syndromes (see Note 1) **MEETS COVERAGE CRITERIA** only when a positive test result would lead to changes in at least **one** of the following:

a) Medication management.

b) Diagnostic testing (alternative potentially invasive tests are avoided).

c) Reproductive decision making.

2) For individuals with an epilepsy syndrome with a suspected genetic cause, targeted mutation testing **MEETS COVERAGE CRITERIA** to assess for the following clinical situations:

a) *ALDH7A1* testing for pyridoxine-related epilepsy.

b) *BTD* testing for biotinidase deficiency-related epilepsy.

c) *FOLR1* testing for cerebral folate deficiency-related epilepsy.

d) *GRIN2A* testing for GRIN2A-related epileptic encephalopathy.

e) *KCNQ2* testing for KCNQ2-related epileptic encephalopathy.

f) *KCNT1* testing for KCNT1-related migrating partial epilepsy of infancy.

g) *PCDH19* testing for epilepsy female-restricted with mental retardation (EFMR).

h) *PHGDH*, *PSAT1,* and *PSPH* testing for epilepsy due to serine biosynthesis defects.

i) *SCN1A* testing for SCN1A-related seizure disorders.

j) *SCN8A* testing for SCN8A-related epileptic encephalopathy.

k) *SLC2A1* testing for glucose transporter type 1 deficiency syndrome.

l) *SLC6A8, GATM,* and *GAMT* testing for epilepsy due to creatine deficiency syndromes.

m) *TSC1* and *TSC2* testing for tuberous sclerosis complex-related epilepsy.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

3) For all other situations and/or mutations not described above, genetic testing for epilepsy **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
AAN	American Academy of Neurology
ALDH7A1	*Aldehyde dehydrogenase 7 family member A1*
AMA	Antiseizure medication
ANK2	*Ankyrin 2*
ASD	Autism spectrum disorders
BTD	*Biotinidase*
CACNA1A	*Calcium voltage-gated channel subunit alpha 1A*
CACNA1AE	*Calcium voltage-gated channel subunit alpha 1AE*
CACNA1D	*Calcium voltage-gated channel subunit alpha 1D*
CACNA1H	*Calcium voltage-gated channel subunit alpha 1H*
CACNA2D3	*Calcium voltage-gated channel subunit alpha 1A*
CDKL5	Calcium voltage-gated channel subunit alpha2delta3
CGH	Comparative genomic hybridization
CHD2	*Chromodomain helicase DNA binding protein 2*
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMA	Chromosomal microarray analysis
CMS	Centers for Medicare and Medicaid Services
CNS	Child Neurology Society
CNV	Copy number variant
DEE	Developmental and epileptic encephalopathy
DLG4	*Discs large homolog 4*
DS	Dravet syndrome
EE	Epileptic encephalopathy
EEG	Electroencephalography
EFMR	Epilepsy female-restricted with mental retardation
EFNS	European Federation of Neurological Societies
EP	Epileptic panels
FGF12	*Fibroblast growth factor 12*
FOLR1	*Folate receptor 1*
GABRA1	*Gamma-aminobutyric acid type A receptor alpha1*
GABRG2	*Gamma-aminobutyric acid type A receptor gamma2*
GAMT	*Guanidinoacetate n-methyltransferase*
GATM	*Glycine amidinotransferase*
GGE	Genetic generalized epilepsy
Glut1DS	Glucose transporter 1 deficiency syndrome
GRIA2	*Glutamate ionotropic receptor AMPA type subunit 2*
GRIN2A	*Glutamate ionotropic receptor NMDA type subunit 2a*
GRIN2B	*Glutamate ionotropic receptor NMDA type subunit 2b*

GS	Genome sequencing
GTAC	Genetic Testing Advisory Committee
HNRNPU	*Heterogeneous nuclear ribonucleoprotein U*
IC-CODE	International Classification of Cognitive Disorders in Epilepsy
ID	Intellectual disability
ILAE	International League Against Epilepsy
KANSL1	*KAT8 regulatory NSL complex subunit 1*
KB	Kilo-base pair
KCNC1	*Potassium voltage-gated channel subfamily C member 1*
KCNQ2	*Potassium voltage-gated channel subfamily Q member 2*
KCNT1	*Potassium sodium-activated channel subfamily T member 1*
KD	Ketogenic diet
LDTs	Laboratory-developed tests
MECP2	*Methyl-CpG binding protein 2*
MGP	Multigene panel
mTOR	Mammalian target of rapamycin
NDD	Neurodevelopmental disorders
NGS	Next-generation sequencing
NICE	National Institute for Health and Care Excellence
PCDH19	*Protocadherin 19*
PHGDH	*Phosphoglycerate dehydrogenase*
PNPO	*Pyridoxamine 5'-phosphate oxidase*
PRRT2	Proline-rich transmembrane protein 2
PSAT1	*Phosphoserine aminotransferase 1*
PSPH	*Phosphoserine phosphatase*
PURA	*Pur-alpha*
SCN1A	*Sodium voltage-gated channel alpha subunit 1*
SCN2A	*Sodium voltage-gated channel type 2 alpha subunit*
SCN8A	*Sodium voltage-gated channel type 8 alpha subunit*
SE	Status epilepticus
SIGN	Scottish Intercollegiate Guidelines Network
SLC2A1	*Solute carrier family 2 member 1*
SLC6A1	*Solute carrier family 6 member 1*
SLC6A8	*Solute carrier family 6 member 8*
SMEI	Severe myoclonic epilepsy of infancy
STX1B	*Syntaxin 1B*
STXBP1	Syntaxin-binding protein 1
TCF4	Transcription factor 4
TSC	Tuberous sclerosis complex

TSC1	Tuberous sclerosis complex 1
TSC2	Tuberous sclerosis complex 2
UBE3A	*Ubiquitin protein ligase E3A*
WES	Whole exome sequencing
WGS	Whole genome sequencing
YWHAG	*Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein gamma*

V. Scientific Background

Epilepsy, defined as having two or more unprovoked seizures, is a common neurologic disorder, affecting an estimated three million adults and 470,000 children in the United States (CDC, 2020). The biological and genetic mechanisms that disturb the balance between excitatory and inhibitory neuronal circuits to result in epilepsy are extremely heterogeneous (Ottman et al., 2010; Williams & Battaglia, 2013). Approximately 20–30% of epilepsy diagnoses can be attributed to other primary conditions, such as stroke, tumor or head injury, but the remaining 70–80% of cases are believed to be due to one or more genetic factors (Hildebrand et al., 2013; Myers & Mefford, 2015).

The epilepsies can be classified by multiple approaches. Clinically, they can be broadly grouped into three classes: genetic generalized epilepsy (GGE), focal epilepsy, and epileptic encephalopathy (EE). GGE is characterized by generalized seizures that involve both sides of the brain, start in childhood or adolescence, are usually associated with normal development and intellect, and include juvenile myoclonic epilepsy and childhood absence epilepsy, among others. Focal seizures originate in one hemisphere of the brain and include temporal lobe epilepsy and autosomal dominant nocturnal frontal lobe epilepsy. EE are severe, early onset conditions characterized by refractory seizures, developmental delay or regression associated with ongoing epileptic activity, and generally poor prognosis such as Dravet, Ohtahara, and West syndromes. Epilepsy is often a comorbid condition with intellectual disability (ID), autism, or schizophrenia, and may be a feature of many metabolic conditions and genetic syndromes (Myers & Mefford, 2015).

The International League Against Epilepsy (ILAE) classifies epilepsies at three levels: seizure types, epilepsy types, and epilepsy syndromes. The classification starts with seizure type as the least specific, epilepsy type as more specific, and epilepsy syndromes as the most specific classification. Seizure type is based on onset, of which there are three categories: focal, generalized, and unknown. The second category, epilepsy type, is divided into four categories; focal, generalized, combined generalized & focal, and unknown. This level of classification typically requires electroencephalography (EEG) data. The “combined generalized & focal” group is new

to the 2017 edition of the ILAE classification, and it was created to include patients with both types of seizures, such as patients with Dravet Syndrome. Finally, the "Unknown" category refers to a case where there is not enough information to determine the epilepsy type. This can occur for numerous reasons, such as the unavailability of an EEG. The third level of classification is "epilepsy syndromes." A syndrome refers to a cluster of features incorporating items such as seizure types, EEG results, and imaging results that tend to occur together. Other distinctive features, such as intellectual disability, may be a part of a syndrome (Scheffer et al., 2017).

Some conditions have well-known mutations caused by a singular gene. For example, in Dravet syndrome, (DS, previously known as severe myoclonic epilepsy of infancy or SMEI) the occurrence of *SCN1A* mutations is 70%-80%. About 90% of these mutations are *de novo*. Truncating mutations have found to be more severe (earlier onset of symptoms, faster cognitive decline) compared to missense mutations. *PCDH19*-related epilepsy is a rare syndrome characterized by focal and/or generalized seizures, which are commonly fever-induced and in clusters. Previously referred to as epilepsy female-restricted with mental retardation (EFMR), *PCDH19*-related epilepsy occurs primarily in females and has an early onset. A mutation in *PCDH19* can cause both DS and *PCDH19*-related epilepsy as *PCDH19* is thought to account for 5% of patients with DS (Andrade & Nascimento, 2023). Another example is tuberous sclerosis complex (TSC); as many as 89% of cases have mutations in either the *TSC1* or *TSC2* gene (Randle, 2023, 2024a, 2024b). Other mutations include *KCNQ2* with EE, *SLC2A1* with glucose transporter deficiency syndrome, *PRRT2* with general infantile convulsions, and *ALDH7A1* and *PNPO* with severe early-onset epilepsy (Poduri et al., 2014). Missense variants in *SLC32A1* can cause genetic epilepsy with febrile seizures plus (GEFS+) and idiopathic generalizes epilepsy (IGE) (Heron et al., 2021).

Epilepsy is genetically heterogeneous, and extensive phenotypic heterogeneity has been observed even in many monogenic epilepsies. This heterogeneity makes testing each gene individually through Sanger sequencing impractical despite its status as the gold standard (Lemke et al., 2012). Mutations in ion channels, chromatin remodeling, transcriptional regulation, and regulation of the mammalian target of rapamycin (mTOR) protein have been implicated in the etiology of epilepsy (Moller et al., 2016).

Clinical Utility and Validity

The DNA sequencing methods for epilepsy diagnosis have high analytic validity; the methods are well-established in laboratories. Sequencing bi-directionally has been evaluated at over 98% sensitivity (Stenhouse et al., 2013). Next generation sequencing (NGS) allows for parallel sequencing of any number of genes, thereby allowing for far superior testing of heterogenous conditions such as epilepsy. Since the number of new

epilepsy genes continues to grow and the phenotypes associated with mutations in each gene are so variable, NGS is a desirable option to diagnose epilepsy patients (Mefford, 2015).

New test options for epilepsy diagnosis have an increased yield of molecular diagnosis, particularly in patients with severe, early-onset epilepsies. For example, a study by Lemke et al. (2012) focused on targeted resequencing of 265 candidate genes, which identified mutations that were presumed to be pathogenic in 16 of 33 patients. Many of these patients had severe epilepsies associated with intellectual disability. This NGS method detected mutations that had been missed by the previous gold standard of Sanger sequencing (with at least one mutation missed due to artifacts from Sanger sequencing). Furthermore, the authors concluded that a patient with more than one possible gene responsible for their phenotype would benefit from this sequencing method compared to Sanger. The authors noted that NGS is only suited for conditions with at least one major gene effect and that whole exome sequencing (WES) would be a better option for more complex genetic conditions (Lemke et al., 2012).

In 2016, a gene panel targeting 46 epilepsy genes was used on 216 patients representing a wide spectrum of epilepsies with age of onset spanning from the neonatal period to adulthood. A presumed pathogenic variant was identified in 49 (23%) patients. The variants were found in 19 different genes including *SCN1A, STXBP1,* and *CDKL5*. Patients with neonatal-onset epilepsies had the highest rate of positive findings (57%). The overall yield for patients with EEs was 32%, compared to 17% among patients with generalized epilepsies and 16% in patients with focal or multifocal (Moller et al., 2016). Another study focusing on the diagnostic use of microarray was performed by Hrabik et al. (2015). Approximately 17.7% (26/147) of participants between the ages of birth to 23 years who were diagnosed with epilepsy and had a SNP microarray performed had an abnormal microarray as defined by laboratory guidelines (Hrabik et al., 2015). Gene panels and exome sequencing for clinical diagnostics provide better and less expensive options for testing and could be implemented early in the diagnostic process. Chromosome microarrays may be considered in severe cases and in GGE with co-morbid features (Myers & Mefford, 2015). Overall, sequencing of many genes at once may provide major benefits to those with epilepsy as epilepsy's genetic profile is widespread.

Copy number abnormalities also play an important role in patients with epilepsy. Of 973 patients who had chromosomal microarray (CMA) and ICD-9 codes for epilepsy or seizures, 805 patients satisfied criteria for epilepsy. 437 copy number variants (CNVs) in 323 patients (1-4 per patient), including 185 (42%) deletions and 252 (58%) duplications were observed. Forty (9%) were confirmed de novo, 186 (43%) were inherited, and parental data were unavailable for 211 (48%). Because the diagnostic

yield of CMA for epilepsy patients is similar to the yield in autism spectrum disorders (ASD) and in prenatal diagnosis (which published guidelines recommending testing with CMA), the authors recommended implementation of CMA in the evaluation of unexplained epilepsy (Olson et al., 2014).

Berg et al. (2017) conducted a prospective cohort study of 775 children with newly diagnosed epilepsy with an onset at less than three years of age to determine the role of genetic testing in the initial evaluation of early life epilepsies. Ninety-five children had brain injury, and of the other 680, 327 underwent genetic testing, such as microarrays, WES, and gene panels. In total, 132 (40.4%) children were found to have pathogenic variants. A total of 446 children were deemed to have an unknown etiology without genetic testing, and 180 of these children were tested. Pathogenic variants were found in 48 or 26.7% of the children, however, epilepsy gene-sequencing panels and whole-exome sequencing (WES) had substantially greater diagnostic yields than CMA. Epilepsy panels detected 28 of 96 (29.2%), WES detected five of 18 or (27.8%), and CMA detected eight for 101 or (7.9%). Without a clinically identified cause, testing yields were greater than 15% and as high as 47% depending on patient subgroups. The authors concluded that broad genetic sequencing methods have high diagnostic yields in diagnosing early-life epilepsies regardless of clinical features. Sequencing tests should be incorporated into the initial evaluation of newly presenting early-life epilepsies and not just reserved for those with severe presentations (Berg et al., 2017).

Gene panels including the most mutated genes have a 10%-50% diagnostic rate (depending on the panel used and the population) although it is possible that a greater number of genes in the panel does not necessarily equate to a higher diagnostic rate. A 67 gene panel and a 265 gene panel were found to have nearly equivalent diagnostic rates; however, the study using the 67 gene panel only included nineteen patients (Dunn et al., 2018; Mefford, 2015). Exome sequencing has been evaluated at a 25% diagnostic rate (without a prior diagnosis), and whole-genome sequencing (WGS) has been evaluated at as high as 60% with smaller studies and known phenotypes. The weaker sequencing depth contributes to the difficulty of locating copy number variants, which are a significant cause of epilepsy-related conditions. Nonetheless, the authors conclude that WES and WGS will become part of the regularly used tools for clinicians once their cost decreases (Dunn et al., 2018).

Stosser et al. (2017) conducted a retrospective analysis of "893 probands with epilepsy who had an epilepsy panel or WES performed and were positive for a pathogenic or likely pathogenic variant in one of nine genes (*CDKL5, GABRA1, GABRG2, GRIN2B, KCNQ2, MECP2, PCDH19, SCN1A*, or *SCN2A*)," which found "mosaic pathogenic variants… at an overall frequency of 3.5% (95% CI, 2.4%-4.9%) in nine genes associated with epilepsy-related disorders…. Mosaicism was most common in the *CDKL5,*

PCDH19, SCN2A, and *SCN1A* genes. Mosaicism was observed in *GABRA1, GABRG2,* and *GRIN2B*, which were not reported to have mosaicism prior to this study, and in *KCNQ2* and *MECP2*. Parental mosaicism was observed for pathogenic variants in multiple genes including *KCNQ2, MECP2, SCN1A*, and *SCN2A*." The authors concluded that patients with epilepsy who previously tested negative for pathogenic variants may benefit from an NGS test, which is superior at detecting mosaic variants. The authors also noted that targeted testing of parents of probands may use NGS to better assess risk and mosaicism (Stosser et al., 2017).

"Epilepsy-plus" patients tend to have highest yield for diagnostic testing; that is, patients with other symptoms such as autism in addition to their epilepsy. Still, even the diagnostic yield of these individuals only reached 50% with panel or exome sequencing (Poduri, 2017). The potential clinical utility of genetic testing for these syndromes is in avoiding further diagnostic testing, directing medication management, and assisting in reproductive decision making. Establishing the genetic basis of epilepsy in a given patient will help in making treatment decisions, genetic counseling, and more (Poduri et al., 2014). For instance, mutations in *ALDH7A1* or in the *PNPO* gene may lead to seizures not treated with typical antiepileptic drugs, but with pyridoxine (Falsaperla & Corsello, 2017). On the other hand, sodium channel agents may be avoided in patients with an *SCNA1* mutation. Genetic counseling revolving around topics such as recurrence or heritability risk for pregnancies or testing of relatives is also helpful, and proper care must be taken to ensure that the information is clearly explained to the patient and their family (Poduri et al., 2014).

Genetic testing for epilepsy could also be useful in diagnosing ASD. Peng et al. (2021) discussed, in their research on developing a multiplex gene and phenotype network to identify shared genes between epilepsy and autism, that mutations in genes for subunits of ion channels were related to epilepsy, and that general ion channel dysfunctions "are also linked to susceptibility to autism, as well as bipolar disorder, schizophrenia, and other neuropsychiatric disorders." The researchers "prioritize *ANK2, CACNA1AE, CACNA2D3, GRIA2,* and *DLG4*… as candidate epilepsy genes because of their overlap with the epilepsy-focused module 2 of the WES [whole-exome sequencing] network;" these genes originated as having association only with autism prior to this study (Peng et al., 2021). A study with similar intention done on co-occurring epilepsy and ASD in Chinese children found *SCN1A* and *MECP2* gene mutations to be the most common; *SCN1A* was more associated with epilepsy, while *MECP2* was more associated with Rett Syndrome. Mutations in *SCN2A, CACNA1A, CACNA1H, CACNA1D,* and *KCNQ2* were also identified, although individual cases for each gene were small in the latter set of genes (Long et al., 2019). Both studies contribute to the understanding of the beneficial utility for genetic testing of epilepsy in the context of other comorbidities, such as ASD.

Sanchez Fernandez et al. (2019) evaluated the cost-effectiveness of genetic testing in patients with epilepsy of unknown etiology. 20 studies were included, with eight evaluating chromosomal microarray (CMA), nine evaluating epileptic panels (EP) with deletion/duplication testing, and six evaluating whole exome sequencing (WES). The authors found WES to have the highest diagnostic yield at 0.45, followed by EP at 0.23 and then CMA at 0.08. EP was found to be the most cost-effective, at $15,848 per diagnosis. Although cost-effectiveness of strategies overlapped, the authors found CMA to be consistently less cost-effective than WES and EP (Sanchez Fernandez et al., 2019).

Borlot et al. (2019) analyzed the results of epilepsy gene panels from 64 patients. Up to 185 genes were tested. 14 probands were found to have "pathogenic or likely pathogenic" variants in the following genes: "*SCN1A, GABRB3, UBE3A, KANSL1, SLC2A1, KCNQ2, SLC6A1, HNRNPU, STX1B, SCN2A, PURA, and CHD2*". The authors also identified six mutations arising *de novo*, with unknown inheritance for eight mutations. Overall, the authors concluded that a commercial gene panel for epilepsy may be useful, as it detected etiology in 22% of patients with epilepsy and intellectual disability (Borlot et al., 2019).

Kim et al. (2021) analyzed the clinical utility of whole-exome sequencing (WES) for patients with infantile-onset epilepsy that had tested negative on gene panel tests for epilepsy. The study included 59 patients. Following WES, 55.4% of the participants received genetic conformation of epilepsy, indicating that WES increased diagnostic yield by 8%. Three epilepsy-causing genes (that were not included on the original gene panel) were identified: *YWHAG, KCNC1,* and *FGF12*. The authors conclude that WES could be an important way to reanalyze novel epilepsy-linked genes without updated gene panels (Kim et al., 2021).

Willimsky et al. (2021) studied the use of next generation sequencing (NGS) by comping the diagnostic yield of small and large gene panels. The authors completed a retrospective study of 190 patients under the age of 18 years who were diagnosed with epilepsy of unknown etiology. Small gene panels were defined as those under 25 kilo-base pair (kb), and large gene panels were defined as those over 25 kb. Diagnostic yield was defined as detection of pathogenic or likely pathogenic variants. The authors found that the diagnostic yield of large panels (29%) was significantly larger than small panels (13%). The authors then analyzed the differences in diagnostic yield in developmental and epileptic encephalography (DEE) and non-DEE. The authors found that the significant increase of diagnostic yield in large panels was only significant for non-DEE patients, and not for DEE patients. The authors conclude that large epilepsy gene panels have significantly higher diagnostic yield for non-DEE patients but note that small gene panels (a maximum of 10 genes) is sufficient for DEE patients

(Willimsky et al., 2021).

Stefanski et al. (2021) completed a meta-analysis and systematic review on the success rate of genetic testing of neurodevelopmental disorders (NDD). The study measured the diagnostic yield, defined as the "percent of pathogenic variant carriers identified in a cohort." The study included 103 clinical sequencing studies that used NGS in a total of 32,331 people with epilepsy, ASD, or ID. The diagnostic yield for epilepsy was 24%. The epilepsy subtypes with the highest diagnostic yield were epilepsy with ID (27.9%) and early onset seizures (36.9%). The authors then studied the diagnostic yield of each sequencing technology and found a diagnostic yield of 27.2% for exome sequencing and a diagnostic yield of 22.6% for target gene sequencing panels (Stefanski et al., 2021).

Sheidley et al. (2021) completed a similar meta-analysis and systematic review to Stefanski et al. (2021), except on 5985 studies on genetic testing of patients with epilepsy. "The overall diagnostic yield across all test modalities was 17%, with the highest yield for GS [genome sequencing] (48%), followed by ES [exome sequencing] (24%), MGP [multigene panel] (19%), and [genome-wide comparative genomic hybridization/chromosomal microarray] CGH/CMA (9%)." The authors also studied non-yield outcomes. 24 studies reported on changes in treatment based on genetic testing; "treatment changes were reported in 12-18% of patients with a genetic diagnosis, including avoiding, stopping, or initiating specific antiseizure medications (AMAs) or ketogenic diet (KD) and halting a plan for surgery in the presence of a specific genetic diagnosis" (Sheidley et al., 2021).

Beyond developing panels to identify genetic variants among epileptic patients, recent advancements in the study of epigenetics could potentially be of use with epilepsy symptoms but not a clear-cut genetic basis. In the patients with neurodevelopmental disorders an epilepsy phenotype, 23% of cases had rare differential methylations (Barbosa et al., 2018). "When the parents were able to be tested, ~40% of the methylation variants were *de novo*, suggesting that methylation abnormalities may be causative in 5-10% of their cohort. When identified, the underlying causes of the methylation changes were varied and included CNVs (copy number variants), sequence variants in regularly elements, or repeat expansions, each of which is easily missed by conventional (even next-generation) sequencing methods" (Hebbar & Mefford, 2020). Genetic and epigenetic testing could further help shape precision medicine in the best types of pharmaceutical drugs used to treat specific epilepsies, such as by understanding individual responses to antiepileptic drug treatment and alterations in drug pharmacokinetics and pharmacodynamics that could guide safer and more effective treatment strategies (Balestrini & Sisodiya, 2018).

McKnight et al. (2022) conducted a study on the diagnostic yield of genetic tests performed on adults with epilepsy. The study included 2,008 individuals and the authors analyzed their NGS targeted testing results of 89-189 genes and clinical information. Diagnostic yield was defined as a definitive molecular diagnosis. In total, 218 adults (10.9%) had clinically actionable findings. The authors compared the diagnostic yield with age of seizure onset and found that "the highest diagnostic yield was in adults with seizure onset during infancy (29.6%, 0–1 year), followed by in early childhood (13.6%, 2–4 years), late childhood (7.0%, 5–10 years), adolescence (2.4%, 11–17 years), and adulthood (3.7%, ≥18 years)." The authors also reported that "comorbid intellectual disability (ID) or developmental delay resulted in a high diagnostic yield (16.0%)." Overall, the authors conclude that genetic testing could impact clinical management and outcomes (McKnight et al., 2022).

VI. Guidelines and Recommendations

International League Against Epilepsy (ILAE)

In 2015, the ILAE Commission of Pediatrics issued a task force report which included the following related to genetic testing in epilepsy.

- "Genetic screening should not be undertaken at a primary or secondary level of care."
- "Standard care should permit genetic counseling by trained personnel to be undertaken at all levels of care (primary to quaternary)."
- "Genetic evaluation for Dravet syndrome and other infantile-onset epileptic encephalopathies should be available at tertiary and quaternary levels of care (optimal intervention would permit an extended genetic evaluation)."
- "Early diagnosis of some mitochondrial conditions may alter long-term outcome, but whether screening at quaternary level is beneficial is unknown." (Wilmshurst et al., 2015)

In 2017, the ILAE released a position paper containing a stance on the genetic etiologies of epilepsies. The ILAE states that "the epilepsies in which a genetic etiology has been implicated are quite diverse and, in most cases, the underlying genes are not yet known". The ILAE also notes that genetic is not equivalent to inherited; de novo mutations are being identified with increasing frequency, thereby making an epileptic syndrome not inherited. Furthermore, the ILAE states that environmental contributions to an epileptic syndrome should not be minimized and that a genetic etiology should be of significant effect in causing epilepsy (Scheffer et al., 2017).

In the 2021 report, the ILAE and the International Neuropsychological Society address the lack of an international taxonomy for cognitive disorders in epilepsy and proposes

the International Classification of Cognitive Disorders in Epilepsy (IC-CODE), stating that "a taxonomy built on the alignment of genetic, neuroimaging, and cognitive processes promises high precision for the diagnosis of cognitive disorders in epilepsy and their treatment" (Norman et al., 2021).

In 2022, ILAE released a position statement on epilepsy syndromes with onset in neonates and infants. The statement notes that "genetic testing [for Dravet syndrome, previously known as Severe Myoclonic Epilepsy of Infancy] is recommended at all ages, including in adults in whom the diagnosis is suspected but details of history in infancy may be difficult to access. A pathogenic variant in SCN1A is present in more than 80%–85% of cases." The statement did not mention recommendations on genetic testing for any other form of epilepsy (Zuberi et al., 2022).

In 2022, the ILAE released a position statement on idiopathic generalized epilepsy syndromes. Genetic testing is mentioned throughout the statement in relation to epilepsy occurring in individuals with intellectual disabilities. In patients suspected of childhood absence epilepsy or generalized tonic–clonic seizures alone, "investigations, including genetic testing, to exclude other etiologies should be considered" in individuals with intellectual disabilities. All other mentions of genetic testing for the diagnosis of epilepsy note that genetic testing is not currently part of the routine diagnostic evaluation but may be used soon, as more genetic determinants are identified (Hirsch et al., 2022).

European Federation of Neurological Societies (EFNS)

In 2010, EFNS issued the following recommendations pertaining to epilepsy. The EFNS noted that molecular investigations may be useful for diagnosis but cannot be considered routine with the large number of mutations present and state that clinical or physiological methods are generally superior. However, they note severe myoclonic epilepsy of infancy (SMEI) has a mutation in the gene *SCN1A* in 80% of patients. Since 2010, this organization has been renamed to the European Academy of Neurology, but no new updates on epilepsy have been released (Burgunder et al., 2010).

American Academy of Neurology (AAN) and Child Neurology Society (CNS)

The AAN and CNS issued guidelines for clinicians on diagnostic assessment of the child with status epilepticus (SE) in 2006, reaffirmed 2019. The recommendations provided guidance for the assessment of laboratory studies, metabolic and genetic studies, electroencephalography, and neuroimaging in children with SE. The expert panel concluded that "there are insufficient data to support or refute whether genetic testing (chromosomal or molecular studies) should be done routinely in children with SE" (Riviello et al., 2006).

American Epilepsy Society Annual Meeting

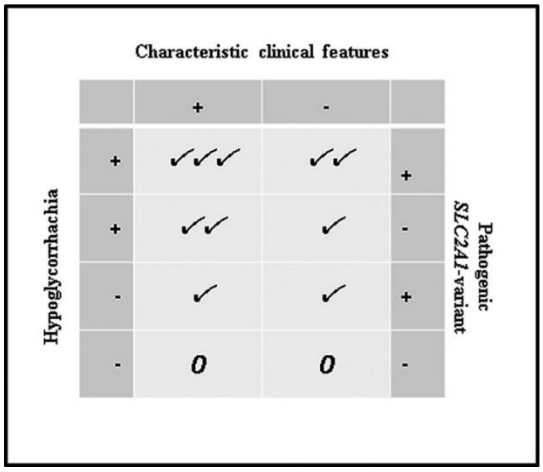
In 2015, a talk presented at the American Epilepsy Society Annual Meeting focused on reviewing the genetics of epilepsy, which groups were likely to benefit from genetic testing, and how to approach genetic testing in the current evolution of medicine. Regarding who would benefit most, "it would make sense to focus testing at this time on those in whom there is a high likelihood of a diagnostic finding and on those whose refractory epilepsy may be influenced by a precise genetic diagnosis that can guide treatment." This study concluded that genetic testing is needed to determine if treatment regimens will be affected, especially in younger children and infants.

With regards to the proper way to approach genetic testing, "we should start with a clinical approach, defining a specific epilepsy syndrome when relevant, to guide the nature and sequence of genetic testing. Pre- and post- testing counseling, whether provided by a physician or genetic counselor, is critical to maintaining clear communication with patients about the implications and limitations of their genetic testing." The types of testing include chromosomal microarray analysis (CMA), complete genome sequencing, and exome sequencing (Poduri, 2017).

International Glut1DS Study Group

The International Glut1DS (glucose transporter 1 deficiency syndrome) put forth recommendations on the diagnosis and treatment of Glut1DS in 2020, which involves "characteristic clinical features, definite hypoglycorrhachia, and pathogenic *SLC2A1* variants." Hypoglycorrhachia is defined as a low glucose level in cerebrospinal fluid in

Symbol	diagnosis of Glut1DS	start KDT
✓✓✓	confirmed	yes
✓✓	probable	yes
✓	possible	consider
0	negative	not required



the setting normal glucose levels in blood, or normoglycemia. Their strength of support for a combination of the three features is shown below.

On genetic testing, the International Glut1DS group also stated that "the absence of *SLC2A1* pathogenic variants does not always exclude Glut1DS…*SLC2A1*-negative patients can be diagnosed on the basis of hypoglycorrhachia and distinctive clinical features, especially when responsive to KDT [ketogenic diet therapies]" (Klepper et al., 2020).

North American Consensus Panel on Dravet Syndrome

This panel was convened to establish standards for evaluation of Dravet Syndrome. The panel recommended that genetic testing be performed in patients whose symptoms indicated Dravet syndrome; the panel noted that either specific *SCN1A* sequencing or a larger gene panel may be performed, and a chromosomal microarray was not necessary.

The panel also notes that genetic testing should be pursued in a child <12 months and with ≥2 febrile seizures >15 minutes as well as a child 12-35 months old with at least one febrile seizure over 15 minutes. However, genetic testing should not be performed for a child <12 months with only one focal or generalized febrile seizure.

Genetic counseling should be provided, particularly information about heritability and risk of epilepsy in siblings. Other information about *SCN1A* mutations may also be provided (Wirrell et al., 2017).

Scottish Intercollegiate Guidelines Network (SIGN)

SIGN lists features suggesting genetic generalized epilepsies, which are as follows:

- childhood or teenage onset
- triggered by sleep deprivation and alcohol
- early morning tonic-clonic seizures or myoclonic jerks
- short absence seizures
- photoparoxysmal response on electroencephalography (EEG)
- generalized three per second spike and wave or polyspike and wave on EEG.

Genetics services are recommended for patients with a "very strong" family history of epilepsy, or with "a clinical phenotype suggestive of a monogenic epilepsy syndrome" (SIGN, 2018).

National Institute for Health and Care Excellence (NICE)

The NICE clinical guideline on epilepsies, in children, young people, and adults was updated in 2022 to include recommendations on genetic testing.

- Discuss with a neurologist or geneticist any uncertainties about whether to offer

genetic testing or which tests to offer to a person with epilepsy.

- Before carrying out genetic tests:
  - discuss the purpose of testing and the possible implications of the results with the person with epilepsy, and their family and carers if appropriate
  - obtain informed consent with appropriate genetic counselling in line with the NHS Genomic Medicine Service.
- Consider whole-genome sequencing for people with epilepsy of unknown cause who:
  - were aged under two years when epilepsy started **or**
  - have clinical features suggestive of a specific genetic epilepsy syndrome (for example, Dravet syndrome) **or**
  - have additional clinical features such as:
    - a learning disability
    - autism spectrum disorder
    - a structural abnormality (for example, dysmorphism or congenital malformation)
    - unexplained cognitive or memory decline.
- Consider whole-genome sequencing for people with epilepsy of unknown cause who were aged between two and three years when epilepsy started, if clinically agreed by a specialist multidisciplinary team (NICE, 2022).

United Kingdom Expert Group on Tuberous Sclerosis Complex

The expert group recommended, with consensus, to offer a genetic test at baseline to patients with definite or probable TSC. The group noted that a genetic test may clarify the diagnosis of TSC for patients that do not fulfill clinical criteria for the condition (Amin et al., 2018).

In 2019, the group released recommendations for provision of coordinated care. They state, "with wider availability of genetic testing, identification of pathogenic mutations in TSC1 or TSC2 is now sufficient to establish a diagnosis, regardless of the presence of clinical features, and is particularly useful in confirming a suspected diagnosis, as many clinical TSC manifestations are infrequent in young patients." The group further recommends that:

- For patients with newly diagnoses or suspected TSC:
  - "Obtain three-generation family history to assess for additional family members at risk of TSC.
  - Offer genetic testing for family counseling or when TSC diagnosis is in question but cannot be clinically confirmed."
- For patients already diagnosed with definite or possible TSC:

- "Offer genetic testing for family counseling or when TSC diagnosis is in question but cannot be clinically confirmed" (Annear et al., 2019)

Genetic Testing Advisory Committee (GTAC) for Ontario, Canada

These guidelines recommend the following as indications for genetic testing:

- "When the clinical features (age of onset, seizure semiology and EEG features) are consistent with a distinct epilepsy syndrome as defined by the International League Against Epilepsy (ILAE), with the exception of syndromes outlined in the following section."
- "When the prognosis based on clinical and EEG findings is poor or the likelihood of lethal outcome is high."
- "When epileptic seizures are refractory to medical treatment as defined by the ILAE12 (with no apparent acquired cause)."
- "When epilepsy is associated with features suggestive of inborn errors of metabolism."
- "When epilepsy is associated with distinctive patterns of malformations of cortical development identified on neuroimaging studies."
- "When epilepsy is associated with clinical signs of neurodegeneration."
- "When epilepsy is associated with paroxysmal neurological features such as paroxysmal dyskinesias, episodic ataxias and hemiplegic migraine."
- "When epilepsy is associated with additional syndromic features such as developmental delay/intellectual disability, multiple congenital anomalies and dysmorphic features."
- "When familial epilepsy is present, defined as at least two first- degree family members with related epilepsy syndromes, unless the epilepsy syndrome is benign."

The guidelines also recommend against genetic testing in the following situations:

- "Recognizable seizure syndrome with benign course."
- "Childhood epilepsy with centro-temporal spikes (previously termed benign rolandic epilepsy)."
- "Isolated mesial temporal lobe epilepsy with hippocampal sclerosis."
- "Typical childhood Absence epilepsy (although if it is early-onset or medically refractory epilepsy, one should consider and test for GLUT1 deficiency)."
- "Juvenile myoclonic epilepsy, which is well controlled on medications and without intellectual disability or any signs of neurodegeneration."
- "Acquired epilepsy."

The guideline also provides a list of "potentially treatable" genetic/metabolic

epilepsies, shown below

Table 3: Potentially treatable genetic/metabolic epilepsies

Effect on ion channels
Dravet syndrome (*SCN1A*)—avoidance of sodium-channel blockers
SCN8A-related epileptic encephalopathy—carbamazepine, phenytoin [sodium-channel blockers]
KCNQ2-related epileptic encephalopathy—retigabine [potassium-channel openers] or carbamazepine [sodium-channel blockers]
KCNT1-related migrating partial epilepsy of infancy—quinidine [gain-of-function mutations being treated by partial channel antagonist]
GRIN2A-related epileptic encephalopathy—memantine [NMDA (N-methyl-d-aspartate) receptor antagonists]
Alternative energy source
GLUT-1 deficiency syndrome (*SLC2A1* gene)—ketogenic diet
Modulate epileptogenesis
Tuberous sclerosis complex (*TSC1/2*)—mTOR inhibitors (Rapamycin/analogs)
Modulating biochemical pathways
Pyridoxine dependency (*ALDH7A1*)—B6 vitamin
Biotinidase deficiency (*BTD*)—biotin
Cerebral folate deficiency (*FOLR1*)—folinic acid
Creatine deficiency syndromes (*SLC6A8, GATM, GAMT*)—creatine, other amino acid supplementation (glycine, arginine, ornithine) or restriction (arginine)
Serine biosynthesis defects (*PHGDH, PSAT1, PSPH*)—serine, glycine

Finally, the guideline also makes recommendations for panel testing. Some situations in which a gene panel may be considered are if:

- epilepsy phenotyping is broad and does not easily classify the patient into a distinct group.
- "if the clinical diagnosis is clear and genetic heterogeneity is high, but clinical diagnosis is not indicative of a treatable condition."
- "if clinical diagnosis is unclear and genetic heterogeneity is unknown."

The guidelines further list examples of these situations, such as seizures with fever as a major trigger and idiopathic generalized epilepsy refractory to treatment (Jain et al., 2019).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by

the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	CPT Description
81404	Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons)
81407	Molecular pathology procedure, Level 8 (eg, analysis of 26-50 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of >50 exons, sequence analysis of multiple genes on one platform)
81419	Epilepsy genomic sequence analysis panel, must include analyses for ALDH7A1, CACNA1A, CDKL5, CHD2, GABRG2, GRIN2A, KCNQ2, MECP2, PCDH19, POLG, PRRT2, SCN1A, SCN1B, SCN2A, SCN8A, SLC2A1, SLC9A6, STXBP1, SYNGAP1, TCF4, TPP1, TSC1, TSC2, and ZEB2
81479	Unlisted molecular pathology procedure

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Amin, S., Kingswood, J. C., Bolton, P. F., Elmslie, F., Gale, D. P., Harland, C., Johnson, S. R., Parker, A., Sampson, J. R., Smeaton, M., Wright, I., & O'Callaghan, F. J. (2018). The UK guidelines for management and surveillance of Tuberous Sclerosis Complex. *QJM: An International Journal of Medicine*, *112*(3), 171-182. https://doi.org/10.1093/qjmed/hcy215

Andrade, D., & Nascimento, F. (2023, September 29). *Dravet syndrome: Genetics, clinical features, and diagnosis*. https://www.uptodate.com/contents/dravet-syndrome-genetics-clinical-features-and-diagnosis

Annear, N. M. P., Appleton, R. E., Bassi, Z., Bhatt, R., Bolton, P. F., Crawford, P., Crowe, A., Tossi, M., Elmslie, F., Finlay, E., Gale, D. P., Henderson, A., Jones, E. A., Johnson, S. R.,

Joss, S., Kerecuk, L., Lipkin, G., Morrison, P. J., O'Callaghan, F. J., . . . Kingswood, J. C. (2019). Tuberous Sclerosis Complex (TSC): Expert Recommendations for Provision of Coordinated Care. *Front Neurol*, *10*, 1116. https://doi.org/10.3389/fneur.2019.01116

Balestrini, S., & Sisodiya, S. M. (2018). Pharmacogenomics in epilepsy. *Neurosci Lett*, *667*, 27-39. https://doi.org/10.1016/j.neulet.2017.01.014

Barbosa, M., Joshi, R. S., Garg, P., Martin-Trujillo, A., Patel, N., Jadhav, B., Watson, C. T., Gibson, W., Chetnik, K., Tessereau, C., Mei, H., De Rubeis, S., Reichert, J., Lopes, F., Vissers, L., Kleefstra, T., Grice, D. E., Edelmann, L., Soares, G., . . . Sharp, A. J. (2018). Identification of rare de novo epigenetic variations in congenital disorders. *Nat Commun*, *9*(1), 2064. https://doi.org/10.1038/s41467-018-04540-x

Berg, A. T., Berkovic, S. F., Brodie, M. J., Buchhalter, J., Cross, J. H., van Emde Boas, W., Engel, J., French, J., Glauser, T. A., Mathern, G. W., Moshe, S. L., Nordli, D., Plouin, P., & Scheffer, I. E. (2010). Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. *Epilepsia*, *51*(4), 676-685. https://doi.org/10.1111/j.1528-1167.2010.02522.x

Berg, A. T., Coryell, J., Saneto, R. P., Grinspan, Z. M., Alexander, J. J., Kekis, M., Sullivan, J. E., Wirrell, E. C., Shellhaas, R. A., Mytinger, J. R., Gaillard, W. D., Kossoff, E. H., Valencia, I., Knupp, K. G., Wusthoff, C., Keator, C., Dobyns, W. B., Ryan, N., Loddenkemper, T., . . . Koh, S. (2017). Early-Life Epilepsies and the Emerging Role of Genetic Testing. *JAMA Pediatr*, *171*(9), 863-871. https://doi.org/10.1001/jamapediatrics.2017.1743

Borlot, F., de Almeida, B. I., Combe, S. L., Andrade, D. M., Filloux, F. M., & Myers, K. A. (2019). Clinical utility of multigene panel testing in adults with epilepsy and intellectual disability. *Epilepsia*, *60*(8), 1661-1669. https://doi.org/10.1111/epi.16273

Burgunder, J. M., Finsterer, J., Szolnoki, Z., Fontaine, B., Baets, J., Van Broeckhoven, C., Di Donato, S., De Jonghe, P., Lynch, T., Mariotti, C., Schöls, L., Spinazzola, A., Tabrizi, S. J., Tallaksen, C., Zeviani, M., Harbo, H. F., & Gasser, T. (2010). EFNS guidelines on the molecular diagnosis of channelopathies, epilepsies, migraine, stroke, and dementias. *European Journal of Neurology*, *17*(5), 641-648. https://doi.org/10.1111/j.1468-1331.2010.02985.x

CDC. (2020). Epilepsy Data and Statistics. https://www.cdc.gov/epilepsy/data/index.html

Dunn, P., Albury, C. L., Maksemous, N., Benton, M. C., Sutherland, H. G., Smith, R. A., Haupt, L. M., & Griffiths, L. R. (2018). Next Generation Sequencing Methods for Diagnosis of Epilepsy Syndromes. *Front Genet*, *9*, 20. https://doi.org/10.3389/fgene.2018.00020

Falsaperla, R., & Corsello, G. (2017). Pyridoxine dependent epilepsies: new therapeutical point of view. *Ital J Pediatr*, *43*(1), 68. https://doi.org/10.1186/s13052-017-0387-3

Hebbar, M., & Mefford, H. C. (2020). Recent advances in epilepsy genomics and genetic testing. *F1000Res*, *9*. https://doi.org/10.12688/f1000research.21366.1

Heron, S. E., Regan, B. M., Harris, R. V., Gardner, A. E., Coleman, M. J., Bennett, M. F., Grinton, B. E., Helbig, K. L., Sperling, M. R., Haut, S., Geller, E. B., Widdess-Walsh, P., Pelekanos, J. T., Bahlo, M., Petrovski, S., Heinzen, E. L., Hildebrand, M. S., Corbett, M. A., Scheffer, I. E., . . . Berkovic, S. F. (2021). Association of SLC32A1 Missense Variants With Genetic Epilepsy With Febrile Seizures Plus. *Neurology*, *96*(18), e2251-e2260. https://doi.org/10.1212/wnl.0000000000011855

Hildebrand, M. S., Dahl, H. H., Damiano, J. A., Smith, R. J., Scheffer, I. E., & Berkovic, S. F. (2013). Recent advances in the molecular genetics of epilepsy. *J Med Genet*, *50*(5), 271-279. https://doi.org/10.1136/jmedgenet-2012-101448

Hirsch, E., French, J., Scheffer, I. E., Bogacz, A., Alsaadi, T., Sperling, M. R., Abdulla, F., Zuberi, S. M., Trinka, E., Specchio, N., Somerville, E., Samia, P., Riney, K., Nabbout, R., Jain, S., Wilmshurst, J. M., Auvin, S., Wiebe, S., Perucca, E., . . . Wirrell, E. C. (2022). ILAE definition of the Idiopathic Generalized Epilepsy Syndromes: Position statement by the ILAE Task Force on Nosology and Definitions. *Epilepsia*, *63*(6), 1475-1499. https://doi.org/https://doi.org/10.1111/epi.17236

Hrabik, S. A., Standridge, S. M., Greiner, H. M., Neilson, D. E., Pilipenko, V. V., Zimmerman, S. L., Connor, J. A., & Spaeth, C. G. (2015). The Clinical Utility of a Single-Nucleotide Polymorphism Microarray in Patients With Epilepsy at a Tertiary Medical Center. *J Child Neurol*, *30*(13), 1770-1777. https://doi.org/10.1177/0883073815579972

Jain, P., Andrade, D., Donner, E., Dyment, D., Prasad, A. N., Goobie, S., Boycott, K., Lines, M., & Snead, O. C. (2019). Development of Criteria for Epilepsy Genetic Testing in Ontario, Canada. *Can J Neurol Sci*, *46*(1), 7-13. https://doi.org/10.1017/cjn.2018.341

Kim, S. Y., Jang, S. S., Kim, H., Hwang, H., Choi, J. E., Chae, J.-H., Kim, K. J., & Lim, B. C. (2021). Genetic diagnosis of infantile-onset epilepsy in the clinic: Application of whole-exome sequencing following epilepsy gene panel testing. *Clinical Genetics*, *99*(3), 418-424. https://doi.org/https://doi.org/10.1111/cge.13903

Klepper, J., Akman, C., Armeno, M., Auvin, S., Cervenka, M., Cross, H. J., De Giorgis, V., Della Marina, A., Engelstad, K., Heussinger, N., Kossoff, E. H., Leen, W. G., Leiendecker, B., Monani, U. R., Oguni, H., Neal, E., Pascual, J. M., Pearson, T. S., Pons, R., . . . De Vivo, D. C. (2020). Glut1 Deficiency Syndrome (Glut1DS): State of the art in 2020 and recommendations of the international Glut1DS study group. *Epilepsia Open*, *5*(3), 354-365. https://doi.org/10.1002/epi4.12414

Lemke, J. R., Riesch, E., Scheurenbrand, T., Schubach, M., Wilhelm, C., Steiner, I., Hansen, J., Courage, C., Gallati, S., Burki, S., Strozzi, S., Simonetti, B. G., Grunt, S., Steinlin, M., Alber, M., Wolff, M., Klopstock, T., Prott, E. C., Lorenz, R., . . . Biskup, S. (2012). Targeted next generation sequencing as a diagnostic tool in epileptic disorders. *Epilepsia*, *53*(8), 1387-1398. https://doi.org/10.1111/j.1528-1167.2012.03516.x

Long, S., Zhou, H., Li, S., Wang, T., Ma, Y., Li, C., Zhou, Y., Zhou, S., Wu, B., & Wang, Y. (2019). The Clinical and Genetic Features of Co-occurring Epilepsy and Autism

Spectrum Disorder in Chinese Children. *Front Neurol*, *10*, 505. https://doi.org/10.3389/fneur.2019.00505
McKnight, D., Bristow, S. L., Truty, R. M., Morales, A., Stetler, M., Westbrook, M. J., Robinson, K., Riethmaier, D., Borlot, F., & Kellogg, M. (2022). Multigene panel testing in a large cohort of adults with epilepsy: diagnostic yield and clinically actionable genetic findings. *Neurology Genetics*, *8*(1).
Mefford, H. C. (2015). Clinical Genetic Testing in Epilepsy. *Epilepsy Curr*, *15*(4), 197-201. https://doi.org/10.5698/1535-7511-15.4.197
Moller, R. S., Larsen, L. H., Johannesen, K. M., Talvik, I., Talvik, T., Vaher, U., Miranda, M. J., Farooq, M., Nielsen, J. E., Svendsen, L. L., Kjelgaard, D. B., Linnet, K. M., Hao, Q., Uldall, P., Frangu, M., Tommerup, N., Baig, S. M., Abdullah, U., Born, A. P., . . . Dahl, H. A. (2016). Gene Panel Testing in Epileptic Encephalopathies and Familial Epilepsies. *Mol Syndromol*, *7*(4), 210-219. https://doi.org/10.1159/000448369
Myers, C. T., & Mefford, H. C. (2015). Advancing epilepsy genetics in the genomic era. *Genome Med*, *7*, 91. https://doi.org/10.1186/s13073-015-0214-7
NICE. (2022, April 27). *Epilepsies in children, young people and adults*. https://www.nice.org.uk/guidance/ng217
Norman, M., Wilson, S. J., Baxendale, S., Barr, W., Block, C., Busch, R. M., Fernandez, A., Hessen, E., Loring, D. W., McDonald, C. R., & Hermann, B. P. (2021). Addressing neuropsychological diagnostics in adults with epilepsy: Introducing the International Classification of Cognitive Disorders in Epilepsy: The IC CODE Initiative. *Epilepsia Open*, *6*(2), 266-275. https://doi.org/10.1002/epi4.12478
Olson, H., Shen, Y., Avallone, J., Sheidley, B. R., Pinsky, R., Bergin, A. M., Berry, G. T., Duffy, F. H., Eksioglu, Y., Harris, D. J., Hisama, F. M., Ho, E., Irons, M., Jacobsen, C. M., James, P., Kothare, S., Khwaja, O., Lipton, J., Loddenkemper, T., . . . Poduri, A. (2014). Copy number variation plays an important role in clinical epilepsy. *Ann Neurol*, *75*(6), 943-958. https://doi.org/10.1002/ana.24178
Ottman, R., Hirose, S., Jain, S., Lerche, H., Lopes-Cendes, I., Noebels, J. L., Serratosa, J., Zara, F., & Scheffer, I. E. (2010). Genetic testing in the epilepsies--report of the ILAE Genetics Commission. *Epilepsia*, *51*(4), 655-670. https://doi.org/10.1111/j.1528-1167.2009.02429.x
Peng, J., Zhou, Y., & Wang, K. (2021). Multiplex gene and phenotype network to characterize shared genetic pathways of epilepsy and autism. *Sci Rep*, *11*(1), 952. https://doi.org/10.1038/s41598-020-78654-y
Poduri, A. (2017). When Should Genetic Testing Be Performed in Epilepsy Patients? *Epilepsy Curr*, *17*(1), 16-22. https://doi.org/10.5698/1535-7511-17.1.16
Poduri, A., Sheidley, B. R., Shostak, S., & Ottman, R. (2014). Genetic testing in the epilepsies —developments and dilemmas. *Nat Rev Neurol*, *10*(5), 293-299. https://doi.org/10.1038/nrneurol.2014.60

Randle, S. (2023, December 6). *Tuberous sclerosis complex: Genetics and pathogenesis*. https://www.uptodate.com/contents/tuberous-sclerosis-complex-genetics-and-pathogenesis

Randle, S. (2024a, January 8). *Tuberous sclerosis complex: Clinical features*. https://www.uptodate.com/contents/tuberous-sclerosis-complex-clinical-features

Randle, S. (2024b, February 29). *Tuberous sclerosis complex: Evaluation and diagnosis*. https://www.uptodate.com/contents/tuberous-sclerosis-complex-evaluation-and-diagnosis

Riviello, J. J., Ashwal, S., Hirtz, D., Glauser, T., Ballaban-Gil, K., Kelley, K., Morton, L. D., Phillips, S., Sloan, E., & Shinnar, S. (2006). Practice Parameter: Diagnostic assessment of the child with status epilepticus (an evidence-based review). *Neurology*, *67*(9), 1542. https://doi.org/10.1212/01.wnl.0000243197.05519.3d

Sanchez Fernandez, I., Loddenkemper, T., Gainza-Lein, M., Sheidley, B. R., & Poduri, A. (2019). Diagnostic yield of genetic tests in epilepsy: A meta-analysis and cost-effectiveness study. *Neurology*. https://doi.org/10.1212/wnl.0000000000006850

Scheffer, I. E., Berkovic, S., Capovilla, G., Connolly, M. B., French, J., Guilhoto, L., Hirsch, E., Jain, S., Mathern, G. W., Moshé, S. L., Nordli, D. R., Perucca, E., Tomson, T., Wiebe, S., Zhang, Y.-H., & Zuberi, S. M. (2017). ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. *Epilepsia*, *58*(4), 512-521. https://doi.org/10.1111/epi.13709

Sheidley, B. R., Malinowski, J., Bergner, A. L., Bier, L., Gloss, D. S., Mu, W., Mulhern, M. M., Partack, E. J., & Poduri, A. (2021). Genetic testing for the epilepsies: A systematic review. *Epilepsia*. https://doi.org/10.1111/epi.17141

SIGN. (2018). *Diagnosis and management of epilepsy in adults*. https://www.sign.ac.uk/media/1079/sign143_2018.pdf

Stefanski, A., Calle-López, Y., Leu, C., Pérez-Palma, E., Pestana-Knight, E., & Lal, D. (2021). Clinical sequencing yield in epilepsy, autism spectrum disorder, and intellectual disability: A systematic review and meta-analysis. *Epilepsia*, *62*(1), 143-151. https://doi.org/10.1111/epi.16755

Stenhouse, S. A., Ellis, R., & Zuberi, S. (2013). SCN1A Genetic Test for Dravet Syndrome (Severe Myoclonic Epilepsy of Infancy and its Clinical Subtypes) for use in the Diagnosis, Prognosis, Treatment and Management of Dravet Syndrome. *PLoS Curr*, *5*. https://doi.org/10.1371/currents.eogt.c553b83d745dd79bfb61eaf35e522b0b

Stosser, M. B., Lindy, A. S., Butler, E., Retterer, K., Piccirillo-Stosser, C. M., Richard, G., & McKnight, D. A. (2017). High frequency of mosaic pathogenic variants in genes causing epilepsy-related neurodevelopmental disorders. *Genet Med*. https://doi.org/10.1038/gim.2017.114

Williams, C. A., & Battaglia, A. (2013). Molecular biology of epilepsy genes. *Exp Neurol*, *244*, 51-58. https://doi.org/10.1016/j.expneurol.2011.12.001

Willimsky, E. K., Munzig, A., Mayer, K., Biskup, S., Abicht, A., Hoertnagel, K., Voss, H. V., Klein, H. G., Rost, I., Larsen, L. H. G., Dahl, H. A., Hoelz, H., Stuelpnagel, C. V., & Borggraefe, I. (2021). Next Generation Sequencing in Pediatric Epilepsy Using Customized Panels: Size Matters. *Neuropediatrics*, *52*(2), 92-97. https://doi.org/10.1055/s-0040-1712488

Wilmshurst, J. M., Gaillard, W. D., Vinayan, K. P., Tsuchida, T. N., Plouin, P., Van Bogaert, P., Carrizosa, J., Elia, M., Craiu, D., Jovic, N. J., Nordli, D., Hirtz, D., Wong, V., Glauser, T., Mizrahi, E. M., & Cross, J. H. (2015). Summary of recommendations for the management of infantile seizures: Task Force Report for the ILAE Commission of Pediatrics. *Epilepsia*, *56*(8), 1185-1197. https://doi.org/10.1111/epi.13057

Wirrell, E. C., Laux, L., Donner, E., Jette, N., Knupp, K., Meskis, M. A., Miller, I., Sullivan, J., Welborn, M., & Berg, A. T. (2017). Optimizing the Diagnosis and Management of Dravet Syndrome: Recommendations From a North American Consensus Panel. *Pediatr Neurol*, *68*, 18-34.e13. https://doi.org/10.1016/j.pediatrneurol.2017.01.025

Zuberi, S. M., Wirrell, E., Yozawitz, E., Wilmshurst, J. M., Specchio, N., Riney, K., Pressler, R., Auvin, S., Samia, P., Hirsch, E., Galicchio, S., Triki, C., Snead, O. C., Wiebe, S., Cross, J. H., Tinuper, P., Scheffer, I. E., Perucca, E., Moshé, S. L., & Nabbout, R. (2022). ILAE classification and definition of epilepsy syndromes with onset in neonates and infants: Position statement by the ILAE Task Force on Nosology and Definitions. *Epilepsia*, *63*(6), 1349-1397. https://doi.org/10.1111/epi.17239

Genetic Testing for Familial Alzheimer Disease

Policy Number: AHS – M2038 – Genetic Testing for Familial Alzheimer Disease	Prior Policy Name and Number, as applicable: • AHS – M2038 – Genetic Testing for Familial Alzheimer's Disease
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Alzheimer disease (AD) is a neurodegenerative disease defined by a gradual decline in memory, cognitive functions, gross atrophy of the brain, and accumulation of extracellular amyloid plaques and intracellular neurofibrillary tangles (Karch et al., 2014).

Familial Alzheimer disease (FAD) is a rare, inherited form of AD. FAD has an earlier onset than other forms of Alzheimer disease with symptoms developing in individuals in their thirties or forties. Genetic counseling is strongly recommended for individuals pursuing genetic testing for FAD.

For recommendations on testing of *APOE* ε4 prior to medication administration, please refer to AHS-M2021-Pharmacogenetic Testing.

II. Related Policies

Policy Number	Policy Title
AHS-M2021	Pharmacogenetic Testing
AHS-M2145	General Genetic Testing, Germline Disorders
AHS-M2146	General Genetic Testing, Somatic Disorders

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) When the results of the testing will inform reproductive decision making, genetic testing for *APP, PSEN1,* and *PSEN2* **MEETS COVERAGE CRITERIA** for individuals with **one** of the following conditions:
   a) For individuals with a family history of autosomal dominant dementia (one or more instances of early-onset Alzheimer disease [AD] [see Note 1]).
   b) For individuals with a first-degree (see Note 2) relative with a known mutation in the *PSEN1, PSEN2,* or *APP* genes.
   c) For symptomatic individuals (suspected early-onset AD [see Note 1]) with an unknown family history (e.g., adoption).

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

2) Outside of situations addressed in AHS-M2021-Pharmacogenetic Testing, testing of *APOE* **DOES NOT MEET COVERAGE CRITERIA.**

3) For all purposes other than reproductive decision making, genetic testing for AD **DOES NOT MEET COVERAGE CRITERIA**, including **any** of the following situations:
   a) Testing to confirm a diagnosis of Alzheimer disease (any type).
   b) Testing for familial Alzheimer disease in individuals less than 18 years of age.
   c) Testing of any other genes not listed above.
   d) Testing for purposes of Alzheimer disease risk assessment.
   e) Screening asymptomatic individuals.

NOTES:

Note 1: Early-onset Alzheimer disease is Alzheimer disease that developed before the individual was 65 years of age.

Note 2: First-degree relatives include parents, full siblings, and children of the individual.

Note 3: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
AAN	American Academy of Neurology
AAO	Age at onset
ACMG	American College of Medical Genetics and Genomics
AD	Alzheimer disease
AMP	Association for Molecular Pathology
APOE	*Apolipoprotein E*
APP	*Amyloid precursor protein*
CASP7	*Caspase-7*
CLIA'88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CSF	Cerebrospinal fluid
DTC	Direct to consumer
ECRG4	*Esophageal cancer-related gene 4*
EFNS	European Federation of Neurological Societies
ENS	European Neurological Society
EOAD	Early-onset autosomal dominant Alzheimer disease
EOFAD	Early onset familial Alzheimer disease mutations
FAD	Familial Alzheimer disease
FDA	Food And Drug Administration
FTLD	Frontotemporal lobar degeneration
GRN	*Granulin precursor*
GRS	Genetic risk score
GWAS	Genome-wide association studies
HDAC9	*Histone deacetylase 9*
LDT	Laboratory-developed test
LOAD	Late-onset Alzheimer disease
MAPT	*Microtubule-associated protein tau*
MPS	Massive parallel resequencing
NGS	Next-generation sequencing
NIA	National Institute on Aging
NSGC	National Society of Genetic Counselors
PGS	Personal genome service
PSEN1	*Presenilin 1*
PSEN2	*Presenilin 2*

USPSTF	United States Preventive Services Task Force

V. Scientific Background

Alzheimer disease (AD) is a devastating neurodegenerative disease with a strong genetic component and is considered the predominant form of dementia. There are more than 55 million people living with dementia worldwide (WHO, 2023).This number is estimated to increase to 131.5 million by 2050 (Prince, 2016). The average lifetime risk of developing AD is 10–12%. This risk at least doubles with the presence of a first-degree relative with the disorder (Goldman et al., 2011). The genetic predisposition of AD, even for late-onset AD patients, is estimated to be 60–80% (Gatz et al., 2006).

Most patients develop clinical symptoms after the age of 65 (spontaneous or late-onset AD); however, up to 10% of patients have an earlier onset of disease (early-onset AD) (Kumar, 2018). AD is characterized by severe neuronal loss, aggregation of extracellular amyloid β plaques, and intraneuronal tau protein tangles resulting in progressive deterioration of memory and cognitive functions (Keene, 2023). Enormous burden on public health is due to the high costs associated with care and treatment. Aside from drugs that temporarily relieve symptoms, no treatment currently exists for AD (Van Cauwenberghe et al., 2016).

Autosomal dominant AD is rare (<1%), but the discovery of fully penetrant pathogenic mutations of *Amyloid precursor protein (APP)* (Goate et al., 1991; St George-Hyslop et al., 1987), *Presenilin 1 (PSEN1)* (Sherrington et al., 1995; Van Broeckhoven et al., 1992), and *Presenilin 2 (PSEN2)* (Sherrington et al., 1996), inherited in an autosomal dominant fashion, has identified molecular mechanisms and pathways involved in AD pathogenesis and valuable targets currently used in diagnosis and drug development (Schneider et al., 2014; Van Cauwenberghe et al., 2016).

One of the primary features of AD is the buildup of amyloid-β protein in the brain. This protein is poisonous to neurons and is normally cleaved by secretases. However, certain genetic mutations may cause these clearing mechanisms to weaken, leading to an overall increase in amyloid-β production. As amyloid-β starts to aggregate in the brain, it creates fibrils that ultimately cause neurological damage such as the characteristic dementia (Keene, 2023).

Amyloid precursor protein (APP) is proteolytically processed in the constitutive pathway by α- and γ-secretases, resulting in nonpathogenic fragments. However, in the amyloidogenic pathway, subsequent proteolysis of APP by β-secretase and γ-secretase gives rise to a mixture of Aβ peptides with different lengths, of which $A\beta_{1-42}$ are more aggregation-prone and are predominantly present in amyloid plaques in

brains of AD patients. A total of 39 *APP* mutations have been described; all of which affect proteolysis of APP in favor of $A\beta_{1–42}$ (Cruts et al., 2012).

Presenilin 1 (PSEN1) and *Presenilin 2 (PSEN2)* are highly homologous genes. Both proteins encoded by these genes are essential components of the γ-secretase complex, which catalyzes the cleavage of membrane proteins, including APP. Mutations in *PSEN1* and *PSEN2* impair the γ-secretase-mediated cleavage of APP, resulting in an increased proportion of $A\beta_{1–42}$ (Cruts & Van Broeckhoven, 1998) (Cruts & Van Broeckhoven, 1998). *PSEN1* is located on chromosome 14 whereas *PSEN2* is located on chromosome 1. However, *PSEN1* is generally associated with a worse prognosis; it has full penetrance compared to 95% penetrance for *PSEN2*, and age of onset was over 10 years earlier for *PSEN1* mutations compared to *PSEN2* (Ryman et al., 2014; Sherva & Kowall, 2023).

Late-onset AD is considered a multifactorial with a strong but complex genetic predisposition (Gatz et al., 2006) involving gene mutations and polymorphisms that may interact with each other or with environmental factors. The ε4 allele of *APOE* was the only major gene known to increase disease risk for both early-onset and late-onset AD. More recently, genome-wide association studies (GWAS) and massive parallel resequencing (MPS) efforts have identified of at least 21 additional genetic risk loci. These loci, shown in the table below from Van Cauwenberghe et al. (2016), are estimated to explain about 28% of the heritability of liability, 30% of familial risk, and over 50% of sibling recurrence risk of developing AD (Van Cauwenberghe et al., 2016). Researchers have recently identified a rare missense variant in the *CASP7* gene that

Gene	Genes in locus	Possible candidate genes	Function	Pathway	Effect on APP or tau
MS4A4A/MS4A6E locus (chr11:59,268,00-60,480,00)	17 genes	*MS4A2, MS4A3, MS4A4A, MS4A4E, MS4A6A, MS4A6E*	Signal transduction	Immune response	—
HLA-DRB5/HLA-DRB1 locus (chr6:3,609,009-4,535,100)	17 genes	Not defined due to the complex genetic organization of the locus	Immunocompetence and histocompatibility	Immune response	—
ZCWPW1 locus (chr7:99,905, 955-100,093,149)	10 genes	*ZCWPW1*; *NYAP1*: affecting brain size, neurite elongation, neuronal morphogenesis	Epistatic regulation (*ZCWPW1*); brain and neural development (*NYAP1*)	Neural development	—
SLC24A4/RIN3 locus (chr14:92,789,411-93,176,224)	2 genes	*SLC24A4*: brain expression; *RIN3*: known interactor of *BIN1* gene product	Neural development and regulation of blood pressure and hypertension	Neural development and synapse function	—
NME8 locus (chr7:37,779, 803-37,992,860)	4 genes	*NME8*: association signal adjacent to the gene	Ciliary functions	Cytoskeletal function and axonal transport	—
CELF1 locus (chr11:47,291, 161-47,666,021)	10 genes	*CELF1; MADD*: long-term neuronal viability in AD	RNA splicing, editing, and translation (*CELF1*); long-term neuronal viability (*MADD*)	Cytoskeletal function and axonal transport	Tau toxicity

For each locus, the number of genes in each locus is shown with the possible candidate genes. The pathway, function, and effect on APP or tau pathway are reported for each locus.

APP, amyloid precursor protein; GWAS, genome-wide association studies.

may be associated with familial late-onset AD (Zhang et al., 2019), as well as a T allele of the CD33 rs3865444 polymorphism also associated with late-onset AD (Mehdizadeh et al., 2019).

The *APOE* gene has several alleles, with the ε4 allele contributing to an increased risk of late-onset AD and the ε2 allele contributing to a decreased risk of late-onset AD compared to the common *APOE* ε3 allele (Yamazaki et al., 2019). Researchers now report that *APOE* influences tau pathology as well as neurodegeneration mediated by tau and microglial responses to AD pathologies; further, *APOE* ε4 is "either pathogenic or shows reduced efficiency in multiple brain homeostatic pathways, including lipid transport, synaptic integrity and plasticity, glucose metabolism and cerebrovascular function" (Yamazaki et al., 2019).

In 2023, the FDA approved Lecanemab (brand name Leqembi), an amyloid beta-directed antibody, for the treatment of Alzheimer Disease in adult patients (FDA, 2023). One potential side effect of Leqembi is amyloid related imaging abnormalities (ARIA), which may be more likely to occur in people who are homozygous *APOE* ε4 carriers (Leqembi, 2024). The FDA cautions that "the prescribing information states that testing for ApoE ε4 status should be performed before starting treatment with Leqembi to inform the risk of developing ARIA" (FDA, 2023).

Proprietary Testing

Early-Onset Alzheimer Disease

Several companies have developed hereditary AD panels. The Invitae Hereditary Alzheimer Disease Panel tests for three genes associated with early-onset hereditary AD: *APP, PSEN1* and *PSEN2*. This test may utilize a blood, DNA or saliva sample and has a 10-21 day turnaround time (Invitae, 2023). The ADmark® Early Onset Alzheimer's Evaluation also tests for the three known early-onset hereditary AD genes: *APP, PSEN1* and *PSEN2*. This test detects sequence variants in these genes, as well as duplications in the *APP* gene. A whole blood sample is required, and a turnaround time of 21-28 days can be expected (Athena, 2023b).

Another panel by Fulgent, termed the Parkinson-Alzheimer-Dementia NGS panel, tests for 35 genes that are associated with developing Parkinson disease, Alzheimer disease and dementia . Some of the genes tested in this panel include *APOE, APP, PSEN1* and *PSEN2*. This test also requires a blood sample or buccal swab and has a three to five week turnaround time (Fulgent, 2023).

Roche has developed the Elecsys® Amyloid Plasma Panel, which has received FDA Breakthrough Device Designation. According to Roche, it is the first qualitative test that combines the result of phosphorylated Tau (pTau) 181 protein assay and APOE ε4 in human plasma. This can leader to earlier diagnosis of Alzheimer's disease as patients "testing negative with the Elecsys Amyloid Plasma Panel are unlikely to be amyloid

positive and should be investigated for other causes of cognitive decline" (Roche, 2024).

Late-Onset Alzheimer Disease

Athena diagnostics developed the ADmark® ApoE Genotype Analysis and Interpretation test which detects *APOE* ε2, ε3, ε4 alleles using restriction fragment length polymorphism (Athena, 2023a). Athena will not perform this test on individuals younger than 18 years of age and recommends pre and post-test genetic counseling; a whole blood sample is required, and a turnaround time of 7-14 days can be expected.

Clinical Utility and Validity

Early-Onset Alzheimer Disease

Comprehensive genetic counseling protocols are available for AD diagnostic and predictive testing to provide a framework for clinicians and geneticists to evaluate which patients may benefit from genetic testing. Available genetic diagnostic and predictive screening for causal mutations of early-onset AD in *APP*, *PSEN1*, and *PSEN2* are only responsible for a small portion of AD patients' risk. They account for approximately 60%-70% of familial autosomal dominant AD, but less than 10 percent of early-onset AD and less than one percent of AD overall (Sherva & Kowall, 2023). For a significant number of patients for whom genetic diagnostic screening is requested, the tests will be negative without excluding a genetic cause of disease (Van Cauwenberghe et al., 2016). Furthermore, the identification of a mutation is not a certain predictor of disease or onset age, given that these mutations can vary in terms of penetrance and gene expression. Nevertheless, the ability to identify an explanation for the clustering of AD in a family and the ability to use this toward predictive testing in subsequent generations provide an important step toward autonomy of patients and at-risk individuals (Van Cauwenberghe et al., 2016). Testing for these highly penetrant mutations often carries significant personal and familial utility which the ACMG (American College of Medical Genetics) has recently supported as important clinical utilities (ACMG, 2015). New mutations in the *APP*, *PSEN1*, and *PSEN2* genes are constantly being identified. For example, two probable pathogenic variants, *PSEN2* p.A415S and p.M174I, were identified by Wong et al. (2020).

Janssen et al. (2003) aimed to determine the proportion of patients with early-onset AD with a positive family history that had mutations in the amyloid precursor protein (APP), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*) genes. A mutational analysis was performed in 31 probands with probable or definite AD from UK families (age at onset <61 years). A total of 23 patients fulfilled criteria for autosomal dominant inheritance. In 17 (55%) probands the authors identified eight novel *PSEN1* sequence variants and

eight recognized pathogenic mutations. In four (13%) probands the authors identified one novel *APP* sequence variant (H677R) and two recognized mutations. Further, 21 of 31 (68%) probands were associated with a sequence variant in *APP* or *PSEN1*. Nine of the 11 (82%) probands with neuropathologically confirmed AD who additionally fulfilled recognized criteria for autosomal dominant inheritance were associated with a sequence variant in *APP* or *PSEN1*. The 10 patients in whom the authors were unable to identify a mutation in *APP*, *PSEN1*, or *PSEN2* were older than the probands with sequence variants (55.4 vs 44.7 years, respectively). The authors concluded that sequence variants in *APP* and *PSEN1* accounted for the majority of neuropathologically confirmed autosomal dominant early-onset AD (Janssen et al., 2003).

Shea et al. (2016) conducted a study to assess the differences in clinical presentations of different genotypes of FAD. A total of 658 pedigrees were evaluated. The authors found that patients with *PSEN1* mutations tended to have earlier age of onset than either *PSEN2* or *APP* mutations. Patients with *PSEN1* were also more commonly affected by symptoms such as seizures or myoclonus, whereas patients with *PSEN2* mutations were more commonly affected by disorientation. Patients with *APP* mutations were more likely to present with aggression or apraxia (Shea et al., 2016).

Lanoiselee et al. (2017) completed a large genetic screening study of familial and sporadic cases of *APP*, *PSEN1*, and *PSEN2* mutations in early-onset AD. Data was taken from 23 French hospitals from 1993 onward; the total number of families identified with mutations was 170 (these families were required to have two first-degree relatives with early-onset AD with an age of onset ≤65 years). One hundred and twenty-nine sporadic cases were also screened with an age of onset ≤51 years. The authors not that "*APP*, *PSEN1*, or *PSEN2* mutations were identified in 53 novel AD-EOAD [early-onset AD] families. Of the 129 sporadic cases screened, 17 carried a *PSEN1* mutation and one carried an *APP* duplication (13%); this led to the conclusion that a portion of *PSEN1* mutations occur de novo (Lanoiselee et al., 2017).

Giau et al. (2019) screened 67 *de novo* early-onset AD cases by next-generation sequencing (NGS) to identify pathogenic variants linked to neurodegenerative disorders in the Korean population. They were able to find three missense mutations in *PSEN1* and a variant in *PSEN2* within 6% of the cases with early onset AD, but also found "67 missense mutations in susceptibility genes for late-onset AD… which may be involved in cholesterol transport, inflammatory response, and β-amyloid modulation." They also found "70 additional novel and missense variants in other genes, such as *MAPT*, *GRN*, *CSF1R*, and *PRNP*, related to neurodegenerative diseases, which may represent overlapping clinical and neuropathological features with AD." Multiple rare variants were found among this patient population as well (Giau et al., 2019).

Qin et al. (2020) conducted an analysis on the genotype and phenotype correlation for early onset familial AD in a Chinese population. With respect to specific mutations, the researchers found that for *APP* mutations, the clinical phenotype was relatively heterogeneous, with an average age at onset ranging from the 40s-50s and clinical presentations of "cognitive dysfunction, especially executive dysfunction and disorientation. Extrapyramidal signs, behavioral, and psychiatric symptoms could also be detected in Chinese *APP* EOFAD [early onset familial Alzheimer disease mutations]." For those with *PSEN1* mutations, the age at onset was in the early 40s, and with an amnestic cognitive profile, as well as myoclonus and seizures. "Extrapyramidal signs, behavioral, and psychiatric symptoms (anxiety, hallucinations, delusions) and ataxia are significantly more frequently found in EOFAD with *PSEN1* mutations." *PSEN2* mutations were found to be the least common, and "compared to PSEN1 mutation carriers, carriers with PSEN2 mutations have a later AAO, relatively longer disease duration and a more variable disease expression." Overall, the researchers found that most of the mutations in China were novel in comparison to pathogenic variants among Caucasians (Qin et al., 2020).

Late-Onset Alzheimer Disease

The primary gene associated with late-onset AD is the apolipoprotein E (*APOE*) gene on chromosome 19, particularly its epsilon (ε) allele. This apolipoprotein is thought to play a role in cholesterol homeostasis and aid in removal of the amyloid- β protein that is at the core of AD. There are three isoforms of this allele: ε2, ε3, and ε4. The ε4 allele binds much more rapidly to the amyloid protein; however, it is less efficient than the other two alleles in protein transfer. These characteristics combined have made the ε4 allele a potential genetic risk factor of AD (Sherva & Kowall, 2023).

The role of genetics in diagnosis and risk prediction in late-onset AD is much less straightforward. *APOE* ε4 is associated with changes in lipid metabolomics in AD patients, and is likely a factor in even the early stages of AD development (Pena-Bautista et al., 2020). Further, it has been suggested that *APOE* ε4 is a selective risk factor, affecting memory-related AD manifestations of the disease more than language-related implications (Weintraub et al., 2020). Despite the established evidence of *APOE* ε4 as a risk factor for AD, its value in disease prediction in a clinical setting is limited, and the relevance of clinical testing for common genetic variations identified in GWAS is even more limited. Combining multiple susceptibility loci into a global genetic risk score (GRS) might improve the prediction of individuals at risk. However, the most comprehensive risk prediction model developed to date only achieved a sensitivity of 55% and a specificity of 78%, impeding use in clinical practice (de Calignon et al., 2012; Van Cauwenberghe et al., 2016).

Naj et al. (2014) assessed the effect of *APOE* alleles on average age of onset in AD patients. Fourteen studies containing 9,162 patients were examined, and the *APOE* allele was found to contribute 3.9% of the variation of age of onset. Each copy of the ε4 was found to reduce the age of onset by 2.45 years (Naj et al., 2014).

Cohn-Hokke et al. (2017) examined the social and personal effects of testing for hereditary neurodegenerative diseases from 74 patient survey responds. The authors concluded that "the result of predictive testing on adult-onset neurodegenerative diseases does not have a large negative effect on social and personal life, although these observations should be interpreted with caution because of the small number of participants and low response rate" (Cohn-Hokke et al., 2017).

The ancestral *APOE* ε4 risk of AD has been studied across Puerto Rican and African American populations. A total of 1,986 participants with late-onset AD (1,766 African Americans and 220 Puerto Ricans) and 3,899 healthy controls older than 65 years of age (3,730 African Americans and 169 Puerto Ricans) participated in this study. The authors note that "*APOE* ε4 alleles on an African background conferred a lower risk than those with a European ancestral background, regardless of population" (Rajabli et al., 2018). This study shows that the risk conferred by the *APOE* ε4 allele differs across populations; the cause of this risk is unknown but may be due to genetic variation, environmental factors, or cultural factors associated with ancestry. Stevenson-Hoare et al. (2023) studied the plasma biomarkers and genetics used for AD diagnosis. The study included 1439 people with AD, with a mean age of 68 years, and 508 controls, with a mean age of 82 years. The authors measured plasma concentrations of 40 and 42 amino acid-long amyloid β fragments, tau phosphorylated at amino acid 181, neurofilament light, and glial fibrillary acidic protein. "The prediction accuracy of Alzheimer's disease clinical diagnosis by the combination of all biomarkers, APOE and polygenic risk score reached area under receiver operating characteristic curve (AUC) = 0.81" The genetic risk was compared to age of onset and disease duration. "All biomarkers were significantly associated with age in cases and controls." The authors conclude that "biomarker-based diagnosis is not perfect because the biomarker measurements in older controls are similar to those in younger clinically diagnosed AD cases" but, "blood plasma biomarkers can only be a useful tool for the assessment and prediction of AD in the context of other genetic and/or clinical information" (Stevenson-Hoare et al., 2023).

There is an ethical concern for mental and personal wellbeing after testing for late-onset AD. Pavarini et al. (2021) interviewed 31 people aged 16 to 26 whose grandparents had late-onset AD. The interviews focused on the participant's moral attitudes and motivations towards online and direct-to-consumer genetic testing for late-onset AD. The authors found that participants agreed that "people should have

the right to access these services," but were concerned about "potential distress in response to learning about risk." Overall, face-to-face services were preferred over online. The authors suggest that these results highlight the importance of support, particularly to young people, before and after testing (Pavarini et al., 2021).

Pavarini et al. (2021) studied the psychological impact of genetic testing on healthy individuals at risk for AD. This study included individuals that underwent counseling and testing protocol. The authors performed a psychological assessment at zero, six and 12 months following genetic test results on 24 participants from 13 families. The authors found that carriers of pathogenic variants (*PSEN1, PSEN2, GRN*) showed higher scores than noncarriers on the Resilience Scale for Adults, social competence, and the Multidimensional Health Locus of Control. The authors conclude that "at-risk individuals undergoing predictive testing showed benefit on personal life and no detrimental impact on a broad range of psychological outcomes" (Galluzzi et al., 2022).

Largent et al. (2021) studied the ethical, social, and legal concerns regarding sharing AD genetic testing results. The authors predict that genetic testing for AD in people who are not yet cognitively impaired will become more common in clinical practice. The authors analyzed the decisions to share genetic information of participants in the A4 Study and the API Generation Program, two clinical trials that shared genetic results with the participants. Participants with no elevated amyloid or no APOE ε4 alleles did not pay close attention to the decision regarding sharing this information, and they felt it was good or no news. Participants with elevated amyloid or APOE ε4 alleles felt the information was sensitive and burdensome to decide if they should share or hide it from others. These individuals felt more worried about stigmatization and discrimination, even though they were not currently cognitively impaired. Additionally, close friends and family members of these individuals are more likely to take up a "pre-caregiver" role, potentially changing their relationship; "12% of participants at increased risk for dementia stated that they disclosed their result so that they could 'count on other people to tell me if I'm changing.'" The authors state that these results and concerns "highlight the need for additional legal protections and policy changes in anticipation of the coming transformation of AD clinical care" (Largent et al., 2021).

VI. Guidelines and Recommendations

American College of Medical Genetics and Genomics (ACMG) and National Society of Genetic Counselors (NSGC)

The American College of Medical Genetics and Genomics (ACMG) and the National Society of Genetic Counselors (NSGC) issued joint practice guidelines related to the genetic assessment of AD. These guidelines include the following recommendations (Goldman et al., 2011):

- "Pediatric testing for AD should not occur."
- "Prenatal testing for AD is not advised if the patient intends to continue a pregnancy with a mutation."
- "Genetic testing for AD should only occur in the context of genetic counseling (in-person or through videoconference) and support by someone with expertise in this area. Symptomatic patients: Genetic counseling for symptomatic patients should be performed in the presence of the individual's legal guardian or family member."
- "DTC (direct to consumer) *APOE* testing is not advised."
- "A risk assessment should be performed by pedigree analysis to determine whether the family history is consistent with EOAD [early-onset AD] or LOAD (late-onset AD) and with autosomal dominant (with or without complete penetrance), familial or sporadic inheritance."

For families in which an autosomal dominant AD gene mutation is a possibility:

- "Testing for genes associated with early-onset autosomal dominant AD should be offered in the following situations:
  - "A symptomatic individual with EOAD in the setting of a family history of dementia or in the setting of an unknown family history (e.g., adoption).
  - "Autosomal dominant family history of dementia with one or more cases of EOAD."
  - "A relative with a mutation consistent with EOAD (currently *PSEN1/2* or *APP*)."
- "Ideally, an affected family member should be tested first. If no affected family member is available for testing and an asymptomatic individual remains interested in testing despite counseling about the low likelihood of an informative result (a positive result for a pathogenic mutation), he/she should be counseled according to the recommended protocol. If the affected relative, or their next of kin, is uninterested in pursuing tested, the option of DNA banking should be discussed."

For families in which an autosomal dominant AD is unlikely:

- "Discuss that both sporadic and familial cases can be due to a genetic susceptibility. Risk estimates are only available for first-degree relatives of an affected individual in sporadic or familial cases."
- "Genetic testing for susceptibility loci (e.g., *APOE*) is not clinically recommended due to limited clinical utility and poor predictive value. If a patient wishes to pursue testing despite genetic counseling and recommendations to the contrary, testing may be considered at the clinician's discretion."

Finally, the authors comment that "in general, clear genotype-phenotype correlations cannot typically be made for the three causative genes, and age of onset can vary more than 20 years within the same family" (Goldman et al., 2011).

In 2019, an addendum was published for the 2011 guidelines. The ACMB board of directors reaffirmed these guidelines (as of June 25, 2018) with two changes:

- "To use the phrase "pathogenic variant" rather than the word "mutation" in discussing pathogenic variants related to autosomal dominant early-onset Alzheimer disease. This would be consistent with current ACMG/AMP Guidelines for Variant Interpretation and Reporting.
- Because this document no longer meets the criteria for an evidence-based practice guideline by either the American College of Medical Genetics and Genomics (ACMG) or National Society of Genetic Counselors (NSGC), NSGC reclassified this document as a Practice Resource in 2016, and ACMG is also classifying it as a Practice Resource as of this reaffirmation" (Goldman et al., 2019).

American College of Medical Genetics and Genomics (ACMG)

In the Choosing Wisely Initiative, the ACMG recommended "Don't order *APOE* genetic testing as a predictive test for Alzheimer's disease." The rationale for the recommendation is that "*APOE* is a susceptibility gene for later-onset Alzheimer disease (AD), the most common cause of dementia. The presence of an ε4 allele is neither necessary nor sufficient to cause AD. The relative risk conferred by the ε4 allele is confounded by the presence of other risk alleles, gender, environment and possibly ethnicity. *APOE* genotyping for AD risk prediction has limited clinical utility and poor predictive value" (ACMG, 2016).

American Academy of Neurology (AAN)

In 2001 (reaffirmed in 2004), AAN made the following recommendation on the use of genetic testing for Alzheimer's disease:

- Routine use of *APOE* genotyping in patients with suspected AD is not recommended at this time (Guideline).
- There are no other genetic markers recommended for routine use in the diagnosis of AD (Guideline) (Knopman et al., 2001).

National Institute on Aging (NIA)

In 2011, Alzheimer's Disease diagnostic guidelines were revised including latest research results and better scientific understanding of the disease. The development of

the new guidelines was led by the National Institute of Health and the Alzheimer's Association. Diagnostic criteria for Alzheimer's disease were re-defined. In respect to genetic testing, NIA issued following guidance and recommendations: "A rare type of familial Alzheimer's disease, called Early-Onset Alzheimer's Disease (EOAD), is caused by mutations in the amyloid precursor protein, presenilin 1, or presenilin 2 genes. A person who inherits any of these mutations from a parent will almost surely develop Alzheimer's dementia before age 65. Genetic testing for the disease is common in families with a history of EOAD"; "The major genetic risk factor for the more common, sporadic form of the disease, or Late-Onset Alzheimer's disease (LOAD), is the ε4 allele of the APOE gene. But carrying this allele by itself does not mean a person has or will develop Alzheimer's dementia, so genetic testing for APOE ε4 is not recommended outside of a research setting" (NIH, 2022).

The NIA and Alzheimer's Association released a joint research framework in 2018. In that framework, they state that "Genetics is not formally included in the research framework because our concept of disease rests on neuropathologic change (that can be detected by biomarkers). In contrast, gene variants do not measure pathologic change but rather indicate an individual's risk for developing pathologic change" (Jack et al., 2018).

European Federation of Neurological Societies (EFNS) and European Neurological Society (ENS)

The EFNS and ENS have developed guidelines for the diagnosis and management of disorders associated with dementia. Regarding genetic testing, these guidelines state that "No studies have addressed the value of genetic counselling for patients with dementia or their families when autosomal-dominant disease is suspected. Because the genetics of dementing illnesses is a very young field, expertise in genetic counselling for the dementias of the elderly is likely to be found only in specialized dementia research centres (Good Practice Point). Screening for known pathogenic mutations can be undertaken in patients with appropriate phenotype or a family history of an autosomal-dominant dementia. This should only be undertaken in specialist centres with appropriate counselling of the patient and family caregivers, and with consent (Good Practice Point). Pre-symptomatic testing may be performed in adults where there is a clear family history, and when there is a known mutation in an affected individual to ensure that a negative result is clinically significant. (Good Practice Point)" (Sorbi et al., 2012).

European Federation of Neurological Sciences (EFNS)

In 2010, EFNS published revised recommendations on the diagnosis and management of Alzheimer disease. It stated that "the ApoE 4 allele is the only genetic factor

consistently implicated in late-onset AD, but it is neither necessary nor sufficient for development of the disease. Hence, there is no evidence to suggest ApoE testing is useful in a diagnostic setting." The EFNS recommended that "screening for known pathogenic mutations can be undertaken in patients with appropriate phenotype or a family history of an autosomal dominant dementia. Routine Apo E genotyping is not recommended" (Hort et al., 2010).

United States Preventive Services Task Force (USPSTF)

The USPSTF has concluded that "the current evidence is insufficient to assess the balance of benefits and harms of screening for cognitive impairment in older adults" (Owens et al., 2020).

Alzheimer's Disease and Related Disorders Therapeutics Work Group

In 2023, a workgroup developed and published information on Lecanemab (Leqembi®) as an approved treatment for Alzheimer disease (AD). The drug can be initiated in early Alzheimer disease for those with confirmed brain amyloid pathology. The working group recommends that patients requiring anticoagulants should not receive lecanemab until more data regarding the interaction with anticoagulants is available. This is because adverse events of amyloid related imaging abnormalities (ARIA) can result with lecanemab and these may involve microhemorrhages and rare macrohemorrhages; in other words, anticoagulation may increase the risk of hemorrhage.

Other recommendations:

- "We recommend that all patients being considered for lecanemab therapy meet diagnostic criteria for mild cognitive impairment (MCI) due to AD or probable mild AD dementia with biomarker evidence (amyloid PET or CSF) of the AD pathophysiological process."
- "Individuals with depression who scored >8 on the Geriatric Depression Scale or had a body mass index (BMI) greater than 35 or less than 17 were excluded from both the phase 2 and 3 studies. We recommend adopting these same criteria while allowing medical judgment for individual circumstances when the impact of these modifications has been considered."
- "Patients who are apolipoprotein E ε4 (*APOE4*) gene carriers, especially *APOE4* homozygotes, are at higher risk for ARIA, and the AUR recommends *APOE* genotyping to better inform risk discussions with patients who are lecanemab candidates."
- "We recommend excluding patients with any history of seizures until additional data are available."

- "Cerebral amyloid angiopathy-related inflammation/amyloid beta-related angiitis (CAA-ri/ABRA) increase the risk for ARIA (discussed below) and should exclude patients as treatment candidates."
- "Patients should not be given lecanemab if they are receiving aducanumab or had severe or recurrent ARIA with use of aducanumab."
- "Participants with clotting disorders should be excluded."
- "Evidence of cerebral contusion, encephalomalacia, brain aneurysms or other vascular malformations, central nervous system (CNS) infection, and brain tumors other than meningioma or arachnoid cysts excluded patients from phase 3 trial participation. These same restrictions should apply when considering patients for treatment with lecanemab" (Cummings et al., 2023).

International Working Group (IWG)

The IWG released recommendations for the clinical diagnosis of AD in their "Personal View" article responding to the National Institute of Aging and the Alzheimer Association biologically defining AD based on biomarkers. The IWG contests this definition, stating that some cognitively unimpaired people with AD biomarkers may never develop clinical symptoms of AD, and that AD can develop as a co-morbidity of other brain diseases. The IWG goes on to recommend how biomarkers should and should not be used to clinically diagnose AD. "We recommend that AD diagnosis be restricted to the occurrence of positive biomarkers together with specific AD phenotypes, whereas biomarker positive cognitively unimpaired individuals should be considered only at-risk for progression to AD" (Dubois et al., 2021). The IWG recommends:

- A "clinical-biological" diagnosis of AD requiring the presence of both a clinical phenotype and a biomarker.
- The common clinical phenotypes for AD are "the amnestic syndrome of the hippocampal type (typical), the posterior cortical atrophy variant, and the logopenic variant primary progressive aphasia." Less common phenotypes include "behavioural/dysexecutive variant7, the cortico-basal variant, and the other variants of primary progressive aphasia."
- Both positive amyloid and tau biomarkers must both be present, with the common phenotypes, for AD diagnosis. Uncommon phenotypes with positive AD markers do not ensure an AD diagnosis, a clinician must determine that AD is the dominant pathology.
- "Recommended biomarker measures for amyloid pathology are low CSF Aβ42, increased CSF Aβ40/42 ratio, or increased tracer retention in amyloid PET; for tau pathology, we recommend high CSF p-tau (not total tau [t-tau] due to its lack of specificity) or increased ligand retention in tau PET."

- The diagnosing clinician must be an expert in critically assessing both clinical and biomarker results.
- "CSF investigation is prioritized as it provides simultaneous information on the 2 types of biomarkers (amyloid and tau) and is less expensive than amyloid-PET and/or tau-PET. Where lumbar puncture is contraindicated, PET investigations are an alternative consideration."
- Clinical pathological biomarker investigation is not recommended for cognitively unimpaired individuals. This may change in the future with prevention programs.
- When biomarker investigation has been performed on cognitively unimpaired individuals, the IWG proposed a risk stratification system to distinguish an absolute risk group, a high-risk group, and an undefined risk group. Individuals should be counselled before AD biomarker investigation and if they are at-risk for progression to prodromal AD or AD dementia.
- Isolated subjective memory complaints or cognitive decline that are not supported by cognitive impairment are not enough to be part an AD phenotype.
- When AD is co-morbid with other brain pathologies, it is recommended that physicians rely more on the phenotype and follow-up for the diagnosis.
- Physicians should objectively evaluate the added-value of biomarker investigation in symptomatic patients based on the clinical situation, life context, patient wishes, the possibility of participating in disease-modifying trials, and the current global appreciation of how this information can change patient management.
- When pathophysiological biomarkers are unavailable, patients should have a clinical syndrome diagnosis and more attention should be given to rule out non-degenerative causes (Dubois et al., 2021).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

On April 6, 2017 the FDA approved the 23andMe PGS Genetic Health Risk Report for Late-onset Alzheimer Disease, indicated for reporting of the ε4 variant in the *APOE*

gene. The report describes if a person's genetic result is associated with an increased risk of developing Late-onset Alzheimer Disease, but it does not describe a person's overall risk of developing Alzheimer Disease. The ε4 variant included in this report is found and has been studied in many ethnicities. Detailed risk estimates have been studied the most in people of European descent (FDA, 2017a).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	CPT Description
81401	Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons)
S3852	DNA analysis for APOE epsilon 4 allele for susceptibility to alzheimer's disease

Current Procedural Terminology© American Medical Association. All Rights reserved.
Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

ACMG. (2015). Clinical utility of genetic and genomic services: a position statement of the American College of Medical Genetics and Genomics. *Genet Med*, *17*(6), 505-507. https://doi.org/10.1038/gim.2015.41

ACMG. (2016, September 15). *Don't order APOE genetic testing as a predictive test for Alzheimer disease.* https://www.aafp.org/pubs/afp/collections/choosing-wisely/282.html

Athena. (2023a). *ADmark® ApoE Genotype Analysis and Interpretation (Symptomatic)*. https://www.athenadiagnostics.com/view-full-catalog/admark-apoe-genotype-analysis-and-interpretation-symptomatic1

Athena. (2023b). *ADmark® Early Onset Alzheimer's Evaluation*. https://www.athenadiagnostics.com/view-full-catalog/admark-alzheimers-evaluation1

Cohn-Hokke, P. E., van Swieten, J. C., Pijnenburg, Y. A. L., Tibben, A., Meijers-Heijboer, H., & Kievit, A. (2017). The Effect of Predictive Testing in Adult-Onset Neurodegenerative Diseases on Social and Personal Life. *J Genet Couns*. https://doi.org/10.1007/s10897-017-0195-3

Cruts, M., Theuns, J., & Van Broeckhoven, C. (2012). Locus-specific mutation databases for neurodegenerative brain diseases. *Hum Mutat*, *33*(9), 1340-1344. https://doi.org/10.1002/humu.22117

Cruts, M., & Van Broeckhoven, C. (1998). Presenilin mutations in Alzheimer's disease. *Hum Mutat*, *11*(3), 183-190. https://pubmed.ncbi.nlm.nih.gov/9521418/

Cummings, J., Apostolova, L., Rabinovici, G. D., Atri, A., Aisen, P., Greenberg, S., Hendrix, S., Selkoe, D., Weiner, M., Petersen, R. C., & Salloway, S. (2023). Lecanemab: Appropriate Use Recommendations. *J Prev Alzheimers Dis*, *10*(3), 362-377. https://doi.org/10.14283/jpad.2023.30

de Calignon, A., Polydoro, M., Suarez-Calvet, M., William, C., Adamowicz, D. H., Kopeikina, K. J., Pitstick, R., Sahara, N., Ashe, K. H., Carlson, G. A., Spires-Jones, T. L., & Hyman, B. T. (2012). Propagation of tau pathology in a model of early Alzheimer's disease. *Neuron*, *73*(4), 685-697. https://doi.org/10.1016/j.neuron.2011.11.033

Dubois, B., Villain, N., Frisoni, G. B., Rabinovici, G. D., Sabbagh, M., Cappa, S., Bejanin, A., Bombois, S., Epelbaum, S., Teichmann, M., Habert, M. O., Nordberg, A., Blennow, K., Galasko, D., Stern, Y., Rowe, C. C., Salloway, S., Schneider, L. S., Cummings, J. L., & Feldman, H. H. (2021). Clinical diagnosis of Alzheimer's disease: recommendations of the International Working Group. *Lancet Neurol*, *20*(6), 484-496. https://doi.org/10.1016/s1474-4422(21)00066-1

FDA. (2017a). *DECISION SUMMARY*. https://www.accessdata.fda.gov/cdrh_docs/reviews/den160026.pdf

FDA. (2017b). *Decision Summary for 23andMe PGS Genetic Health Risk Report*. U.S. Food and Drug Administration Retrieved from https://www.accessdata.fda.gov/cdrh_docs/reviews/DEN160026.pdf

Fulgent. (2023). *Parkinson-Alzheimer-Dementia NGS Panel*. https://www.fulgentgenetics.com/Parkinson-Alzheimer-Dementia

Galluzzi, S., Mega, A., Di Fede, G., Muscio, C., Fascendini, S., Benussi, L., Tagliavini, F., Frisoni, G. B., & Di Maria, E. (2022). Psychological Impact of Predictive Genetic Testing for Inherited Alzheimer Disease and Frontotemporal Dementia: The IT-DIAfN

Protocol. *Alzheimer Dis Assoc Disord*. https://doi.org/10.1097/wad.0000000000000494

Gatz, M., Reynolds, C. A., Fratiglioni, L., Johansson, B., Mortimer, J. A., Berg, S., Fiske, A., & Pedersen, N. L. (2006). Role of genes and environments for explaining Alzheimer disease. *Arch Gen Psychiatry*, *63*(2), 168-174. https://doi.org/10.1001/archpsyc.63.2.168

Giau, V. V., Bagyinszky, E., Yang, Y. S., Youn, Y. C., An, S. S. A., & Kim, S. Y. (2019). Genetic analyses of early-onset Alzheimer's disease using next generation sequencing. *Scientific reports*, *9*(1), 8368-8368. https://doi.org/10.1038/s41598-019-44848-2

Goate, A., Chartier-Harlin, M. C., Mullan, M., Brown, J., Crawford, F., Fidani, L., Giuffra, L., Haynes, A., Irving, N., James, L., & et al. (1991). Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature*, *349*(6311), 704-706. https://doi.org/10.1038/349704a0

Goldman, J. S., Hahn, S. E., Catania, J. W., LaRusse-Eckert, S., Butson, M. B., Rumbaugh, M., Strecker, M. N., Roberts, J. S., Burke, W., Mayeux, R., & Bird, T. (2011). Genetic counseling and testing for Alzheimer disease: Joint practice guidelines of the American College of Medical Genetics and the National Society of Genetic Counselors. *Genet Med*, *13*(6), 597-605. https://doi.org/10.1097/GIM.0b013e31821d69b8

Goldman, J. S., Hahn, S. E., Catania, J. W., LaRusse-Eckert, S., Butson, M. B., Rumbaugh, M., Strecker, M. N., Roberts, J. S., Burke, W., Mayeux, R., & Bird, T. (2019). ADDENDUM: Genetic counseling and testing for Alzheimer disease: joint practice guidelines of the American College of Medical Genetics and the National Society of Genetic Counselors. *Genet Med*, *21*(10), 2404. https://doi.org/10.1038/s41436-019-0559-1

Hort, J., O'Brien, J. T., Gainotti, G., Pirttila, T., Popescu, B. O., Rektorova, I., Sorbi, S., & Scheltens, P. (2010). EFNS guidelines for the diagnosis and management of Alzheimer's disease. *Eur J Neurol*, *17*(10), 1236-1248. https://doi.org/10.1111/j.1468-1331.2010.03040.x

Invitae. (2023). *Invitae Hereditary Alzheimer's Disease Panel*. https://www.invitae.com/en/physician/tests/03504/

Jack, C. R., Jr., Bennett, D. A., Blennow, K., Carrillo, M. C., Dunn, B., Haeberlein, S. B., Holtzman, D. M., Jagust, W., Jessen, F., Karlawish, J., Liu, E., Molinuevo, J. L., Montine, T., Phelps, C., Rankin, K. P., Rowe, C. C., Scheltens, P., Siemers, E., Snyder, H. M., . . . Silverberg, N. (2018). NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association*, *14*(4), 535-562. https://doi.org/10.1016/j.jalz.2018.02.018

Janssen, J. C., Beck, J. A., Campbell, T. A., Dickinson, A., Fox, N. C., Harvey, R. J., Houlden, H., Rossor, M. N., & Collinge, J. (2003). Early onset familial Alzheimer's disease:

Mutation frequency in 31 families. *Neurology*, *60*(2), 235-239. http://n.neurology.org/content/60/2/235.long

Karch, C. M., Cruchaga, C., & Goate, A. M. (2014). Alzheimer's disease genetics: from the bench to the clinic. *Neuron*, *83*(1), 11-26. https://doi.org/10.1016/j.neuron.2014.05.041

Keene, C. D., Montine, Thomas, Kuller, Lewis. (2023, January 19). *Epidemiology, pathology, and pathogenesis of Alzheimer disease*. https://www.uptodate.com/contents/epidemiology-pathology-and-pathogenesis-of-alzheimer-disease

Knopman, D. S., DeKosky, S. T., Cummings, J. L., Chui, H., Corey-Bloom, J., Relkin, N., Small, G. W., Miller, B., & Stevens, J. C. (2001). Practice parameter: diagnosis of dementia (an evidence-based review). Report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology*, *56*(9), 1143-1153. https://doi.org/10.1212/wnl.56.9.1143

Kumar, A., Tsao, Jack. (2018). Alzheimer Disease. https://www.ncbi.nlm.nih.gov/books/NBK499922/

Lanoiselee, H. M., Nicolas, G., Wallon, D., Rovelet-Lecrux, A., Lacour, M., Rousseau, S., Richard, A. C., Pasquier, F., Rollin-Sillaire, A., Martinaud, O., Quillard-Muraine, M., de la Sayette, V., Boutoleau-Bretonniere, C., Etcharry-Bouyx, F., Chauvire, V., Sarazin, M., le Ber, I., Epelbaum, S., Jonveaux, T., . . . Campion, D. (2017). APP, PSEN1, and PSEN2 mutations in early-onset Alzheimer disease: A genetic screening study of familial and sporadic cases. *PLoS Med*, *14*(3), e1002270. https://doi.org/10.1371/journal.pmed.1002270

Largent, E. A., Stites, S. D., Harkins, K., & Karlawish, J. (2021). 'That would be dreadful': The ethical, legal, and social challenges of sharing your Alzheimer's disease biomarker and genetic testing results with others. *J Law Biosci*, *8*(1), lsab004. https://doi.org/10.1093/jlb/lsab004

Mehdizadeh, E., Khalaj-Kondori, M., Shaghaghi-Tarakdari, Z., Sadigh-Eteghad, S., Talebi, M., & Andalib, S. (2019). Association of MS4A6A, CD33, and TREM2 gene polymorphisms with the late-onset Alzheimer's disease. *Bioimpacts*, *9*(4), 219-225. https://doi.org/10.15171/bi.2019.27

Naj, A. C., Jun, G., Reitz, C., Kunkle, B. W., Perry, W., Park, Y. S., Beecham, G. W., Rajbhandary, R. A., Hamilton-Nelson, K. L., Wang, L. S., Kauwe, J. S., Huentelman, M. J., Myers, A. J., Bird, T. D., Boeve, B. F., Baldwin, C. T., Jarvik, G. P., Crane, P. K., Rogaeva, E., . . . Yu, L. (2014). Effects of multiple genetic loci on age at onset in late-onset Alzheimer disease: a genome-wide association study. *JAMA Neurol*, *71*(11), 1394-1404. https://doi.org/10.1001/jamaneurol.2014.1491

NIH. (2022). *Alzheimer's Disease Diagnostic Guidelines*. https://www.nia.nih.gov/health/alzheimers-disease-diagnostic-guidelines

Owens, D. K., Davidson, K. W., Krist, A. H., Barry, M. J., Cabana, M., Caughey, A. B., Doubeni, C. A., Epling, J. W., Jr., Kubik, M., Landefeld, C. S., Mangione, C. M., Pbert, L., Silverstein, M., Simon, M. A., Tseng, C. W., & Wong, J. B. (2020). Screening for Cognitive Impairment in Older Adults: US Preventive Services Task Force Recommendation Statement. *Jama*, *323*(8), 757-763. https://doi.org/10.1001/jama.2020.0435

Pavarini, G., Hamdi, L., Lorimer, J., & Singh, I. (2021). Young people's moral attitudes and motivations towards direct-to-consumer genetic testing for inherited risk of Alzheimer disease. *Eur J Med Genet*, *64*(6), 104180. https://doi.org/10.1016/j.ejmg.2021.104180

Pena-Bautista, C., Roca, M., Lopez-Cuevas, R., Baquero, M., Vento, M., & Chafer-Pericas, C. (2020). Metabolomics study to identify plasma biomarkers in alzheimer disease: ApoE genotype effect. *J Pharm Biomed Anal*, *180*, 113088. https://doi.org/10.1016/j.jpba.2019.113088

Prince, M. (2016). *World Alzheimer Report 2015*. A. s. D. International. https://www.alzint.org/u/WorldAlzheimerReport2015.pdf

Qin, Q., Yin, Y., Wang, Y., Lu, Y., Tang, Y., & Jia, J. (2020). Gene mutations associated with early onset familial Alzheimer's disease in China: An overview and current status. *Molecular genetics & genomic medicine*, *8*(10), e1443-e1443. https://doi.org/10.1002/mgg3.1443

Rajabli, F., Feliciano, B. E., Celis, K., Hamilton-Nelson, K. L., Whitehead, P. L., Adams, L. D., Bussies, P. L., Manrique, C. P., Rodriguez, A., Rodriguez, V., Starks, T., Byfield, G. E., Sierra Lopez, C. B., McCauley, J. L., Acosta, H., Chinea, A., Kunkle, B. W., Reitz, C., Farrer, L. A., . . . Pericak-Vance, M. A. (2018). Ancestral origin of ApoE epsilon4 Alzheimer disease risk in Puerto Rican and African American populations. *PLoS Genet*, *14*(12), e1007791. https://doi.org/10.1371/journal.pgen.1007791

Roche. (2024). *Roche's Elecsys Amyloid Plasma Panel Granted FDA Breakthrough Device Designation to Enable a Timely Diagnosis of Alzheimer's Disease*. https://diagnostics.roche.com/us/en/news-listing/2022/elecsys-amyloid-plasma-panel-fda-breakthrough-device-designation-alzheimers.html

Ryman, D. C., Acosta-Baena, N., Aisen, P. S., Bird, T., Danek, A., Fox, N. C., Goate, A., Frommelt, P., Ghetti, B., Langbaum, J. B., Lopera, F., Martins, R., Masters, C. L., Mayeux, R. P., McDade, E., Moreno, S., Reiman, E. M., Ringman, J. M., Salloway, S., . . . Bateman, R. J. (2014). Symptom onset in autosomal dominant Alzheimer disease: a systematic review and meta-analysis. *Neurology*, *83*(3), 253-260. https://doi.org/10.1212/wnl.0000000000000596

Schneider, L. S., Mangialasche, F., Andreasen, N., Feldman, H., Giacobini, E., Jones, R., Mantua, V., Mecocci, P., Pani, L., Winblad, B., & Kivipelto, M. (2014). Clinical trials and late-stage drug development for Alzheimer's disease: an appraisal from 1984 to 2014. *J Intern Med*, *275*(3), 251-283. https://doi.org/10.1111/joim.12191

Shea, Y. F., Chu, L. W., Chan, A. O., Ha, J., Li, Y., & Song, Y. Q. (2016). A systematic review of familial Alzheimer's disease: Differences in presentation of clinical features among three mutated genes and potential ethnic differences. *J Formos Med Assoc*, *115*(2), 67-75. https://doi.org/10.1016/j.jfma.2015.08.004

Sherrington, R., Froelich, S., Sorbi, S., Campion, D., Chi, H., Rogaeva, E. A., Levesque, G., Rogaev, E. I., Lin, C., Liang, Y., Ikeda, M., Mar, L., Brice, A., Agid, Y., Percy, M. E., Clerget-Darpoux, F., Piacentini, S., Marcon, G., Nacmias, B., . . . St George-Hyslop, P. H. (1996). Alzheimer's disease associated with mutations in presenilin 2 is rare and variably penetrant. *Hum Mol Genet*, *5*(7), 985-988.

Sherrington, R., Rogaev, E. I., Liang, Y., Rogaeva, E. A., Levesque, G., Ikeda, M., Chi, H., Lin, C., Li, G., Holman, K., Tsuda, T., Mar, L., Foncin, J. F., Bruni, A. C., Montesi, M. P., Sorbi, S., Rainero, I., Pinessi, L., Nee, L., . . . St George-Hyslop, P. H. (1995). Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature*, *375*(6534), 754-760. https://doi.org/10.1038/375754a0

Sherva, R., & Kowall, N. W. (2023, July 23). *Genetics of Alzheimer disease*. https://www.uptodate.com/contents/genetics-of-alzheimer-disease

Sorbi, S., Hort, J., Erkinjuntti, T., Fladby, T., Gainotti, G., Gurvit, H., Nacmias, B., Pasquier, F., Popescu, B. O., Rektorova, I., Religa, D., Rusina, R., Rossor, M., Schmidt, R., Stefanova, E., Warren, J. D., & Scheltens, P. (2012). EFNS-ENS Guidelines on the diagnosis and management of disorders associated with dementia. *Eur J Neurol*, *19*(9), 1159-1179. https://doi.org/10.1111/j.1468-1331.2012.03784.x

St George-Hyslop, P. H., Tanzi, R. E., Polinsky, R. J., Haines, J. L., Nee, L., Watkins, P. C., Myers, R. H., Feldman, R. G., Pollen, D., Drachman, D., & et al. (1987). The genetic defect causing familial Alzheimer's disease maps on chromosome 21. *Science*, *235*(4791), 885-890.

Stevenson-Hoare, J., Heslegrave, A., Leonenko, G., Fathalla, D., Bellou, E., Luckcuck, L., Marshall, R., Sims, R., Morgan, B. P., Hardy, J., de Strooper, B., Williams, J., Zetterberg, H., & Escott-Price, V. (2023). Plasma biomarkers and genetics in the diagnosis and prediction of Alzheimer's disease. *Brain*, *146*(2), 690-699. https://doi.org/10.1093/brain/awac128

Van Broeckhoven, C., Backhovens, H., Cruts, M., De Winter, G., Bruyland, M., Cras, P., & Martin, J. J. (1992). Mapping of a gene predisposing to early-onset Alzheimer's disease to chromosome 14q24.3. *Nat Genet*, *2*(4), 335-339. https://doi.org/10.1038/ng1292-335

Van Cauwenberghe, C., Van Broeckhoven, C., & Sleegers, K. (2016). The genetic landscape of Alzheimer disease: clinical implications and perspectives. *Genet Med*, *18*(5), 421-430. https://doi.org/10.1038/gim.2015.117

Weintraub, S., Teylan, M., Rader, B., Chan, K. C. G., Bollenbeck, M., Kukull, W. A., Coventry, C., Rogalski, E., Bigio, E., & Mesulam, M. M. (2020). APOE is a correlate of phenotypic

heterogeneity in Alzheimer disease in a national cohort. *Neurology*, *94*(6), e607-e612. https://doi.org/10.1212/wnl.0000000000008666

WHO. (2023). *Dementia*. https://www.who.int/news-room/fact-sheets/detail/dementia

Wong, T. H., Seelaar, H., Melhem, S., Rozemuller, A. J. M., & van Swieten, J. C. (2020). Genetic screening in early-onset Alzheimer's disease identified three novel presenilin mutations. *Neurobiol Aging*, *86*, 201.e209-201.e214. https://doi.org/10.1016/j.neurobiolaging.2019.01.015

Yamazaki, Y., Zhao, N., Caulfield, T. R., Liu, C. C., & Bu, G. (2019). Apolipoprotein E and Alzheimer disease: pathobiology and targeting strategies. *Nat Rev Neurol*, *15*(9), 501-518. https://doi.org/10.1038/s41582-019-0228-7

Zhang, X., Zhu, C., Beecham, G., Vardarajan, B. N., Ma, Y., Lancour, D., Farrell, J. J., Chung, J., Mayeux, R., Haines, J. L., Schellenberg, G. D., Pericak-Vance, M. A., Lunetta, K. L., & Farrer, L. A. (2019). A rare missense variant of CASP7 is associated with familial late-onset Alzheimer's disease. *Alzheimers Dement*, *15*(3), 441-452. https://doi.org/10.1016/j.jalz.2018.10.005

Genetic Testing for Familial Hypercholesterolemia

Policy Number: AHS – M2137 – Genetic Testing for Familial Hypercholesterolemia	Prior Policy Name and Number, as applicable: • AHS-M2137-Genetic Testing for Heterozygous Familial Hypercholesterolemia
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Familial hypercholesterolemia (FH) is a genetic condition that results in premature atherosclerotic cardiovascular disease due to lifelong exposure to elevated low-density lipoprotein cholesterol (LDL-C) levels (Sturm et al., 2018). FH encompasses multiple clinical phenotypes associated with distinct molecular etiologies. The most common is an autosomal dominant disorder caused by mutations in one of three genes, low-density lipoprotein receptor (*LDLR*), *apolipoprotein B-100* (*APOB*), and proprotein convertase subtilisin-like kexin type 9 (*PCSK9*) (Ahmad et al., 2016; Goldberg et al., 2011). Rare autosomal recessive disease results from mutation in low-density lipoprotein receptor adaptor protein (*LDLRAP*) (Garcia et al., 2001).

Genetic counseling is strongly recommended for individuals pursuing genetic testing for familial hypercholesterolemia.

II. Related Policies

Policy Number	Policy Title
AHS-M2180	Genetic Markers for Assessing Risk of Cardiovascular Disease

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Genetic testing to establish a molecular diagnosis of familial hypercholesterolemia (FH) **MEETS COVERAGE CRITERIA** when **both** of the following criteria are met:

   a) When FH is clinically suspected (based on clinical features, family history, physical exam, lipid levels, etc.) and a definitive diagnosis is required;

   b) When the result of the test will directly impact the treatment being delivered to the individual.

2) For asymptomatic close blood relatives (see Note 1) of an individual affected with FH, genetic testing for a known familial mutation associated with FH **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

3) For all other situations not described above, genetic testing to confirm a diagnosis of FH **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Close blood relatives include 1st-degree relatives (e.g., parents, siblings, and children), 2nd-degree relatives (e.g., grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings), and 3rd-degree relatives (great-grandparents, great-aunts, great-uncles, great-grandchildren, and first cousins), all of whom are on the same side of the family.

Note 2: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
AACE	American Association of Clinical Endocrinology
ACC	American College of Cardiology
ACE	American College of Endocrinology
AHA	American Heart Association
APOB	*Apolipoprotein B-100*
ASCVD	Atherosclerotic cardiovascular disease
CAD	Coronary artery disease
CCS	Canadian Cardiovascular Society

CHD	Coronary heart disease
CMS	Centers for Medicare and Medicaid Services
DLCN	Dutch Lipid Clinic Network
EAS	European Atherosclerosis Society
ESC	European Society of Cardiology
FH	Familial hypercholesterolemia
HEART UK	Hyperlipidaemia Education and Atherosclerosis Research Trust United Kingdom
HoFH	Homozygous hypercholesterolemia
IMT	Intima-media thickness
LDL-C	Low-density lipoprotein cholesterol
LDLR	*Low-density lipoprotein receptor*
LDLRAP	Low-density lipoprotein receptor adaptor protein
MEDPED	Make early diagnosis to prevent early death
NICE	National Institute for Health and Care Excellence
NLA	National lipid association
PCSK9	*Proprotein convertase subtilisin-like kexin type 9*
USPSTF	United States Preventive Services Task Force

V. Scientific Background

Familial Hypercholesterolemia (FH) is considered the most common inherited cardiovascular disease, affecting up to one in 200 people. FH's signature clinical sign is extremely elevated levels of low-density lipoprotein (LDL) cholesterol, which often leads to early-onset atherosclerotic cardiovascular disease (ASCVD) (Rosenson & Durrington, 2023). FH likely accounts for up to 3% of myocardial infarctions for individuals under 60 (Youngblom et al., 2016). Although affected individuals have a 20-fold increased risk of premature ASCVD (Austin et al., 2004), early diagnosis and treatment with lipid-lowering drugs can reduce the risk of coronary heart disease (CHD) to rates comparable to the general population (Ahmad et al., 2016; Knowles et al., 2014; Versmissen et al., 2008).

The primary pathogenic mechanism of FH is the impairment of LDL-receptor mediated catabolism of LDL. Mutations in any of the three main genes (*APOB*, *LDLR*, *PCSK9*) typically cause this impairment, and mutations can be detected in up to 80% of patients with "definite" FH and up to 30% with "possible" FH. Of the three mutations, *LDLR* is the most common, composing 85-90% of the total mutations. *PCSK9* consists of 2-4% of the total, and *APOB* consists of 1-12%. The severity of clinical phenotype depends on the extent to which LDL metabolism is affected. *LDLR* mutations reduce the efficacy of LDL receptors to clear LDL particles, *APOB* mutations impair binding of

LDL particles to the LDL receptor, and *PCSK9* mutations lead to decreased *LDLR* expression (fewer LDL receptors). Other factors (unrelated genetic conditions, diet, et al.) may affect LDL levels as well (Rosenson & Durrington, 2023). Several proprietary gene panels exist for assessment of FH. These typically include the three primary genes, but they may also include rarer genes, such as *LDLRAP* (Ambry, 2020; Invitae, 2020). Some panels intended for broader conditions, such as hyperlipidemia, may also include FH-related genes, such as BluePrint Genetics' 20 gene panel (BluePrint, 2022).

At least three current diagnostic criteria have been developed (Simon Broome, Dutch Lipid Clinic Network (DLCN), and the US Make Early Diagnosis to Prevent Early Death [MEDPED]). These criteria have been able to identify patients with FH-causing mutations with >80% sensitivity or specificity (Civeira, 2004; Marks et al., 2003; Williams et al., 1993). The Simon Broome and DLCN diagnostic criteria consider DNA-based evidence of mutations in any of *APOB, LDLR,* or *PCSK9* to be suitable evidence for a "definite" diagnosis of FH (Rosenson & Durrington, 2023).

However, <10% of FH cases are identified (O'Brien et al., 2014), despite an estimated prevalence of 1:200 to 1:500 (Ahmad et al., 2016; De Backer et al., 2015; Do et al., 2015; Nordestgaard et al., 2013). Ahmad et al. noted the heterogeneity of clinical application of FH diagnostic criteria, observing that the most commonly used formal criteria was Simon–Broome only (21%), followed by multiple diagnostic criteria (16%), MEDPED only (7%), DLCN only (1%), and other (0.5%) (Ahmad et al., 2016).

Clinical Utility and Validity

Wald et al. (2016) assessed the efficacy and feasibility of child-parent screening for familial hypercholesterolemia in primary care practice. 10095 children provided capillary blood samples, and the authors measured their cholesterol. Children were considered positive for FH if their levels were at or above the 99.2% percentile (1.53 times the median level). 28 (0.3%) children were considered positive for FH, and 20 children were classified as carriers of an FH mutation. Another 17 children with levels under the 1.53 median were found to have an FH mutation. Overall, the mutation prevalence was 1/273 children (37/10095). The authors concluded that "Child-parent screening was feasible in primary care practices at routine child immunization visits. For every 1000 children screened, 8 persons (4 children and 4 parents) were identified as having positive screening results for familial hypercholesterolemia and were consequently at high risk for cardiovascular disease" (Wald et al., 2016).

Khera et al. (2016) evaluated the prevalence of an FH mutation among those with "severe" hypercholesterolemia and determined whether coronary artery disease (CAD) risk varies with mutation status. Three genes causative of FH (*LDLR*, *APOB*, and *PCSK9*) were sequenced in 26025 patients from seven case-control studies (5540 with CAD,

8577 controls without) and five prospective cohort studies (n = 11908). Out of the 20485 prospective cohort and CAD-free patients, 1386 were found to have LDL cholesterol levels of ≥190 mg/dL, and only 24 of these carried an FH mutation. Patients with LDL cholesterol ≥190 mg/dL and no FH mutation were found to have a 6-fold higher risk for CAD compared to patients with LDL <130 mg/dL, but patients with both LDL cholesterol ≥190 mg/dL and an FH mutation were found to have a 22-fold higher risk (Khera et al., 2016).

Braamskamp et al. (2017) performed a study assessing the effect of two-year treatment with rosuvastatin on carotid intima-media thickness (IMT) in children with heterozygous familial hypercholesterolemia (HeFH). 197 children with HeFH were provided rosuvastatin for two years, and carotid IMT was assessed at baseline, one year, and two years. The authors noted that at baseline, carotid IMT was greater in HeFH than affected siblings, but rosuvastatin treatment resulted in "significantly less progression of increased carotid IMT in children with HeFH than untreated unaffected siblings", even suggesting that "no difference in carotid IMT could be detected between the 2 groups after 2 years of rosuvastatin." The authors concluded that "these findings support the value of early initiation of statin treatment for low-density lipoprotein cholesterol reduction in children with HeFH" (Braamskamp et al., 2017).

Elbitar et al. (2018) showed the identification new mutations in FH through use of exome sequencing of *LDLR*, *APOB*, *PCSK9,* and *APOE*. 13 French families with "autosomal dominant hypercholesterolemia" had an exome sequencing performed. Several new mutations were identified, such as "p.Arg50Gln mutation in the *APOB* gene, a p.Ala3396Thr mutation of *APOB*, and one patient with a severe phenotype carrying also a mutation in *PCSK9*: p.Arg96Cys." The authors stated that this study provided the first known case of a compound heterozygote with a mutation in *APOB* and *PCSK9* and suggested that identifying these new mutations "lead to better diagnosis and treatment of ADH" (Elbitar et al., 2018).

Lee et al. (2019) performed a meta-analysis on the impact of genetic testing for FH on "1) diagnosis of 'definite familial hypercholesterolemia', 2) initiation and adherence of lipid-lowering therapy and 3) risk of ASCVD." The authors included 56 studies. Genetic testing was found to have provided confirmation of FH in 28-80% of cases over clinical criteria alone. The authors also identified a 76751-individual cohort that indicated that an FH-causing variant was found in only 1.7%-2.5% of subjects with LDL >190 mg/dL. Molecular diagnosis was found to increase lipid-lowering therapy adherence (4181 definite FH subjects). A loss-of-function *LDLR* variant was found to increase risk of myocardial infarction by 6.77-fold, and even a milder pathogenic *LDLR* variant still increased risk by 4.4-fold. The authors concluded that "DNA sequencing confirms the diagnosis of FH but has a poor yield in unselected patients whose sole criterion is an

elevated LDL-C. Initiation and adherence to treatment is improved. The risk of ASCVD is 4.4- to 6.8-fold increased in patients with an FH-causing variant compared with controls, depending on the severity of the DNA change" (Lee et al., 2019).

Trinder et al. (2019) evaluated the risk of premature (defined as <55 years old) cardiovascular events in patients with clinically diagnosed familial hypercholesterolemia. Monogenic (defined as mutations in *LDLR, APOB,* or *PCSK9,* comprising up to 80% of cases) and polygenic causes of FH were compared. 626 patients were included, and both targeted sequencing and genetic variant analysis were performed to identify patients for both cohorts. Patients with polygenic scores above the 80th percentile were considered to have polygenic FH. Risk of several cardiovascular events (unstable angina, myocardial infarction, coronary revascularization, or stroke) were assessed. Monogenic causes of FH were associated with a 1.96-fold increase of CVD, whereas the polygenic cohort saw no significant increase compared to patients without any genetic cause of FH at all. The authors also found that an elevated low-density lipoprotein polygenic risk score increased the CVD risk for monogenic patients to 3.06-fold. The authors concluded that "genetic testing for FH provides important prognostic information that is independent of LDL-C levels" (Trinder et al., 2019).

Trinder et al. (2020) compared the risk of CVD events between three cohorts; monogenic FH (277 patients), polygenic FH (2379), and hypercholesterolemia with "undetermined cause" (2232). The authors defined polygenic FH as a "polygenic score >95th percentile based on 223 single-nucleotide variants." The authors found that patients with monogenic FH were three times more likely than polygenic FH to experience a CVD event before age 55 (6.1% vs 2.0%) and that both genetically-based types of FH were more likely to experience a CVD event compared to patients with hypercholesterolemia of unknown cause. The authors concluded that "genetic determinants of LDL-C levels may impose additional risk of CVD" and that "understanding the possible genetic cause of hypercholesterolemia may provide important prognostic information to treat patients" (Trinder et al., 2020).

Sturm et al. (2021) conducted a cross-sectional study comparing limited-variant screening and comprehensive next-generation (NGS) genetic testing for diagnosing FH. In the patient cohort, the researchers found that the limited-variant screen would have only yielded a positive detection rate of 8.4%, compared to the 27.0% positive detection rate with the comprehensive test, meaning that 68.9% of individuals with a FH-associated gene would've been missed by the limited screen. Individuals of self-reported Black/African American and Hispanic descent were more likely to be missed by the limited-variant screen. This demonstrates the need to conduct a full evaluation

via genetic screening, and how it is a useful modality for diagnosing FH (Sturm et al., 2021).

Reeskamp et al. (2020) performed a study that investigated the role of NGS in clinical FH. For the diagnostic yield of NGS, the researchers stated that "a FH-causing genetic variant was identified in only 14.9% of FH patients with LDL-cholesterol levels of 5 mmol/L or greater;" the mutations being considered were from *LDLR* (80.2%), APOB (14.5%), or PCSK9 (5.3%). "This percentage increased to more than 50% when patients were stratified according to either higher LDL-cholesterol levels or more stringent diagnostic FH criteria ascertained by the DLCN [Dutch Lipid Clinic Network] criteria; 4.8% of FH-mutation negative patients were heterozygous carriers of a pathogenic variant in a minor FH gene." Though this study overall had a lower diagnostic yield using NGS in comparison to other studies, the researchers propose that "stringent use of clinical criteria algorithms is warranted to increase this yield" and thus maximize the clinical utility of NGS (Reeskamp et al., 2020).

Vallejo et al. (2021) conducted a cross-sectional assessment of 42,617 adults 18 years and older with a clinical or genetic diagnosis of heterozygous familial hypercholesterolaemia. The European Atherosclerosis Society Familial Hypercholesterolaemia Studies Collaboration provided a global registry of individuals with FH in World Health Organization regions. Cardiovascular risk factors varied by age and World Health Organization region. The authors found that, "among patients taking lipid-lowering medications, 2.7% had LDL cholesterol lower than 1.8 mmol/L; the use of combination therapy, particularly with three drugs and with proprotein convertase subtilisin-kexin type 9 inhibitors, was associated with a higher proportion and greater odds of having LDL cholesterol lower than 1·8 mmol/L." In addition, the authors advocated for earlier detection and the use of combination therapies (as opposed to single-drug therapy) to achieve guideline-recommended LDL concentrations.

VI. Guidelines and Recommendations

European Atherosclerosis Society (EAS)

Cuchel et al. (2014) published a position paper from the Consensus Panel on Familial Hypercholesterolemia of the EAS that stated that the diagnosis of homozygous familial hypercholesterolemia (HoFH) can be made on clinical or genetic criteria. The authors recommended genetic analysis should be considered to:

- Confirm the clinical diagnosis
- Facilitate testing of family members (reverse cascade screening) – still endorsed in 2023

- Assist in diagnosis where clinical presentation is borderline between that of HoFH and heterozygous FH

In 2023, the EAS provided updates to their clinical guidance for homozygous familial hypercholesterolaemia. In terms of the updated genetic criteria, EAS stated the following:

- "Genetic confirmation of bi-allelic pathogenic/likely pathogenic variants on different chromosomes at the *LDLR, APOB, PCSK9, or LDLRAP1* genes or ≥2 such variants at different loci"
- "Consider only variants reported as 'pathogenic/likely pathogenic' according to recognized criteria as a confirmed genetic diagnosis of HoFH."

Despite these recommendations, the EAS acknowledges that "genetic results can be potentially misunderstood or miscommunicated." When there are privacy concerns for probands or parents, or limited access to testing, the EAS also states that screening of lipid levels is "acceptable" to identify family members with FH, as the LDL-C level/phenotype is what ultimately determines therapy (Cuchel et al., 2023).

Wiegman et al. (2015) published a position paper from the EAS regarding FH in children. In it, they state that "DNA testing establishes the diagnosis," and that "detection of a pathogenic mutation, usually in the *LDLR* gene, is the gold standard for diagnosis of FH." The EAS also observes that "It is best practice to first genetically test a phenotypically affected parent or a second-degree relative in the absence of a parent. If a mutation is identified, genetic testing and counselling should be offered to all family members" (Wiegman et al., 2015).

European Society of Cardiology and European Atherosclerosis Society (ESC/EAS)

The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) published an update in 2019. In it, they recommend that genetic testing should be performed to confirm clinically suspicious cases of FH. The EAS lists the following criteria as evidence for FH:

- "TC ≥8 mmol/L (≥310 mg/dL) without treatment in an adult or adult family member (or >95th percentile by age and gender for country);
- Premature CHD in the patient or a family member;
- Tendon xanthomas in the patient or a family member; or
- Sudden premature cardiac death in a family member."

The EAS also recommends that cascade testing be performed when a causative mutation is known in an index case of heterozygous FH. Moreover, "To improve risk

assessment, the use of imaging techniques to detect asymptomatic atherosclerosis is recommended" (Mach et al., 2019).

American Heart Association (AHA)

In 2015, the AHA released a scientific statement on FH that stated that "identification of all patients with FH is critical, but the optimal screening strategy has not been determined, and the complementary roles of genetic testing, family history, and LDL-C need to be further defined, particularly for children." AHA noted that "In healthcare systems that are less cohesive such as the US system, genetic testing is controversial for individuals in confirming diagnosis, and implementing cascade screening will be more difficult. In most countries, genetic testing remains relatively expensive and has limited availability. A reduction in costs and improved efficiency of genetic testing is likely to increase its broader application in screening families for FH." Regarding testing for family members of patients with FH, AHA stated that "Consenting family members should be offered a standard plasma lipid profile and a genetic test if the family mutation is known, and DNA testing is available." The AHA also recommended that "Genetic counseling for FH can help patients and their families complete their pedigree and understand the inheritance of FH and the personal and familial implications of the diagnosis" (Gidding et al., 2015).

For diagnosing FH, the AHA states that "genetic testing may offer additional insight regarding cardiac risk and diagnosis" and can confirm diagnosis, but "cannot be excluded in the absence of a causative mutation" (McGowan et al., 2019).

The American Heart Association also proposed new diagnostic criteria shown below (Gidding et al., 2015):

***ICD-10* Category**	**Clinical Criteria**	**With Genetic Testing Performed**
Heterozygous FH	LDL-C ≥160 mg/dL (4 mmol/L) for children and ≥190 mg/dL (5 mmol/L) for adults and with one first-degree relative similarly affected or with premature CAD or with positive genetic testing for an LDL-C–raising gene defect (LDL receptor, *APOB*, or *PCSK9*)	Presence of one abnormal LDL-C–raising (LDL receptor, *APOB* or *PCSK9*) gene defect Diagnosed as heterozygous FH if LDL-C–raising

		defect positive and LDL-C <160 mg/dL (4 mmol/L) Occasionally, heterozygotes will have LDL-C >400 mg/dL (10 mmol/L); they should be treated similarly to homozygotes Presence of both abnormal LDL-C–raising (LDL receptor, *APOB* or *PCSK9*) gene defect(s) and LDL-C–lowering gene variant(s) with LDL-C <160 mg/dL (4 mmol/L)
Homozygous FH	LDL-C ≥400 mg/dL (10 mmol/L) and one or both parents having clinically diagnosed familial hypercholesterolemia, positive genetic testing for an LDL-C–raising (LDL receptor, *APOB*, or *PCSK9*) gene defect, or autosomal-recessive FH	Presence of two identical (true homozygous FH) or nonidentical (compound heterozygous FH) abnormal LDL-C–raising (LDL receptor, *APOB* or *PCSK9*) gene defects; includes the rare autosomal-recessive type
	If LDL-C >560 mg/dL (14 mmol/L) or LDL-C >400 mg/dL (10 mmol/L) with	Occasionally, homozygotes will

	aortic valve disease or xanthomata at <20 y of age, homozygous FH highly likely	have LDL-C <400 mg/dL (10 mmol/L)
Family history of FH	LDL-C level not a criterion; presence of a first-degree relative with confirmed FH	Genetic testing not performed

-
-

American College of Cardiology/American Heart Association (ACC/AHA)

The 2018 statement (Sturm et al., 2018) on clinical genetic testing for familial hypercholesterolemia recommends:

Proband (index case)

Genetic testing for FH should be offered to individuals of any age in whom a strong clinical index of suspicion for FH exists based on examination of the patient's clinical and/or family histories. This index of suspicion includes the following:

- "Children with persistent∗ LDL-C levels ≥160 mg/dl or adults with persistent LDL-C levels ≥190 mg/dl without an apparent secondary cause of hypercholesterolemia and with at least 1 first-degree relative similarly affected or with premature CAD‡ or where family history is not available (e.g., adoption)"
- "Children with persistent∗ LDL-C levels ≥190 mg/dl or adults with persistent LDL-C levels ≥250 mg/dl without an apparent secondary cause of hypercholesterolemia, even in the absence of a positive family history"

Genetic testing for FH may be considered in the following clinical scenarios:

- "Children with persistent∗ LDL-C levels ≥160 mg/dl (without an apparent secondary cause of hypercholesterolemia†) with an LDL-C level ≥190 mg/dl in at least 1 parent or a family history of hypercholesterolemia and premature CAD"
- "Adults with no pre-treatment LDL-C levels available but with a personal history of premature CAD and family history of both hypercholesterolemia and premature CAD"
- "Adults with persistent LDL-C levels ≥160 mg/dl (without an apparent secondary cause of hypercholesterolemia) in the setting of a family history of

hypercholesterolemia and either a personal history or a family history of premature CAD"

*Two or more measurements, including assessment after intensive lifestyle modification

†Hypothyroidism, diabetes, renal disease, nephrotic syndrome, liver disease, medications.

If LDL-C values are unavailable, total cholesterol values ≥320, 260, and 230 mg/dl (corresponding to LDL-C levels ≥250, 190, and 160 mg/dl, respectively) could be used.

At-risk relatives

Cascade genetic testing for the specific variant(s) identified in the FH proband (known familial variant testing) should be offered to all first-degree relatives. If first-degree relatives are unavailable, or do not wish to undergo testing, known familial variant testing should be offered to second-degree relatives. Cascade genetic testing should commence throughout the entire extended family until all at-risk individuals have been tested and all known relatives with FH have been identified.

They recommend that "Genetic testing for patients with suspected FH should, at a minimum, include analysis of *LDLR, APOB,* and *PCSK9*. This analysis should include for *LDLR* and *PCSK9* sequencing of all exons and exon/ intron boundaries, as well as *LDLR* deletion/duplication analysis, and for *APOB* the exons encoding the *LDLR* ligand-binding region… Larger, more inclusive, lipid disorder NGS panels are also available that provide evaluation of not only the main FH genes but also the genes causing conditions with phenotypic overlap previously described. These expanded panels should be considered to improve the diagnosis of patients with these 'phenocopy' conditions that may require specific therapies, and they should include the following genes: *LDLR, APOB, PCSK9, LDLRAP1, LIPA, ABCG5, ABCG8,* and *APOE*" (Sturm et al., 2018).

National Lipid Association (NLA)

In 2011, the NLA released guidelines for screening, diagnosing, and managing familial hypercholesterolemia among pediatric and adult patients. This was last reaffirmed in August 2020. With regards to genetic testing/screening, the NLA stated the following:

- "Genetic screening for FH is generally not needed for diagnosis or clinical management but may be useful when the diagnosis is uncertain.
- Identification of a causal mutation may provide additional motivation for some patients to implement appropriate treatment.

- Importantly, a negative genetic test does not exclude FH, since approximately 20% of clinically definite FH patients will not be found to have a mutation despite an exhaustive search using current methods."

On cascade screening, the NLA stated the following:

- "Cascade screening involves testing lipid levels in all first-degree relatives of diagnosed FH patients.
- As cascade screening proceeds, newly identified FH cases provide additional relatives who should be considered for screening.
- Cascade screening is the most cost-effective means of finding previously undiagnosed FH patients and is also cost-effective in terms of cost per year of life saved. General population screening of a young population (before age 16) is similarly cost-effective in terms of cost per year of life saved, given that effective cholesterol-lowering treatment is begun in all those identified."

However, in terms of diagnosis of FH, the NLA states:

- "Untreated fasting lipid levels at which FH may be suspected in children, adolescents and young adults (<20 years) are LDL cholesterol concentration ≥160 mg/dL or non-HDL cholesterol ≥190 mg/dL. These levels are supported by family studies of affected individuals.
- A second lipid profile should be performed to assess response to diet management, to account for regression to the mean, and to accurately classify those with levels close to classification thresholds" (Goldberg et al., 2011).

In 2015, the NLA published guidelines for the management of dyslipidemia (Jacobson et al., 2015) which was reaffirmed in August 2020:

"If LDL-C is ≥190 mg/dL, consider severe hypercholesterolemia phenotype, which includes familial hypercholesterolemia."

The NLA also published a statement on genetic testing for dyslipidemia in 2020. This statement has been reaffirmed in June 2021. The FH-related recommendations are listed below:

- "Genetic testing is reasonable when heterozygous familial hypercholesterolemia is suspected but not definitively diagnosed based on clinical criteria alone."
- "Cascade screening for FH either by lipid profile or genetic testing is recommended in all first-degree relatives (children and siblings) of an individual who has tested genetically positive for FH."

- "Cascade testing [for general genetic dyslipidemias] should begin with first-degree relatives (parents, siblings, and children) and then extend to second- and third-degree relatives."

The Association also remarks that genetic testing for FH can predict clinical outcomes and that identifying specific mutations (such as *LDLR*) may guide targeted therapy in the future. No "polygenic risk scores" have been identified as a "gold standard" (Brown et al., 2020).

American Association of Clinical Endocrinologists (AACE) and American College of Endocrinology (ACE)

The 2017 American Association of Clinical Endocrinologists and American College of Endocrinology Guidelines for Management of Dyslipidemia and Prevention of Cardiovascular Disease include some items regarding FH. These guidelines include the following (Jellinger et al., 2017):

- For children at risk (such as family history) for FH,
  - screening should be at three years of age
  - repeated once between ages nine and 11 years
  - repeated again at age 18
- "Individuals should be screened for familial hypercholesterolemia (FH) when there is a family history of premature ASCVD. . ."
- Individuals should be screened if they have "Elevated cholesterol levels (total, non-HDL and/ or LDL) consistent with FH"
- The authors note, "While genetic testing may identify FH, it is not commonly used in the U.S. due to cost and lack of payer coverage" (Jellinger et al., 2017).

United States Preventive Services Task Force (USPSTF)

According to the 2016 USPSTF guidelines, "Screening can detect FH in children, and lipid-lowering treatment in childhood can reduce lipid concentrations in the short term, with little evidence of harm. There is no evidence for the effect of screening for FH in childhood on lipid concentrations or cardiovascular outcomes in adulthood, or on the long-term benefits or harms of beginning lipid-lowering treatment in childhood" (Lozano et al., 2016).

In a 2023 screening recommendation, "The USPSTF concludes that the current evidence is insufficient to assess the balance of benefits and harms of screening for lipid disorders in children and adolescents 20 years or younger" (Barry et al., 2023).

National Institute for Health and Care Excellence (NICE)

NICE published an update on FH in 2019, and their relevant recommendations include the following:

- "Carry out cascade testing using DNA testing to identify affected first- and second- and, when possible, third-degree biological relatives of people with a genetic diagnosis of FH."
- "Healthcare professionals should consider a clinical diagnosis of homozygous FH in adults with a low-density lipoprotein cholesterol (LDL-C) concentration greater than 13 mmol/l and in children/young people with an LDL-C concentration greater than 11 mmol/l."
- "Use the Simon Broome or Dutch Lipid Clinic Network (DLCN) criteria to make a clinical diagnosis of FH in primary care settings."
- "Refer the person to an FH specialist service for DNA testing if they meet the Simon Broome criteria for possible or definite FH, or they have a DLCN score greater than 5" (NICE, 2019).

Hyperlipidaemia Education and Atherosclerosis Research Trust United Kingdom (HEART UK)

This guideline focuses on homozygous FH. For diagnosis of homozygous FH, HEART-UK recommends that mutation analysis "should be by comprehensive DNA sequencing of introns and exons of the *LDLR, APOB, PCSK9* and *LDLRAP1* gene loci [in an accredited laboratory]" (France et al., 2016).

International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel (IAS)

The panel notes that in addition to the main three genes, mutations in *APOE* or *STAP1* may result in the heterozygous FH phenotype. However, the panel remarks that "…identification of a causative gene variant is not essential for either diagnosis or treatment decisions, since as mentioned these are more appropriately guided by the LDL-C and not by the genotype" (Santos et al., 2016).

New guidance from this society asserts that "Early detection of FH is fundamental to all models of care for FH… However, the best approach to detecting FH in primary care remains uncertain." Nonetheless, they offer the following recommendations for the screening of FH:

Clinical recommendations	Class	Level
1. Multiple screening strategies (for example, selective, opportunistic and/or universal) should ideally be used to detect index cases with FH	1	B
2. Age-specific, sex-specific and country-specific LDL-cholesterol concentrations (estimated in plasma or serum) above the corresponding 95th percentiles for the population should preferably be used to screen for index cases with FH	1	B
3. Selective screening should be used to detect index cases among adults with premature ASCVD, mainly coronary artery disease, and a family history of premature ASCVD and/or hypercholesterolaemia	1	A
4. Opportunistic screening, such as an LDL-cholesterol concentration >4.9 mmol/l (≥190 mg/dl), should be used to detect cases in the community	1	B
5. Universal screening using age-specific and sex-specific criteria for LDL-cholesterol concentration should be considered initially to detect children and adolescents with FH, after which the diagnosis should be formally confirmed and reverse cascade testing offered to parents, as indicated	2	B
6. Cascade testing should be offered to all close relatives of an index case with definite FH and be carried out using phenotypic and genetic methods; if genetic testing is not feasible, LDL-cholesterol testing (based on appropriate age-specific and gender-specific thresholds) should be used	1	A
7. Genome-based population screening of adults may be considered for wider and more accurate detection of FH, but requires careful implementation	3	C
8. After initial detection of potential index cases, the diagnosis of FH should be formally confirmed using country-specific (or internationally accepted) phenotypic criteria and ideally with genetic testing	1	A
9. Children with suspected HoFH (for example, with physical stigmata), or at risk of FH (both parents known to have FH), should be tested as early as possible (at the newborn stage or by 2 years of age), with measurement of LDL-cholesterol concentrations, followed by genetic confirmation	1	B
10. Screening of children at risk of HeFH should be considered using LDL-cholesterol concentrations at or after the age of 5 years, or as early as 2 years in those with a strong family history of premature ASCVD, with confirmation of the diagnosis genetically, as indicated	2	B
11. Non-fasting samples may be considered when screening for FH; the Friedewald equation should be used with caution owing to the confounding effect of hypertriglyceridaemia on the estimation of LDL-cholesterol concentration	3	B
12. Patients with hypertriglyceridaemia > 4.5 mmol/l (>400 mg/dl), in whom FH is strongly suspected, should be re-screened for FH with a 12-h fasting sample and LDL-cholesterol concentration measured using a direct assay	1	A
13. In the absence of a direct assay for LDL-cholesterol concentration, the probability of FH should be reconsidered in patients with very severe hypertriglyceridaemia after therapeutic lowering of triglyceride concentrations to <4.5 mmol/l (<400 mg/dl), or by calculating LDL-cholesterol using a novel equation, if triglycerides are between 4.5 mmol/l and 10.0 mmol/l (400–850 mg/dl)	2	C
14. The effects of cholesterol-lowering medications and acute illness should be accounted for when phenotypically screening for FH; LDL-cholesterol concentrations should be adjusted for the use of statins, ezetimibe, PCSK9 inhibitors and other therapies, particularly if a reliable pretreatment value is unavailable; if the diagnosis of FH is in doubt, LDL-cholesterol measurement should be repeated after full recovery from acute illness	1	B

(Watts et al., 2023).

For the diagnosis of FH, IAS offers the recommendations below.

Clinical recommendations	Class	Level
1. A diagnosis of HeFH or HoFH should be made, whenever possible, using genetic testing that identifies pathogenic variants (such as in *LDLR*, *APOB*, *PCSK9* or *LDLRAP1*) that impair the LDL-receptor pathway; such testing is particularly important when phenotypic features are less obvious, such as in children, and for planning long-term care and cascade testing of family members. Conversely, if the phenotype strongly suggests FH and a pathogenic or likely pathogenic variant is not detected, FH should not be excluded	1	A
2. If genetic testing is not feasible, a clinical diagnosis of FH in adults should be made using country-specific or recognized phenotypic criteria (such as the Dutch Lipid Clinic Network, Simon Broome criteria, MED-PED, AHA, Canadian or Japanese criteria) for index cases (Supplementary Material 2)	1	A
3. A phenotypic diagnosis of FH in adults and children requires exclusion of, or correction for secondary causes of, high LDL-cholesterol concentrations (Supplementary Material 3); in the absence of an untreated value, LDL-cholesterol concentration should be adjusted for concurrent use of cholesterol-lowering medication; LDL-cholesterol concentrations should ideally be measured after fasting and on two occasions	1	A
4. Use of imaging-based detection of subclinical Achilles tendon xanthomas may be considered to increase the specificity and accuracy of the phenotypic diagnosis of FH in adults	3	B
5. A clinical diagnosis of FH in children and adolescents should be considered as highly probable in the presence of an untreated LDL-cholesterol concentration >4.9 mmol/l (>190 mg/dl), recorded on at least two occasions (fasting lipid profile, >2 weeks but <3 months apart), and a parental history of high LDL-cholesterol levels, premature ASCVD or a positive genetic test for FH	2	B
6. After exclusion of secondary causes of high LDL-cholesterol levels (Supplementary Material 3), a clinical diagnosis of FH in children and adolescents should be considered as probable in the presence of an untreated (a) LDL-cholesterol concentration > 4.9 mmol/l (>190 mg/dl; recorded on at least two occasions), even in the absence of a parental history of high LDL-cholesterol concentrations or premature ASCVD; (b) LDL-cholesterol concentration > 4.0 mmol/l (>160 mg/dl; recorded on at least two occasions), with a parental history of high LDL-cholesterol concentrations or premature ASCVD; (c) LDL-cholesterol concentration > 3.5 mmol/l (>135 mg/dl; recorded on at least two occasions), with a parent having a pathogenic gene variant for FH; (d) LDL-cholesterol concentration (recorded on at least two occasions) exceeding a country-specific LDL-cholesterol threshold (lower than the above) and a parental history of elevated LDL-cholesterol concentrations or premature ASCVD	2	B
7. Phenotypic criteria developed for making a diagnosis of HeFH in adult index cases (such as the Dutch Lipid Clinic Network criteria) should not be used in children or adolescents, or when undertaking cascade testing	1	A
8. After excluding secondary causes of high LDL-cholesterol levels (Supplementary Material 3), a clinical diagnosis of HoFH (that is, phenotypic HoFH) should be made in children and adults with an untreated LDL-cholesterol concentration > 10 mmol/l (>400 mg/dl; recorded on two occasions) in the presence of (a) physical stigmata (tendon or cutaneous xanthomas, arcus cornealis) before the age of 10 years and/or (b) untreated LDL-cholesterol concentrations consistent with HeFH in both parents; in the absence of genetic testing and a clear history of FH in both parents, sitosterolaemia and hypercholesterolaemia (cerebrotendinous xanthomatosis) should also be excluded	1	C
9. If cascade testing in the family is recommended, the diagnosis of FH in the proband or index case should ideally be confirmed genetically	1	A
10. The diagnosis of FH during phenotypic cascade testing should be made using age-specific, sex-specific and country-specific LDL-cholesterol concentrations, ideally measured after fasting and on two occasions	1	A

(Watts et al., 2023)

Finally, in asserting that "Genetic testing procedures should be standardized, including informed consent, pre-test and post-test genetic counselling, classification of variants,

reporting and return of results, follow-up of family members for cascade testing, and shared decision-making", IAS created the following table of recommendations:

Clinical recommendations	Class	Level
1. Genetic testing for FH should be offered to all individuals in whom there is a strong suspicion of FH based on clinical and/or family history (for example, phenotypic HoFH, definite or highly probable phenotypic HeFH in an adult, child or adolescent)	1	B
2. Genetic testing should be considered in individuals with a probable phenotypic diagnosis of HeFH	2	B
3. Genetic testing may be considered in individuals with a phenotypic diagnosis of possible HeFH, especially when there is incomplete information to establish a diagnosis and the genetic result affects clinical management	3	C
4. Genetic testing for FH should be carried out using an accredited method in a certified laboratory, using targeted next-generation sequencing of all exons and exon–intron boundaries of *LDLR*, *APOB*, *PCSK9* and *LDLRAP1*, and the exons in *APOB* that encode the LDLR ligand-binding region, as well as analysis for deletions and duplications in *LDLR*	1	A
5. Variants detected by genetic testing should be classified and reported according to contemporary standardized guidelines, for example, those of the ACMG, AMP or ClinGen FH Variant Curation Expert Panel	1	A
6. If a pathogenic or likely pathogenic variant is not detected, FH should not be excluded, particularly if the clinical phenotype is strongly suggestive of FH, because the condition may result from undetected genetic variants	1	A
7. Genetic counselling should be offered, before and after genetic testing, to all individuals suspected of having FH	1	B
8. Genetic counselling should at a minimum include obtaining a three-generation family medical history, risk and psychological assessment, family-based care, enabling of cascade testing, anticipatory guidance and psychological assessment	1	A
9. Pre-conception counselling should be offered to all couples, especially if both partners/parents are known, or suspected, to have FH	1	B
10. Prenatal or pre-implantation genetic testing should be offered if both partners/parents are known to have FH, counselling being particularly important in parents with HeFH who have previously had a child with HoFH	1	C
11. Polygenic scores for hypercholesterolaemia may be useful but are not yet fully standardized, so that they should be used with caution when assessing the differential diagnosis of FH in clinical practice	3	B
12. Cascade genetic testing is highly cost-effective and should be used after a disease-causing variant has been identified in the proband or index case	1	A
13. Pre-test and post-test genetic counselling should be offered to all at-risk relatives as an integral component of cascade testing	1	A
14. Cascade testing should be undertaken using both phenotypic and genotypic approaches (Fig. 1); if genetic testing is not available, a phenotypic approach (that is, a plasma or serum lipid profile, including the LDL-cholesterol concentration) should be used	1	A
15. Cascade genetic testing for the specific variant (variants) identified in the proband (that is, known familial variant testing) should initially be offered to all first-degree relatives; if first-degree relatives are unavailable, or do not wish to undergo testing, known familial variant testing should be offered to at-risk second-degree and then third-degree relatives, with sequential extension to the entire family until all at-risk individuals have been offered testing (Fig. 1)	1	A
16. At-risk children should be offered cascade genetic testing at the earliest opportunity (and more than once if not pursued at the first offer) if an FH-causing variant has been identified in a parent or other first-degree relative	1	A
17. When genetic testing is not feasible, the diagnosis of FH in at-risk relatives should be made phenotypically using age-specific, sex-specific and country-specific LDL-cholesterol concentrations (Fig. 1; Supplementary Material 4); clinical tools for diagnosing FH probands (such as the Dutch Lipid Clinic Network criteria and Simon Broome criteria) are not valid for this purpose. Phenotypic cascade testing should initially be offered to all first-degree relatives. If first-degree relatives are unavailable, or decline testing, phenotypic testing should next be offered to second-degree and then third-degree relatives, with sequential extension to the entire family until all at-risk individuals have been offered testing	1	A
18. 'Reverse' cascade testing (from child to parents) should be offered to parents after a child is identified as a proband with FH, such as after making a diagnosis following a clinical presentation or via a universal or newborn screening programme	1	B

(Watts et al., 2023).

Canadian Cardiovascular Society (CCS)

In 2018, the CCS released a position statement on FH. With regards to genetic testing, screening and diagnosis of FH, the CCS included the following relevant recommendations:

- Diagnosis of FH
  - "We recommend that FH be defined using the DLCNC [Dutch Lipid Clinic Network Criteria], Simon Broome Registry, or FH Canada definition (Strong Recommendation, High-Quality Evidence)."
- Screening for FH
  - "We recommend that cascade screening (lipid profile) protocols be implemented at the local, provincial, and national level in Canada and offered to first-degree relatives of patients with FH (Strong Recommendation, Moderate-Quality Evidence)."
- Genetics
  - "We recommend that genetic testing be offered, when available, to complement a diagnosis of FH and enable cascade screening (Strong Recommendation, High Quality Evidence). The decision to request genetic screening should be made by the treating physician after discussion with the patient."
- ASCVD [atherosclerotic cardiovascular disease] and FH
  - "We suggest that if available, genetic testing should be used to stratify the ASCVD risk in patients with FH (Weak Recommendation, Moderate-Quality Evidence).
    - Values and preferences. An FH-causing genetic variant increases ASCVD risk, beyond that associated with an elevated LDL-C level. Patients should be informed on the high lifetime risk of ASCVD associated with FH."
- Homozygous FH
  - "We recommend that patients with HoFH be referred to a specialized lipid clinic and undergo complete evaluation for genetic analysis, presence of ASCVD, and aggressive lipid-lowering therapies, including consideration for extracorporeal LDL-C removal, lomitapide, and PCSK9 inhibitors (Strong Recommendation, Moderate-Quality Evidence)" (Brunham et al., 2018).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for

Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81401	Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)
81403	Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons)
81407	Molecular pathology procedure, Level 8 (eg, analysis of 26-50 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of >50 exons, sequence analysis of multiple genes on one platform)

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Ahmad, Z. S., Andersen, R. L., Andersen, L. H., O'Brien, E. C., Kindt, I., Shrader, P., Vasandani, C., Newman, C. B., deGoma, E. M., Baum, S. J., Hemphill, L. C., Hudgins, L. C., Ahmed, C. D., Kullo, I. J., Gidding, S. S., Duffy, D., Neal, W., Wilemon, K., Roe, M. T., . . . Knowles, J. W. (2016). US physician practices for diagnosing familial hypercholesterolemia: data from the CASCADE-FH registry. *J Clin Lipidol*, *10*(5), 1223-1229. https://doi.org/10.1016/j.jacl.2016.07.011

Ambry. (2020). FHNext®. https://www.ambrygen.com/clinician/genetic-testing/13/cardiology/fhnext

Austin, M. A., Hutter, C. M., Zimmern, R. L., & Humphries, S. E. (2004). Familial hypercholesterolemia and coronary heart disease: a HuGE association review. *Am J Epidemiol*, *160*(5), 421-429. https://doi.org/10.1093/aje/kwh237

Barry, M. J., Nicholson, W. K., Silverstein, M., Chelmow, D., Coker, T. R., Davis, E. M., Donahue, K. E., Jaén, C. R., Li, L., Ogedegbe, G., Rao, G., Ruiz, J. M., Stevermer, J., Tsevat, J., & Underwood, S. M. (2023). Screening for Lipid Disorders in Children and Adolescents: US Preventive Services Task Force Recommendation Statement. *Jama*, *330*(3), 253-260. https://doi.org/10.1001/jama.2023.11330

Braamskamp, M., Langslet, G., McCrindle, B. W., Cassiman, D., Francis, G. A., Gagne, C., Gaudet, D., Morrison, K. M., Wiegman, A., Turner, T., Miller, E., Kusters, D. M., Raichlen, J. S., Martin, P. D., Stein, E. A., Kastelein, J. J. P., & Hutten, B. A. (2017). Effect of Rosuvastatin on Carotid Intima-Media Thickness in Children With Heterozygous Familial Hypercholesterolemia: The CHARON Study (Hypercholesterolemia in Children and Adolescents Taking Rosuvastatin Open Label). *Circulation*, *136*(4), 359-366. https://doi.org/10.1161/circulationaha.116.025158

Brown, E. E., Sturm, A. C., Cuchel, M., Braun, L. T., Duell, P. B., Underberg, J. A., Jacobson, T. A., & Hegele, R. A. (2020). Genetic testing in dyslipidemia: A scientific statement from the National Lipid Association. *J Clin Lipidol*. https://doi.org/10.1016/j.jacl.2020.04.011

Brunham, L. R., Ruel, I., Aljenedil, S., Rivière, J. B., Baass, A., Tu, J. V., Mancini, G. B. J., Raggi, P., Gupta, M., Couture, P., Pearson, G. J., Bergeron, J., Francis, G. A., McCrindle, B. W., Morrison, K., St-Pierre, J., Henderson, M., Hegele, R. A., Genest, J., . . . Brophy, J. (2018). Canadian Cardiovascular Society Position Statement on Familial Hypercholesterolemia: Update 2018. *Can J Cardiol*, *34*(12), 1553-1563. https://doi.org/10.1016/j.cjca.2018.09.005

Civeira, F. (2004). Guidelines for the diagnosis and management of heterozygous familial hypercholesterolemia. *Atherosclerosis*, *173*(1), 55-68. https://doi.org/10.1016/j.atherosclerosis.2003.11.010

Cuchel, M., Bruckert, E., Ginsberg, H. N., Raal, F. J., Santos, R. D., Hegele, R. A., Kuivenhoven, J. A., Nordestgaard, B. G., Descamps, O. S., Steinhagen-Thiessen, E.,

Tybjærg-Hansen, A., Watts, G. F., Averna, M., Boileau, C., Borén, J., Catapano, A. L., Defesche, J. C., Hovingh, G. K., Humphries, S. E., . . . Chapman, M. J. (2014). Homozygous familial hypercholesterolaemia: new insights and guidance for clinicians to improve detection and clinical management. A position paper from the Consensus Panel on Familial Hypercholesterolaemia of the European Atherosclerosis Society. *Eur Heart J*, *35*(32), 2146-2157. https://doi.org/10.1093/eurheartj/ehu274

Cuchel, M., Raal, F. J., Hegele, R. A., Al-Rasadi, K., Arca, M., Averna, M., Bruckert, E., Freiberger, T., Gaudet, D., Harada-Shiba, M., Hudgins, L. C., Kayikcioglu, M., Masana, L., Parhofer, K. G., Roeters van Lennep, J. E., Santos, R. D., Stroes, E. S. G., Watts, G. F., Wiegman, A., . . . Ray, K. K. (2023). 2023 Update on European Atherosclerosis Society Consensus Statement on Homozygous Familial Hypercholesterolaemia: new treatments and clinical guidance. *Eur Heart J*, *44*(25), 2277-2291. https://doi.org/10.1093/eurheartj/ehad197

De Backer, G., Besseling, J., Chapman, J., Hovingh, G. K., Kastelein, J. J., Kotseva, K., Ray, K., Reiner, Z., Wood, D., & De Bacquer, D. (2015). Prevalence and management of familial hypercholesterolaemia in coronary patients: An analysis of EUROASPIRE IV, a study of the European Society of Cardiology. *Atherosclerosis*, *241*(1), 169-175. https://doi.org/10.1016/j.atherosclerosis.2015.04.809

Do, R., Stitziel, N. O., Won, H. H., Jorgensen, A. B., Duga, S., Angelica Merlini, P., Kiezun, A., Farrall, M., Goel, A., Zuk, O., Guella, I., Asselta, R., Lange, L. A., Peloso, G. M., Auer, P. L., Girelli, D., Martinelli, N., Farlow, D. N., DePristo, M. A., . . . Kathiresan, S. (2015). Exome sequencing identifies rare LDLR and APOA5 alleles conferring risk for myocardial infarction. *Nature*, *518*(7537), 102-106. https://doi.org/10.1038/nature13917

Elbitar, S., Susan-Resiga, D., Ghaleb, Y., El Khoury, P., Peloso, G., Stitziel, N., Rabes, J. P., Carreau, V., Hamelin, J., Ben-Djoudi-Ouadda, A., Bruckert, E., Boileau, C., Seidah, N. G., Varret, M., & Abifadel, M. (2018). New Sequencing technologies help revealing unexpected mutations in Autosomal Dominant Hypercholesterolemia. *Sci Rep*, *8*(1), 1943. https://doi.org/10.1038/s41598-018-20281-9

France, M., Rees, A., Datta, D., Thompson, G., Capps, N., Ferns, G., Ramaswami, U., Seed, M., Neely, D., Cramb, R., Shoulders, C., Barbir, M., Pottle, A., Eatough, R., Martin, S., Bayly, G., Simpson, B., Halcox, J., Edwards, R., . . . Soran, H. (2016). HEART UK statement on the management of homozygous familial hypercholesterolaemia in the United Kingdom. *Atherosclerosis*, *255*, 128-139. https://doi.org/10.1016/j.atherosclerosis.2016.10.017

Garcia, C. K., Wilund, K., Arca, M., Zuliani, G., Fellin, R., Maioli, M., Calandra, S., Bertolini, S., Cossu, F., Grishin, N., Barnes, R., Cohen, J. C., & Hobbs, H. H. (2001). Autosomal recessive hypercholesterolemia caused by mutations in a putative LDL receptor adaptor protein. *Science*, *292*(5520), 1394-1398. https://doi.org/10.1126/science.1060458

Gidding, S. S., Champagne, M. A., de Ferranti, S. D., Defesche, J., Ito, M. K., Knowles, J. W., McCrindle, B., Raal, F., Rader, D., Santos, R. D., Lopes-Virella, M., Watts, G. F., & Wierzbicki, A. S. (2015). The Agenda for Familial Hypercholesterolemia: A Scientific Statement From the American Heart Association. *Circulation*, *132*(22), 2167-2192. https://doi.org/10.1161/cir.0000000000000297

Goldberg, A. C., Hopkins, P. N., Toth, P. P., Ballantyne, C. M., Rader, D. J., Robinson, J. G., Daniels, S. R., Gidding, S. S., de Ferranti, S. D., Ito, M. K., McGowan, M. P., Moriarty, P. M., Cromwell, W. C., Ross, J. L., & Ziajka, P. E. (2011). Familial hypercholesterolemia: screening, diagnosis and management of pediatric and adult patients: clinical guidance from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. *J Clin Lipidol*, *5*(3 Suppl), S1-8. https://doi.org/10.1016/j.jacl.2011.04.003

Invitae. (2020). *Invitae Familial Hypercholesterolemia Panel*. https://www.invitae.com/en/physician/tests/02401/

Jacobson, T. A., Ito, M. K., Maki, K. C., Orringer, C. E., Bays, H. E., Jones, P. H., McKenney, J. M., Grundy, S. M., Gill, E. A., Wild, R. A., Wilson, D. P., & Brown, W. V. (2015). National lipid association recommendations for patient-centered management of dyslipidemia: part 1--full report. *J Clin Lipidol*, *9*(2), 129-169. https://doi.org/10.1016/j.jacl.2015.02.003

Jellinger, P. S., Handelsman, Y., Rosenblit, P. D., Bloomgarden, Z. T., Fonseca, V. A., Garber, A. J., Grunberger, G., Guerin, C. K., Bell, D. S. H., Mechanick, J. I., Pessah-Pollack, R., Wyne, K., Smith, D., Brinton, E. A., Fazio, S., & Davidson, M. (2017). AMERICAN ASSOCIATION OF CLINICAL ENDOCRINOLOGISTS AND AMERICAN COLLEGE OF ENDOCRINOLOGY GUIDELINES FOR MANAGEMENT OF DYSLIPIDEMIA AND PREVENTION OF CARDIOVASCULAR DISEASE. *Endocr Pract*, *23*(Suppl 2), 1-87. https://doi.org/10.4158/ep171764.appgl

Khera, A. V., Won, H. H., Peloso, G. M., Lawson, K. S., Bartz, T. M., Deng, X., van Leeuwen, E. M., Natarajan, P., Emdin, C. A., Bick, A. G., Morrison, A. C., Brody, J. A., Gupta, N., Nomura, A., Kessler, T., Duga, S., Bis, J. C., van Duijn, C. M., Cupples, L. A., . . . Kathiresan, S. (2016). Diagnostic Yield and Clinical Utility of Sequencing Familial Hypercholesterolemia Genes in Patients With Severe Hypercholesterolemia. *J Am Coll Cardiol*, *67*(22), 2578-2589. https://doi.org/10.1016/j.jacc.2016.03.520

Knowles, J. W., O'Brien, E. C., Greendale, K., Wilemon, K., Genest, J., Sperling, L. S., Neal, W. A., Rader, D. J., & Khoury, M. J. (2014). Reducing the burden of disease and death from familial hypercholesterolemia: a call to action. *Am Heart J*, *168*(6), 807-811. https://doi.org/10.1016/j.ahj.2014.09.001

Lee, S., Akioyamen, L. E., Aljenedil, S., Riviere, J. B., Ruel, I., & Genest, J. (2019). Genetic testing for familial hypercholesterolemia: Impact on diagnosis, treatment and cardiovascular risk. *Eur J Prev Cardiol*, *26*(12), 1262-1270. https://doi.org/10.1177/2047487319829746

Lozano, P., Henrikson, N. B., Dunn, J., Morrison, C. C., Nguyen, M., Blasi, P. R., Anderson, M. L., & Whitlock, E. P. (2016). Lipid Screening in Childhood and Adolescence for Detection of Familial Hypercholesterolemia: Evidence Report and Systematic Review for the US Preventive Services Task Force. *Jama*, *316*(6), 645-655. https://doi.org/10.1001/jama.2016.6176

Mach, F., Baigent, C., Catapano, A. L., Koskinas, K. C., Casula, M., Badimon, L., Chapman, M. J., De Backer, G. G., Delgado, V., Ference, B. A., Graham, I. M., Halliday, A., Landmesser, U., Mihaylova, B., Pedersen, T. R., Riccardi, G., Richter, D. J., Sabatine, M. S., Taskinen, M.-R., . . . Group, E. S. C. S. D. (2019). 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk: The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS). *Eur Heart J*, *41*(1), 111-188. https://doi.org/10.1093/eurheartj/ehz455

Marks, D., Thorogood, M., Neil, H. A., & Humphries, S. E. (2003). A review on the diagnosis, natural history, and treatment of familial hypercholesterolaemia. *Atherosclerosis*, *168*(1), 1-14.

McGowan, M. P., Hosseini Dehkordi, S. H., Moriarty, P. M., & Duell, P. B. (2019). Diagnosis and Treatment of Heterozygous Familial Hypercholesterolemia. *Journal of the American Heart Association*, *8*(24), e013225. https://doi.org/10.1161/JAHA.119.013225

NICE. (2019). *Familial hypercholesterolaemia: identification and management*. https://www.nice.org.uk/guidance/cg71/chapter/Recommendations

Nordestgaard, B. G., Chapman, M. J., Humphries, S. E., Ginsberg, H. N., Masana, L., Descamps, O. S., Wiklund, O., Hegele, R. A., Raal, F. J., Defesche, J. C., Wiegman, A., Santos, R. D., Watts, G. F., Parhofer, K. G., Hovingh, G. K., Kovanen, P. T., Boileau, C., Averna, M., Boren, J., . . . Tybjaerg-Hansen, A. (2013). Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur Heart J*, *34*(45), 3478-3490a. https://doi.org/10.1093/eurheartj/eht273

O'Brien, E. C., Roe, M. T., Fraulo, E. S., Peterson, E. D., Ballantyne, C. M., Genest, J., Gidding, S. S., Hammond, E., Hemphill, L. C., Hudgins, L. C., Kindt, I., Moriarty, P. M., Ross, J., Underberg, J. A., Watson, K., Pickhardt, D., Rader, D. J., Wilemon, K., & Knowles, J. W. (2014). Rationale and design of the familial hypercholesterolemia foundation CAscade SCreening for Awareness and DEtection of Familial Hypercholesterolemia registry. *Am Heart J*, *167*(3), 342-349.e317. https://doi.org/10.1016/j.ahj.2013.12.008

Reeskamp, L. F., Tromp, T. R., Defesche, J. C., Grefhorst, A., Stroes, E. S., Hovingh, G. K., & Zuurbier, L. (2020). Next-generation sequencing to confirm clinical familial

hypercholesterolemia. *Eur J Prev Cardiol*, 2047487320942996. https://doi.org/10.1177/2047487320942996

Rosenson, R., & Durrington, P. (2023, December 10). *Familial hypercholesterolemia in adults: Overview*. https://www.uptodate.com/contents/familial-hypercholesterolemia-in-adults-overview

Santos, R. D., Gidding, S. S., Hegele, R. A., Cuchel, M. A., Barter, P. J., Watts, G. F., Baum, S. J., Catapano, A. L., Chapman, M. J., Defesche, J. C., Folco, E., Freiberger, T., Genest, J., Hovingh, G. K., Harada-Shiba, M., Humphries, S. E., Jackson, A. S., Mata, P., Moriarty, P. M., . . . Yamashita, S. (2016). Defining severe familial hypercholesterolaemia and the implications for clinical management: a consensus statement from the International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel. *Lancet Diabetes Endocrinol*, *4*(10), 850-861. https://doi.org/10.1016/s2213-8587(16)30041-9

Sturm, A. C., Knowles, J. W., Gidding, S. S., Ahmad, Z. S., Ahmed, C. D., Ballantyne, C. M., Baum, S. J., Bourbon, M., Carrié, A., Cuchel, M., Ferranti, S. D. d., Defesche, J. C., Freiberger, T., Hershberger, R. E., Hovingh, G. K., Karayan, L., Kastelein, J. J. P., Kindt, I., Lane, S. R., . . . Foundation, C. b. t. F. H. (2018). Clinical Genetic Testing for Familial Hypercholesterolemia. *Journal of the American College of Cardiology*, *72*(6). https://doi.org/10.1016/j.jacc.2018.05.044

Sturm, A. C., Truty, R., Callis, T. E., Aguilar, S., Esplin, E. D., Garcia, S., Haverfield, E. V., Morales, A., Nussbaum, R. L., Rojahn, S., Vatta, M., & Rader, D. J. (2021). Limited-Variant Screening vs Comprehensive Genetic Testing for Familial Hypercholesterolemia Diagnosis. *JAMA Cardiology*, *6*(8).

Trinder, M., Francis, G. A., & Brunham, L. R. (2020). Association of Monogenic vs Polygenic Hypercholesterolemia With Risk of Atherosclerotic Cardiovascular Disease. *JAMA Cardiology*, *5*(4), 390-399. https://doi.org/10.1001/jamacardio.2019.5954

Trinder, M., Li, X., DeCastro, M. L., Cermakova, L., Sadananda, S., Jackson, L. M., Azizi, H., Mancini, G. B. J., Francis, G. A., Frohlich, J., & Brunham, L. R. (2019). Risk of Premature Atherosclerotic Disease in Patients With Monogenic Versus Polygenic Familial Hypercholesterolemia. *J Am Coll Cardiol*, *74*(4), 512-522. https://doi.org/10.1016/j.jacc.2019.05.043

Versmissen, J., Oosterveer, D. M., Yazdanpanah, M., Defesche, J. C., Basart, D. C., Liem, A. H., Heeringa, J., Witteman, J. C., Lansberg, P. J., Kastelein, J. J., & Sijbrands, E. J. (2008). Efficacy of statins in familial hypercholesterolaemia: a long term cohort study. *Bmj*, *337*, a2423. https://doi.org/10.1136/bmj.a2423

Wald, D. S., Bestwick, J. P., Morris, J. K., Whyte, K., Jenkins, L., & Wald, N. J. (2016). Child-Parent Familial Hypercholesterolemia Screening in Primary Care. *N Engl J Med*, *375*(17), 1628-1637. https://doi.org/10.1056/NEJMoa1602777

Watts, G. F., Gidding, S. S., Hegele, R. A., Raal, F. J., Sturm, A. C., Jones, L. K., Sarkies, M. N., Al-Rasadi, K., Blom, D. J., Daccord, M., de Ferranti, S. D., Folco, E., Libby, P., Mata,

P., Nawawi, H. M., Ramaswami, U., Ray, K. K., Stefanutti, C., Yamashita, S., . . . Santos, R. D. (2023). International Atherosclerosis Society guidance for implementing best practice in the care of familial hypercholesterolaemia. *Nat Rev Cardiol*. https://doi.org/10.1038/s41569-023-00892-0

Wiegman, A., for the European Atherosclerosis Society Consensus, P., Gidding, S. S., for the European Atherosclerosis Society Consensus, P., Watts, G. F., for the European Atherosclerosis Society Consensus, P., Chapman, M. J., for the European Atherosclerosis Society Consensus, P., Ginsberg, H. N., for the European Atherosclerosis Society Consensus, P., Cuchel, M., for the European Atherosclerosis Society Consensus, P., Ose, L., for the European Atherosclerosis Society Consensus, P., Averna, M., for the European Atherosclerosis Society Consensus, P., Boileau, C., for the European Atherosclerosis Society Consensus, P., Borén, J., . . . Ginsberg, H. N. (2015). Familial hypercholesterolaemia in children and adolescents: gaining decades of life by optimizing detection and treatment. *Eur Heart J*, *36*(36), 2425-2437. https://doi.org/10.1093/eurheartj/ehv157

Williams, R. R., Hunt, S. C., Schumacher, M. C., Hegele, R. A., Leppert, M. F., Ludwig, E. H., & Hopkins, P. N. (1993). Diagnosing heterozygous familial hypercholesterolemia using new practical criteria validated by molecular genetics. *Am J Cardiol*, *72*(2), 171-176.

Youngblom, E., Pariani, M., & Knowles, J. W. (2016). Familial Hypercholesterolemia [Text]. *GeneReviews*. https://www.ncbi.nlm.nih.gov/books/NBK174884/

Genetic Testing for Fanconi Anemia

Policy Number: AHS – M2077 – Genetic Testing for Fanconi Anemia	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Fanconi anemia (FA) is an inherited disorder in which cells cannot correctly repair inter-strand crosslinks (ICLs), a specific type of DNA damage that results in genomic instability. This can lead to bone marrow failure (such as aplastic anemia), leukemia, and/or solid tumors. FA is rare, occurring in one in 100,000 to 250,000 births, with an increased incidence in populations such as Ashkenazi Jews and South African Afrikaner populations (Olson, 2022).

For guidance on prenatal or preconception screening for FA, please see AHS-M2179-Prenatal Screening (Genetic).

II. Related Policies

Policy Number	Policy Title
AHS-M2145	General Genetic Testing, Germline Mutations
AHS-M2179	Prenatal Screening (Genetic)

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals who have received genetic counseling and who have clinical signs and symptoms of Fanconi anemia (FA), chromosome breakage testing or gene sequencing (single gene or multi-gene panel testing) for the diagnosis of FA **MEETS COVERAGE CRITERIA**.

2) For all other situations not discussed above, genetic testing for the diagnosis of FA **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note: For two or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
ACMG	American College of Medical Genetics and Genomics
ACOG	American College of Obstetricians and Gynecologists
AML	Acute myeloid leukemia
ATM	Ataxia-telangiectasia mutated
ATR	Ataxia-telangiectasia mutated and Rad3-related protein
BRCA2	*Breast cancer 2 gene*
CBT	Chromosome breakage test
CCO	Cancer Care Ontario
CDK	Cyclin-dependent kinase
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CNV	Copy number variants
DEB	Diepoxybutane
DNA	Deoxyribonucleic acid
FA	Fanconi anemia
FA-A	Fanconi anemia complementation group A
FA-C	Fanconi anemia complementation group C
FA-D1	Fanconi anemia complementation group D1 (breast cancer 2 gene related)
FANCA	Fanconi anemia complementation group A
FANCC	Fanconi anemia complementation group C
FANCD1	Fanconi anemia complementation group D1
FANCD2	Fanconi anemia complementation group D2
FANCF	Fanconi anemia complementation group F
FANCG	Fanconi anemia complementation group G
FANCI	Fanconi anemia complementation group I
FANCM	Fanconi anemia complementation group M
FANCP	Fanconi anemia complementation group P

FARF	Fanconi Anemia Research Fund
FDA	Food and Drug Administration
FISH	Fluorescence in situ hybridization
G2	Second growth phase of the cell
HCT	Hematopoietic cell transplant
HSC	Hematopoietic stem cell
ICLs	Inter-strand cross links
IFAR	The International Fanconi Anemia Registry
LDTs	Laboratory-developed tests
MDS	Myelodysplastic syndrome
MMC	Mitomycin C
NCCN	The National Comprehensive Cancer Network
NGS	Next-generation sequencing
NORD	The National Organization for Rare Disorders
ROS	Reactive oxygen species
SLX4	*SLX4 structure-specific endonuclease subunit*
USPSTF	U.S. Preventive Services Task Force
VACTERL-H	Vertebral anomalies, anal atresia, congenital heart disease, tracheoesophageal fistula, esophageal atresia, renal anomalies, limb anomalies, and hydrocephalus
WES	Whole exome sequencing
WGS	Whole-genome sequencing

V. Scientific Background

Primarily inherited as an autosomal recessive disorder, Fanconi anemia (FA) is associated with known mutations in at least 22 FA identified genes (Jung et al., 2020). It is found equally in males and females, as well as in different ethnic groups; approximately 50% of FA patients are diagnosed by age ten (NORD, 2020). Jung et al. (2020) also noted that siblings with FA often have similar hematological courses, potentially attributed to similarity in causative variants and environmental factors but have different presentations of congenital anomalies (except for kidney abnormalities and microcephaly to a moderate degree). The three most mutated genes in FA are *FANCA*, *FANCC*, and *FANCG*; these comprise up to 80-90% of all FA cases, with *FANCA* mutations accounting for approximately 60% of cases worldwide (Bogliolo et al., 2019; Olson, 2022). The main function of this set of proteins is to repair the inter-strand crosslinks (ICL) that typically form during DNA replication and transcription (Olson, 2022). A cell is estimated to repair about 10 ICLs per day, but as few as 20-40 unresolved ICLs can lead to cell death (Sumpter & Levine, 2017).

The FA pathway may also play a role in other functions, such as metabolizing alcohol, ensuring the stability of the replication fork during DNA replication, managing oxidative stress as in providing defense from reactive oxygen species (ROS)-induced cell death, and repairing double strand breaks (Kottemann & Smogorzewska, 2013; Longerich et al., 2014; Milletti et al., 2020; Olson, 2022). For example, a mutation in the *FANCC* gene was found to impede the cell's ability to clear out damaged mitochondria and viruses, which could eventually lower immunity to viral infection and contribute to the characteristic bone marrow failure (Cheung & Taniguchi, 2017; Sumpter et al., 2016).

Fanconi anemia may manifest in several ways with symptoms including short stature, skin findings such as hyper- or hypo- pigmentation and café-au-lait skin lesions, microcephaly, and abnormalities in the thumb, eye, axial skeleton, ear, renal system, or urinary tract. There is also a potential connection between FA and the VACTERL-H association (three or more of the following: vertebral anomalies, anal atresia, congenital heart disease, tracheoesophageal fistula, esophageal atresia, renal anomalies, limb anomalies, and hydrocephalus) as the percentage of FA patients also meeting the criteria for VACTERL-H was much higher than previously found (Alter & Giri, 2016). However, up to 25-40% of FA patients look physically normal (D'Andrea, 2010). At the physiological level, the most common symptoms are bone marrow failure and cytopenias, such as pancytopenia, macrocytic anemia, or thrombocytopenia (Olson, 2022). Bone marrow failure is reportedly the most common primary symptom in FA and presents itself in 70-80% of patients by age ten (Bogliolo et al., 2019). Though the exact mechanism of premature hematopoietic stem cell (HSC) loss in FA remains unclear, it is thought to be impacted by defective DNA repair, causing increased damage and cell cycle arrest, increased levels of reactive oxygen species and inflammatory cytokines, and damage caused by reactive aldehydes in the absence of intact repair pathways (Olson, 2022).

Aplastic anemia, another common FA side effect which causes the body to halt the production of red blood cells, also typically occurs early, either leading to death or to a HSC transplant. Endocrine issues, such as growth hormone deficiency, abnormal glucose/insulin metabolism, dyslipidemia, pubertal delay, and hypothyroidism are also commonly associated with an FA diagnosis (about 80% of FA children and adults have at least one endocrine defect) and often lead to a worsening life quality in FA patients (Milletti et al., 2020; Shimamura & Alter, 2010).

Screening for Fanconi Anemia

The most common screening assay for Fanconi anemia is the chromosome breakage test. A DNA crosslinking agent, such as mitomycin C (MMC) or diepoxybutane (DEB), is

used to induce chromosome breakage, and the cells are evaluated at their respective stages in the cell cycle. FA cells typically have more DNA damage and are forced to arrest in the G2 phase when these cells can be observed. Tests may be positive, negative, or inconclusive; a positive test typically shows about 90% of lymphocytes with increased breakage, a negative test shows no increased breakage, and an inconclusive test cannot provide any definitive answer (Hays, 2014). Normal cells have a mean baseline of <.05 breaks per cell while FA cells may range from 0.02 – 0.85 breaks per cell. DEB (the more sensitive and specific agent) typically has a mean baseline of <.10 breaks per normal cell and from 1.06 to 23.9 breaks per FA cell (Auerbach, 2015). The International Fanconi Anemia Registry (IFAR) found the mean standard error of breaks per cell of 104 FA patients to be 8.96 ± 0.448 and the mean standard error of percentage of cells with breaks to be 85.15% ± 1.99%, compared to 0.06 ± 0.004 breaks and 5.12% ± 0.28% of 224 non-FA patients (Kook et al., 1998).

Inconclusive results are typically due to one of two possibilities—one is "mosaicism," where two separate populations of lymphocytes in the blood occur, and the other is where the patient has another underlying condition causing chromosome breakage. However, a mutation analysis can corroborate a diagnosis or provide further information. This can be particularly helpful in assessing the patient's family members, such as potential carriers, asymptomatic family members, or members who may develop clinical symptoms (FARF, 2020).

More recently, researchers have utilized whole exome sequencing as a diagnostic method for FA. Historically, molecular diagnostics regarding FA have been challenging for the medical community because the disease is caused by hereditary patterns featuring point mutations and large genomic deletions in an estimated 22 genes (Rio et al., 2019). Nonetheless, the whole exome sequencing method used in this study identified 93.3% of deletions and mutated alleles when compared to a previously validated method, leading the researchers to the conclusion that whole exome sequencing is efficient enough to characterize patients with FA (Rio et al., 2019).

Clinical Utility and Validity

Due to the increased instability of an FA patient's genome, it is common to see an increased risk of cancer in patients with FA, particularly bone marrow cancers such as leukemia. A study found the observed to expected ratio of all cancers to be as high as 48 (i.e., the observed rate was 48 times higher than expected after controlling for factors such as age and sex) (Alter, 2014). The same study found the likelihood that an FA patient would develop acute myeloid leukemia (AML) to be 700 times higher than normal and the likelihood to develop any myelodysplastic syndrome (MDS) to be 6000 times higher (Alter, 2014). Underlying FA disease mechanisms may also be causing

patients to develop cancers at a much earlier age than typically observed. A study focusing on 35 FA patients found that when compared to the general population, those afflicted by FA were, on average, diagnosed with head and neck squamous cell carcinoma 31 years earlier than controls (32 years for FA patients, 63 years for general population). FA mutation type may also play a factor in cancer development as another study found that FA patients with *FANCA* mutations developed cancer at a significantly older age than those with other mutations; however, mutation type did not seem to affect the overall survival rates of FA cancer patients (Steinberg-Shemer et al., 2019). Furthermore, the common risk factors, such as tobacco or alcohol consumption, were typically not a factor for the FA patients as is usually seen in the general population (Kutler et al., 2016).

Another example of how intertwined the FA proteins and pathway is with cancer is found in the *FANCD1* (Fanconi anemia complementation group D1) gene. The *FANCD1* gene, also known as *BRCA2*, is a gene whose mutations often lead to a higher risk of breast cancer. The *BRCA2* (-/-) cell reacts the same way an FA cell does when treated with the crosslinking agents and *BRCA2* co-localizes with *FANCD2* at damaged sections of DNA. The patients with heterozygous genotypes of *BRCA2* are historically more likely to have a higher risk of breast and ovarian cancer (D'Andrea, 2010).

Novel studies have further demonstrated the risk of germline mutations in FA complementation group (FANC) of the FA pathway in cancer. *FANCD2* (Fanconi anemia complementation group D2) was found to confer a malignant phenotype in esophageal squamous cell carcinoma, and cyclin-cyclin-dependent kinase (CDK) and ataxia-telangiectasia RAD3-related/ataxia-telangiectasia mutated (ATR/ATM) signaling was shown to help in depletion of FANCD2 protein expression and suppress cancer cell proliferation (Lei et al., 2020). In a different study done on a Han Chinese population, Yu et al. (2020) identified that Fanconi anemia complement group F (*FANCF*), though already known to impact cell proliferation and DNA repair, can increase risk of colorectal cancer if hypomethylated. Aberrant methylation of *FANCF* was also observed in ovarian tumors, non-small-cell lung cancer, cervical cancer, and oral cancer previously in general populations (Yu et al., 2020). This conveys the markedly increased predisposition to cancer via mutations in FA and FA pathway components.

Chang et al. (2021) discusses novel diagnostic approaches for FA by single-cell sequencing and capillary nano-immunoassay. Next-generation sequencing (NGS) has been widely utilized for FA diagnosis but has limitations that may lead to unconfirmed genetic subtypes. Chang et al. (2021) studies one FA cause with *FANCM* mutation by conducting the capillary-nano-immunoassay to analyze the expression profile of FA-associated proteins. This assay is designed to detect 417 blood disease genes, including the 17 known FA-related genes. In this case, Chang et al. (2021) observed

two homozygous mutations of the *FANCM* and *FANCD1* genes and abnormal expression of both genes simultaneously existed, diagnosing the patient as a FA-D1/FA-M dual subtype. According to the author, "compared with mixed cell sequencing, single-cell sequencing data shows more accuracy for the FA subtype evaluation, while the capillary nano-immunoassay is a good method to detect the expression profile of abnormal or modified FA protein" (Chang et al., 2021).

Chan et al. (2021) studied the genetic spectrum of FA-associated genes across populations of varying ancestries to explore potential genotype-phenotype associations in cancer. A total of 3,523 subjects in Singapore, of varying ancestry, were assessed for carrier frequency and variant spectrum of potentially pathogenic variants in 17 FA genes. The data suggested higher germline and somatic mutation burden between *FANCA* and *FANCC* with head and neck and lung squamous cell carcinomas and *FANCI* and *SLX4/FANCP* with uterine cancer. Additionally, Chan et al. (2021) highlighted the differences in carrier frequencies in non-European populations, considering the fact that our knowledge about the clinical and genetic spectrum of FA is derived predominantly from populations of European ancestry. Consequently, "Given the variable distribution of germline pathogenic variant carriers across different ancestries, genetic testing for molecular diagnosis should not be restricted to *FANCA*, *FANCC*, and *FANCG*, which are reported more frequently in FA patients of European descent, but should include all known FA genes."

Alter et al. (2022) classified patients by age of diagnosis as part of a National Cancer Institute IBMFS cohort that included 178 pediatric patients and 26 adult patients. The authors were investigating whether FA cases could be distinguished and placed into subgroups by age diagnosed. The various features that were compared included the cumulative incidences of first adverse events (severe BMF leading to hematopoietic cell transplant or death, leukemia, or solid tumors) between an adult cohort and the pediatric cohort. The adult group did not consistently have the traditional FA features of birth defects, early-onset bone marrow failure or leukemia, and the group had more patients with the *FANCA* genotype. This adult group developed more head and neck squamous cell carcinoma and/or gynecological cancers (as compared to the pediatric group). The overall conclusion is Hematology and Oncology providers should investigate an FA diagnosis in adult patients that present with early-onset head and neck squamous cell carcinoma or gynecological cancer (with or without hematologic issues) (Alter et al., 2022).

VI. Guidelines and Recommendations

American College of Medical Genetics and Genomics (ACMG)

The guidelines for clinical genetics laboratories are specified in the 2018 (revised January 2021) edition of the ***Standards and Guidelines for Clinical Genetics Laboratories*** by the ACMG. The guidelines on FA state that:

- A cytogenetic evaluation for FA should include an induction of breakage with a crosslinking agent such as MMC or DEB (in addition to a baseline chromosome breakage).
- A well-established negative and positive control should be present and multiple cultures are recommended (if there is enough specimen available).
- At least 50 different cells (banded or unbanded) in the metaphase stage of the cell cycle should be analyzed, and the percentage of cells with aberration should reported (Kaiser-Rogers et al., 2021).

In 2021, ACMG released an updated guideline for screening for autosomal recessive and X-linked conditions during pregnancy and preconception. Their practice resource reviews aim to recommend "a consistent and equitable approach for offering carrier screening to all individuals during pregnancy and preconception" and replaces any earlier ACMG position statements on prenatal/preconception expanded carrier screening and provide the following recommendations:

- "Analytical validity of carrier screening is to be established by a laboratory in compliance with CLIA/CAP regulations and adhering to ACMG Laboratory Standards and Guidelines."
- "As evidence evolves, ClinVar and ClinGen continually update pathogenicity of variants and the association between genes and conditions, respectively."
- "Carrier screening enables those screened to consider their reproductive risks, reproductive options, and to make informed decisions."
- "Published evidence supports clinical utility for carrier screening of multiple conditions simultaneously."
- "The phrase 'expanded carrier screening' be replaced by 'carrier screening.'"
- "Adopting a more precise tiered system based on carrier frequency:
  - Tier 4: <1/200 carrier frequency (includes Tier 3) genes/condition will vary by lab
  - Tier 3: ≥ 1/200 carrier frequency (includes Tier 2) includes X-linked conditions
  - Tier 2: ≥1/100 carrier frequency (includes Tier 1)
  - Tier 1: CF [Cystic Fibrosis] + SMA [spinal muscular atrophy] + Risk Based Screening"

Fanconi anemia falls into Tier 2 carrier screening, according to the ACMG recommendation. For purposes of Fanconi anemia screening, this policy focuses on Tier 2 and Tier 3 carrier screening (as Tier 3 is inclusive of Tier 2). ACMG recommends

that

- "Tier 2 carrier screening stems from an ACOG recommendation for conditions that have a severe or moderate phenotype and a carrier frequency of at least 1/100." However, "data demonstrate that carrier screening for two common conditions using a carrier frequency threshold of 1/100 may not be equitable across diverse populations. Others have shown that limiting the carrier frequency to ≥1/100 creates missed opportunities to identify couples at risk for serious conditions."
- "We define Tier 3 screening as carrier screening for conditions with a carrier frequency ≥1/200 . . . Tier 2 and Tier 3 screening prioritize carrier frequency as a way to think about conditions most appropriate for screening in the general population. However, when ACOG proposed this level, they did not specify whether it was thinking about carrier frequency in terms of the global population or subpopulations. We use "carrier frequency" to mean in any ethnic group with reasonable representation in the United States."
- "All pregnant patients and those planning a pregnancy should be offered Tier 3 carrier screening."
- ACMG does NOT recommend:
  - Offering Tier 1 and/or Tier 2 screening, because these do not provide equitable evaluation of all racial/ethnic groups.
  - Routine offering of Tier 4 panels.
- "Carrier screening paradigms should be ethnic and population neutral and more inclusive of diverse populations to promote equity and inclusion."
- "All pregnant patients and those planning a pregnancy should be offered Tier 3 carrier screening for autosomal recessive (Tables 1–5) and X-linked (Table 6) conditions."
- "Reproductive partners of pregnant patients and those planning a pregnancy may be offered Tier 3 carrier screening for autosomal recessive conditions (Tables 1–5) when carrier screening is performed simultaneously with their partner."
- "All XX patients should be offered screening for only those X-linked genes listed in Table 6 as part of Tier 3 screening."
- "When Tier 1 or Tier 2 carrier screening was performed in a prior pregnancy, Tier 3 screening should be offered" (Gregg et al., 2021).

ACMG table 2 lists autosomal recessive genes for screening with carrier frequency and includes Fanconi anemia, complementation C , as reported below.

Table 2. Autosomal recessive genes for screening with carrier frequency <1/50 to ≥1/100.

OMIM gene	OMIM gene name	Maximum carrier frequency[a]	OMIM phenotype	Conditions
610142	*CEP290*	0.014422	610188	Joubert syndrome 5
			611755	Leber congenital amaurosis 10
607839	*GBE1*	0.013799	232500	Glycogen storage disease, type IV
			263570	GBE1-related disorders
606800	*GAA*	0.013565	232300	Glycogen storage disease, type II (Pompe disease)
100725	*CHRNE*	0.013526	100725	Myasthenic syndrome, congenital, 4A, slow-channel
				Myasthenic syndrome, congenital, 4B, fast-channel
613742	*G6PC*	0.013401	232200	Glycogen storage disease type IA
611409	*OCA2*	0.013113	203200	Oculocutaneous albinism brown and type II
120120	*COL7A1*	0.012995	226600	Recessive dystrophic epidermolysis bullosa
600509	*ABCC8*	0.012242	618857	Diabetes mellitus, permanent neonatal 3
612724	*ALDOB*	0.012119	229600	Hereditary fructosuria
613899	*FANCC*	0.011992	227645	Fanconi anemia, complementation group C
604597	*GRIP1*	0.011989	617667	Fraser syndrome
248611	*BCKDHB*	0.011760	245600	Maple syrup urine disease
613726	*ANO10*	0.010781	613728	Spinocerebellar ataxia 10
104170	*NAGA*	0.010637	609241	Schindler disease, type 1
				Schindler disease, type 3
607608	*SMPD1*	0.010259	257200	Niemann–Pick disease, type A
			607616	Niemann–Pick disease, type B
608400	*USH2A*	0.010203	276901	Usher syndrome, type 2A
609058	*MMUT*	0.009999	251000	Methylmalonic aciduria–methylmalonyl–CoA mutase deficiency
600650	*CPT2*	0.009742	600649	Carnitine palmitoyltransferase II deficiency, infantile
			608836	Carnitine palmitoyltransferase II deficiency, lethal neonatal
608894	*AHI1*	0.009740	608629	Joubert syndrome 3

OMIM Online Mendelian Inheritance in Man.[55]
[a]After rounding values are < 0.02 and ≥ 0.01 (two decimal places).

(Gregg et al., 2021).

American College of Obstetricians and Gynecologists (ACOG) Committee Opinion on Carrier Screening for Genetic Conditions

In March 2017, ACOG issued a Committee Opinion on Carrier Screening for Genetic Conditions. Regarding Fanconi anemia, ACOG asserts the following:

"Fanconi anemia can be caused by mutations in at least 15 different genes, but 80–90% of cases are due to mutation in one of three genes: 1) *FANCA*, 2) *FANCC*, and 3) *FANCG*. Affected individuals can experience bone marrow failure; increased risk of cancer, including leukemia and solid tumors; and structural defects such as short stature, skin pigment changes, nervous system abnormalities (including central nervous system malformations), eye and ear malformations and hearing loss, skeletal abnormalities in particular affecting the thumb or forearms, gastrointestinal abnormalities (including effects on the oral cavity), and others. Of note, 25–40% of affected individuals do not have any physical abnormalities" (ACOG, 2017).

These ACOG guidelines were reaffirmed in 2023.

Second Pediatric Blood and Marrow Transplant Consortium International Conference on Late Effects after Pediatric HCT

Due to recent increase in survival following a hematopoietic cell transplant (HCT), the conference recommends continued screening and follow up with a wide variety of specialists, with focus on the late side-effects of HCT. The conference emphasizes the importance of screening for cancer due to the increased cumulative risk (Dietz et al., 2017).

The National Organization for Rare Disorders (NORD)

The National Organization for Rare Disorders has published several recommendations for testing patients with suspected FA. These recommendations state that "FA should be suspected and tested for in any infant born with the thumb and arm abnormalities described previously. Anyone developing aplastic anemia at any age should be tested for FA, even if no other defects are present. Any patient who develops squamous cell carcinoma of the head and neck, gastrointestinal or gynecologic system at an early age with or without a history of tobacco or alcohol use, should be tested for FA. Many FA patients show no other abnormalities. It is essential to test for FA before contemplating stem cell transplantation for aplastic anemia or treatment for cancer, as standard chemotherapy and radiation protocols may prove toxic to FA patients" (NORD, 2020).

"Complementation testing is usually done first in order to identify which FA gene is mutated. Sequence analysis of the appropriate gene can then be done to determine the specific mutation in that gene. If a mutation is not identified, deletion/duplication analysis is available clinically for the genes associated with FA. Targeted mutation analysis is available for the common Ashkenazi Jewish FANCC mutation" (NORD, 2020).

Cancer Care Ontario (CCO)

In December 2016, the CCO published recommendations for malignant hematology conditions. It is stated that patients aged <50 years with suspected aplastic anemia may be tested for FA via a peripheral blood chromosomal breakage analysis, such as the diepoxybutane test (DEB Test). However, "it would also be indicated to screen older patients if FA is clinically suspected. It is difficult to set an upper age limit for FA screening, as anecdotal cases have been diagnosed in the fifth decade (unpublished observations). Screen all patients who are transplant candidates and siblings of FA patients". An abdominal ultrasound scan and echocardiogram is also indicated in some instances as "an enlarged spleen and/or lymph nodes raise the possibility of a malignant haematological disorder as the cause of the pancytopenia. In younger patients, abnormal or anatomically displaced kidneys are features of FA" (CCO, 2016).

The National Comprehensive Cancer Network (NCCN)

As FA often results in higher incidence of cancers, the NCCN has noted some observations regarding this condition. In the guideline for Esophageal and Esophagogastric Junction Cancers, the NCCN stated that

"The genes involved in Fanconi anemia (FA) include FA complementation groups A–E, with FA-A (FANCA) located at 16q24.3; FA-B (FANCB), unknown; FA-C (FANCC) at 9q22.3; FA-D (FANCD) at 3p26–p22; and FA-E (FANCE), unknown. Mutations in FANCA and FANCC have been identified. Individuals are identified by pancytopenia and chromosome breakage and hematologic abnormalities, including anemia, bleeding, and easy bruising. Increased frequency of SCC of the esophagus as well as other squamous epithelium is observed. Karyotyping does not identify individuals with FA, but enhanced chromosome breakage with mitomycin C can identify homozygotes but not heterozygotes" (NCCN, 2024).

United Kingdom National Multidisciplinary Guidelines

These recommendations were specifically made in the context of head and neck cancers. The recommendations for Fanconi anemia (FA) are as follows:

- "FA patients should receive prophylactic vaccination against high-risk HPV virus.
- FA patients should have quarterly screening for head and neck squamous cell carcinoma and an aggressive biopsy policy…treatment for head and neck squamous cell carcinoma with surgery alone where possible".
- FA patients should follow up with a specialty Fanconi clinic (Shaw & Beasley, 2016).

U.S. Preventive Services Task Force (USPSTF)

No U.S. Preventive Services Task Force recommendations for genetic testing for FA have been identified. A search for "Fanconi" on the USPSTF website turned up no results on October 15, 2024.

Fanconi Anemia Research Fund (FARF)

In 2023, the Fanconi Anemia Research Fund updated their recommendations on testing and genetic counseling. For a patient suspected of having FA (FARF, 2023):

- "Any patient suspected of having FA should be referred to a hematologist and/or clinical geneticist or genetic counselor, who can arrange for diagnostic testing. As FA testing is highly specialized, particularly the evaluation of chromosome breakage in response to DNA damage, only laboratories with extensive

experience should undertake this testing."

- "The recommended testing procedures are outlined in the flow chart in Figure 1. The flow chart presents one potential algorithm for testing, starting with chromosome breakage testing and ending with genotyping. However, genetic testing has become increasingly utilized as a first-line diagnostic test, especially for newborns and pediatric patients with multiple congenital anomalies. Regardless of the order of testing, it is important that both chromosome breakage and germline genetic testing be performed to obtain a precise diagnosis in all persons with suspected FA."
- The gold-standard test for diagnosing Fanconi anemia (FA) is the chromosome breakage test using DNA crosslinking agents, specifically diepoxybutane (DEB). Mitomycin C (MMC) may also be used, but it has a higher potential for false-positive results. Dotted line: If chromosome breakage testing is unavailable, a diagnosis of FA can be directly confirmed by genetic testing. If peripheral blood testing is negative but clinical suspicion for FA remains high, the negative result may be a false negative because of somatic mosaicism in the peripheral blood. Therefore, repeat testing on fibroblasts cultured from a skin biopsy should be performed. If the chromosome breakage testing is positive, FA panel testing should be performed. If FA panel testing is negative, whole exome sequencing (WES) and/or research testing should be considered. In cases where chromosome breakage testing on both blood and skin cells is negative, referral for evaluation of conditions that overlap clinically with FA should be considered."
- "Following a positive chromosome breakage test, NGS panel testing for clinically relevant FANC genes should be offered as the next step. Clinical laboratories have evolved to offer two types of panel tests: dedicated panels (laboratory pre-selected genes associated with a patient's phenotype) and custom panels (self-selection of relevant genes from a large list of genes). When selecting a panel, it is important to consider whether the test has been designed to address variant hotspots and/or gene regions known to present reporting challenges. As an example, the FANCD2 gene has two pseudogenes that can complicate the accuracy and interpretation of test results. Because of rapidly evolving knowledge of FA, many laboratories have not yet added the more recently discovered FANC genes to their panels. Thus, most currently available panels evaluate only a subset of the 22 known FANC genes."
- "Following the diagnosis of FA, cytogenetic analysis of chromosomes in bone marrow cells should be performed using standard G-banding methodology. The goal of this analysis is to investigate for the presence of a clone with acquired chromosome abnormalities and, if present, to characterize the observed abnormalities. Identification of a clone, which involves the presence of the same numerical and/or structural chromosomal abnormalities in multiple cells, is an

indication of an abnormal hematologic process. The significance of cytogenetic findings must be interpreted within the context of the clinical findings, bone marrow morphologic findings from hematopathology examination, and immunophenotyping results."

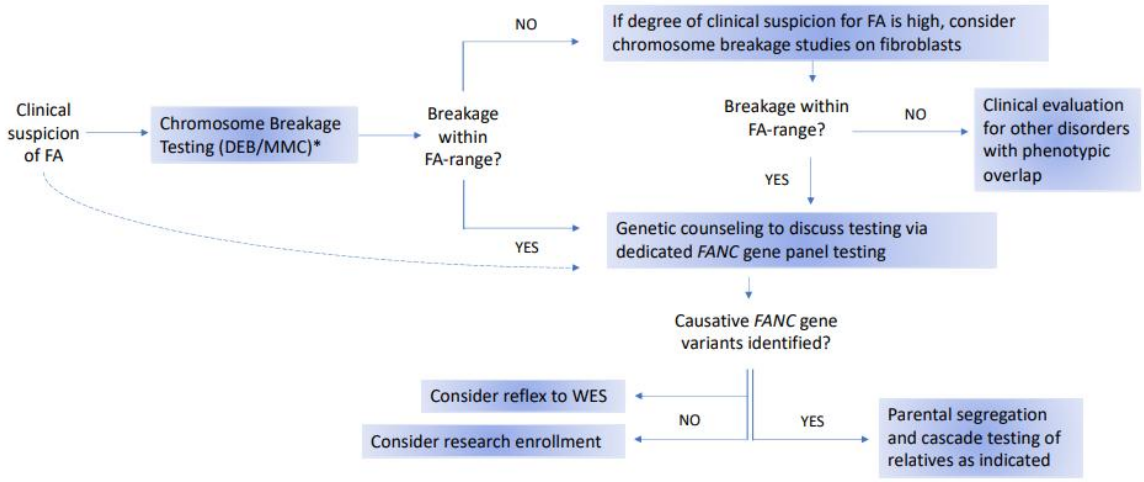


Figure 1: *Schematic representing a suggested algorithm for Fanconi anemia testing* (FARF, 2023).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81242	FANCC (Fanconi Anemia, complementation group C) (e.g.: Fanconi Anemia, type C) gene analysis, common variant (e.g.: IVS4+4A>T)
81412	Ashkenazi Jewish associated disorders (eg, Bloom syndrome, Canavan disease, cystic fibrosis, familial dysautonomia, Fanconi anemia group C, Gaucher disease, Tay-Sachs disease), genomic sequence analysis panel, must include sequencing of at least 9 genes, including ASPA, BLM, CFTR, FANCC, GBA, HEXA, IKBKAP, MCOLN1, and SMPD1
81443	Genetic testing for severe inherited conditions (eg, cystic fibrosis, Ashkenazi Jewish-associated disorders [eg, Bloom syndrome, Canavan disease, Fanconi anemia type C, mucolipidosis type VI, Gaucher disease, Tay-Sachs disease], beta hemoglobinopathies, phenylketonuria, galactosemia), genomic sequence analysis panel, must include sequencing of at least 15 genes (eg, ACADM, ARSA, ASPA, ATP7B, BCKDHA, BCKDHB, BLM, CFTR, DHCR7, FANCC, G6PC, GAA, GALT, GBA, GBE1, HBB, HEXA, IKBKAP, MCOLN1, PAH)
88230	Tissue culture for non-neoplastic disorders; lymphocyte
88248	Chromosome analysis for breakage syndromes; baseline breakage, score 50-100 cells, count 20 cells, 2 karyotypes (eg, for ataxia telangiectasia, Fanconi anemia, fragile X)
88249	Chromosome analysis for breakage syndromes; score 100 cells, clastogen stress (eg, diepoxybutane, mitomycin C, ionizing radiation, UV radiation
88291	Cytogenetics and molecular cytogenetics, interpretation and report

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

ACOG. (2017). Carrier Screening for Genetic Conditions. https://www.acog.org/clinical/clinical-guidance/committee-opinion/articles/2017/03/carrier-screening-for-genetic-conditions

Alter, B. P. (2014). Fanconi anemia and the development of leukemia. *Best Pract Res Clin Haematol*, *27*(3-4), 214-221. https://doi.org/10.1016/j.beha.2014.10.002

Alter, B. P., & Giri, N. (2016). Thinking of VACTERL-H? Rule out Fanconi Anemia according to PHENOS. *Am J Med Genet A*, *170*(6), 1520-1524. https://doi.org/10.1002/ajmg.a.37637

Alter, B. P., Giri, N., McReynolds, L. J., & Altintas, B. (2022). Fanconi anaemia: A syndrome with distinct subgroups. *Br J Haematol*, *197*(4), 467-474. https://doi.org/10.1111/bjh.18091

Auerbach, A. D. (2015). Diagnosis of Fanconi anemia by diepoxybutane analysis. *Curr Protoc Hum Genet*, *85*, 8.7.1-17. https://doi.org/10.1002/0471142905.hg0807s85

Bogliolo, M., Pujol, R., Aza-Carmona, M., Munoz-Subirana, N., Rodriguez-Santiago, B., Casado, J. A., Rio, P., Bauser, C., Reina-Castillon, J., Lopez-Sanchez, M., Gonzalez-Quereda, L., Gallano, P., Catala, A., Ruiz-Llobet, A., Badell, I., Diaz-Heredia, C., Hladun, R., Senent, L., Argiles, B., . . . Surralles, J. (2019). Optimised molecular genetic diagnostics of Fanconi anaemia by whole exome sequencing and functional studies. *J Med Genet*. https://doi.org/10.1136/jmedgenet-2019-106249

CCO. (2016). Consensus Pathology Recommendations for Complex Malignant Hematology. https://www.cancercareontario.ca/en/guidelines-advice/types-of-cancer/43061

Chan, S. H., Ni, Y., Li, S.-T., Teo, J. X., Ishak, N. D. B., Lim, W. K., & Ngeow, J. (2021). Spectrum of Germline Mutations Within Fanconi Anemia–Associated Genes Across Populations of Varying Ancestry. *JNCI Cancer Spectrum*, *5*(1). https://doi.org/10.1093/jncics/pkaa117

Chang, L., Gao, X., Ji, G., Cheng, X., Zou, Y., Cheng, T., Yuan, W., & Zhu, X. (2021). Novel diagnostic approaches for Fanconi anemia (FA) by single-cell sequencing and capillary nano-immunoassay. *Blood Science*, *3*(1), 20-25. https://doi.org/10.1097/bs9.0000000000000065

Cheung, R. S., & Taniguchi, T. (2017). Recent insights into the molecular basis of Fanconi anemia: genes, modifiers, and drivers. *Int J Hematol*, *106*(3), 335-344. https://doi.org/10.1007/s12185-017-2283-4

D'Andrea, A. D. (2010). Susceptibility pathways in Fanconi's anemia and breast cancer. *N Engl J Med*, *362*(20), 1909-1919. https://doi.org/10.1056/NEJMra0809889

Dietz, A. C., Savage, S. A., Vlachos, A., Mehta, P. A., Bresters, D., Tolar, J., Bonfim, C., Dalle, J. H., de la Fuente, J., Skinner, R., Boulad, F., Duncan, C. N., Baker, K. S., Pulsipher, M. A., Lipton, J. M., Wagner, J. E., & Alter, B. P. (2017). Late Effects Screening Guidelines after Hematopoietic Cell Transplantation for Inherited Bone Marrow Failure Syndromes: Consensus Statement From the Second Pediatric Blood and Marrow Transplant Consortium International Conference on Late Effects After Pediatric HCT. *Biol Blood Marrow Transplant*, *23*(9), 1422-1428. https://doi.org/10.1016/j.bbmt.2017.05.022

FARF. (2020). Fanconi Anemia Clinical Care Guidelines, Fifth Edition. https://www.fanconi.org/images/uploads/other/Fanconi_Anemia_Clinical_Care_Guidelines_5thEdition_web.pdf

FARF. (2023). Diagnosis of Fanconi Anemia: Testing and Genetic Counseling. https://fanconi.org/wp-content/uploads/2024/03/Chapter_2._Diagnosis__July_2023_.pdf

Gregg, A. R., Aarabi, M., Klugman, S., Leach, N. T., Bashford, M. T., Goldwaser, T., Chen, E., Sparks, T. N., Reddi, H. V., Rajkovic, A., Dungan, J. S., Practice, A. P., & Guidelines, C. (2021). Screening for autosomal recessive and X-linked conditions during pregnancy and preconception: a practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *23*(10), 1793-1806. https://doi.org/10.1038/s41436-021-01203-z

Hays, L. (2014). *Fanconi Anemia: Guidelines for Diagnosis and Management* (4th ed.). Fanconi Anemia Research Fund. https://www.fanconi.org/images/uploads/other/FARF_Guidelines_Book_interior_lo-res.pdf

Jung, M., Mehta, P. A., Jiang, C. S., Rosti, R. O., Usleaman, G., Correa da Rosa, J. M., Lach, F. P., Goodridge, E., Auerbach, A. D., Davies, S. M., Smogorzewska, A., & Boulad, F. (2020). Comparison of the clinical phenotype and haematological course of siblings with Fanconi anaemia. *Br J Haematol*. https://doi.org/10.1111/bjh.17061

Kaiser-Rogers, K., Astbury, C., & Scott, A. I. (2021). STANDARDS AND GUIDELINES FOR CLINICAL GENETICS LABORATORIES. https://www.acmg.net/PDFLibrary/ACMG%20Technical%20Lab%20Standards%20Section%20A.pdf

Kook, H., Cho, D., Cho, S. H., Hong, W. P., Kim, C. J., Park, J. Y., Yoon, W. S., Ryang, D. W., & Hwang, T. J. (1998). Fanconi anemia screening by diepoxybutane and mitomicin C tests in Korean children with bone marrow failure syndromes. *J Korean Med Sci*, *13*(6), 623-628. https://doi.org/10.3346/jkms.1998.13.6.623

Kottemann, M. C., & Smogorzewska, A. (2013). Fanconi anaemia and the repair of Watson and Crick DNA crosslinks. *Nature*, *493*(7432), 356-363. https://doi.org/10.1038/nature11863

Kutler, D. I., Patel, K. R., Auerbach, A. D., Kennedy, J., Lach, F. P., Sanborn, E., Cohen, M. A., Kuhel, W. I., & Smogorzewska, A. (2016). Natural history and management of Fanconi anemia patients with head and neck cancer: A 10-year follow-up. *Laryngoscope*, *126*(4), 870-879. https://doi.org/10.1002/lary.25726

Lei, L. C., Yu, V. Z., Ko, J. M. Y., Ning, L., & Lung, M. L. (2020). FANCD2 Confers a Malignant Phenotype in Esophageal Squamous Cell Carcinoma by Regulating Cell Cycle Progression. *Cancers (Basel)*, *12*(9). https://doi.org/10.3390/cancers12092545

Longerich, S., Li, J., Xiong, Y., Sung, P., & Kupfer, G. M. (2014). Stress and DNA repair biology of the Fanconi anemia pathway. *Blood*, *124*(18), 2812-2819. https://doi.org/10.1182/blood-2014-04-526293

Milletti, G., Strocchio, L., Pagliara, D., Girardi, K., Carta, R., Mastronuzzi, A., Locatelli, F., & Nazio, F. (2020). Canonical and Noncanonical Roles of Fanconi Anemia Proteins:

Implications in Cancer Predisposition. *Cancers (Basel)*, *12*(9). https://doi.org/10.3390/cancers12092684
NCCN. (2024, July 24, 2024). *Esophageal and Esophagogastric Junction Cancers Version 4.2024 — July 30,2024*. https://www.nccn.org/professionals/physician_gls/pdf/esophageal.pdf
NORD. (2020). Fanconi Anemia. https://rarediseases.org/rare-diseases/fanconi-anemia/
Olson, T. S. (2022, 06/16/2022). *Clinical manifestations and diagnosis of Fanconi anemia*. https://www.uptodate.com/contents/clinical-manifestations-and-diagnosis-of-fanconi-anemia
Rio, P., Navarro, S., Wang, W., Sanchez-Dominguez, R., Pujol, R. M., Segovia, J. C., Bogliolo, M., Merino, E., Wu, N., Salgado, R., Lamana, M. L., Yanez, R. M., Casado, J. A., Gimenez, Y., Roman-Rodriguez, F. J., Alvarez, L., Alberquilla, O., Raimbault, A., Guenechea, G., . . . Bueren, J. A. (2019). Successful engraftment of gene-corrected hematopoietic stem cells in non-conditioned patients with Fanconi anemia. *Nat Med*, *25*(9), 1396-1401. https://doi.org/10.1038/s41591-019-0550-z
Shaw, R., & Beasley, N. (2016). Aetiology and risk factors for head and neck cancer: United Kingdom National Multidisciplinary Guidelines. *J Laryngol Otol*, *130*(S2), S9-s12. https://doi.org/10.1017/s0022215116000360
Shimamura, A., & Alter, B. P. (2010). Pathophysiology and management of inherited bone marrow failure syndromes. *Blood Rev*, *24*(3), 101-122. https://doi.org/10.1016/j.blre.2010.03.002
Steinberg-Shemer, O., Goldberg, T. A., Yacobovich, J., Levin, C., Koren, A., Revel-Vilk, S., Ben-Ami, T., Kuperman, A. A., Shkalim Zemer, V., Toren, A., Kapelushnik, J., Ben-Barak, A., Miskin, H., Krasnov, T., Noy-Lotan, S., Dgany, O., & Tamary, H. (2019). Characterization and genotype-phenotype correlation of patients with Fanconi anemia in a multi-ethnic population. *Haematologica*. https://doi.org/10.3324/haematol.2019.222877
Sumpter, R., Jr., & Levine, B. (2017). Emerging functions of the Fanconi anemia pathway at a glance. *J Cell Sci*, *130*(16), 2657-2662. https://doi.org/10.1242/jcs.204909
Sumpter, R., Jr., Sirasanagandla, S., Fernández Á, F., Wei, Y., Dong, X., Franco, L., Zou, Z., Marchal, C., Lee, M. Y., Clapp, D. W., Hanenberg, H., & Levine, B. (2016). Fanconi Anemia Proteins Function in Mitophagy and Immunity. *Cell*, *165*(4), 867-881. https://doi.org/10.1016/j.cell.2016.04.006
Yu, H., Pan, R., Gao, T., Wu, D., Ying, J., & Duan, S. (2020). FANCF hypomethylation is associated with colorectal cancer in Han Chinese. *Turk J Gastroenterol*, *31*(8), 558-565. https://doi.org/10.5152/tjg.2020.19394

Genetic Testing for *FMR1* Mutations

Policy Number: AHS – M2028 – Genetic Testing for *FMR1* Mutations	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Fragile X syndrome (FXS) is an X-linked disorder resulting from a loss of function mutation of the Fragile X Mental Retardation-1 (*FMR1*) gene (Saul & Tarleton, 1993); FXS is the most common cause of heritable intellectual disability (Coffee et al., 2009). *FMR1*-related disorders include FXS, fragile X-associated tremor/ataxia syndrome (FXTAS), and *FMR1*-related primary ovarian insufficiency (FRPOI). FXS results in a range of physical, cognitive, and behavioral effects of variable severity (Mila et al., 2016), generally characterized by moderate intellectual disability and autistic characteristics in affected males and mild intellectual disability and emotional and/or psychiatric problems in affected females (Mila et al., 2016; Monaghan et al., 2013).

Terms such as male and female are used when necessary to refer to sex assigned at birth. For guidance on prenatal or pre-conception screening for FXS, please see AHS-M2179-Prenatal Screening (Genetic).

II. Related Policies

Policy Number	Policy Title
AHS-M2167	Genetic Testing for Neurodegenerative Disorders
AHS-M2176	Testing for Developmental Delay
AHS-M2179	Prenatal Screening (Genetic)

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals who have received genetic counseling, diagnostic genetic testing for *FMR1* gene CGG repeats (including AGG interruption testing) and methylation status **MEETS COVERAGE CRITERIA** for any of the following conditions:
   a) For individuals with unexplained mental retardation, developmental delay, or autism spectrum disorder.
   b) For symptomatic individuals with features of Fragile X syndrome or a family history of Fragile X syndrome.
   c) For females with unexplained ovarian insufficiency, unexplained ovarian failure, or unexplained elevated FSH prior to 40 years of age.
   d) For individuals with unexplained late-onset tremor-ataxia.
   e) For fetuses and offspring of known *FMR1* premutation or full mutation carriers.
2) Genetic screening for *FMR1* gene CGG repeat length more than once per lifetime **DOES NOT MEET COVERAGE CRITERIA.**
3) Determination of *FMR1* gene point mutations **DOES NOT MEET COVERAGE CRITERIA**.
4) Determination of *FMR1* gene deletion **DOES NOT MEET COVERAGE CRITERIA**.
5) General population screening for Fragile X syndrome **DOES NOT MEET COVERAGE CRITERIA**.
6) Cytogenetic testing for Fragile X syndrome **DOES NOT MEET COVERAGE CRITERIA**.
7) Testing for the FMRP protein **DOES NOT MEET COVERAGE CRITERIA**.

IV. Table of Terminology

Term	Definition
ACMG	American College of Medical Genetics and Genomics
ACOG	The American College of Obstetricians and Gynecologists
CCMG	Canadian College of Medical Geneticists
CI	Confidence interval
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid
CNS	Central nervous system
DBS	Dried blood spots
dTP-PCR	Triplet-primed polymerase chain reaction

EMQN	European Molecular Genetics Quality Network
FDA	Food and Drug Administration
FMR1	*Fragile X mental retardation-1*
FMRP	Protein encoded by *Fragile X mental retardation-1*
FRPOI	*Fragile X mental retardation-1*-Related Primary Ovarian Insufficiency
FSH	Follicle stimulating hormone
FXS	Fragile X syndrome
FXTAS	Fragile X-associated tremor/ataxia syndrome
LDT	Laboratory-developed test
mGluR	Metabotropic glutamate receptor 2
mRNA	Messenger ribonucleic acid
miRNA	Micro ribonucleic acid
MoMe	Methylation mosaic
MoMN	Mosaicism for full mutation and normal alleles
MoMP	Mosaic premutation and full mutation
mRNA	Messenger ribonucleic acid
NORD	National Organization for Rare Disorders\
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
SB	Southern blotting
SNP	Single nucleotide polymorphisms
SOGC	Society of Obstetricians and Gynecologists of Canada

V. Scientific Background

Fragile X Syndrome (FXS) and related disorders affects about one in 4,000 males and one in 6,000 to 8,000 females in America (NORD, 2022). Transmitted as an X-linked dominant trait with reduced penetrance, FXS is associated with a fragile site on the X chromosome (Yu et al., 1991) identified as the *Fragile X Mental Retardation-1* (*FMR1*) gene (Santoro et al., 2012). More than 99% of patients with FXS have a mutation in this gene with over 200 CGG repeats and atypical methylation (NORD, 2022). The protein encoded by *FMR1* (FMRP) is a multifunctional RNA-binding protein that regulates the translation of a subset of dendritic mRNAs and plays a central role in neuronal development and synaptic plasticity (Antar et al., 2006; Ascano et al., 2012; Bechara et al., 2009; Castagnola et al., 2018; Didiot et al., 2008; Kenny et al., 2014; Parvin et al., 2018; Yang et al., 2018). The absence of FMRP results in excessive and persistent mGluR-mediated protein synthesis in postsynaptic dendrites, dysregulation of ion homeostasis, and disruption of calcium ion homeostasis leading to abnormal synaptic signaling and dendritic development (Bear et al., 2004; Castagnola et al., 2018;

Finucane et al., 2012). The typical clinical phenotype includes intellectual disability, social impairment, autism spectrum disorder, speech and language delay, neurological dysfunction (seizures and abnormal sleep patterns), sensory hypersensitivity (Rais et al., 2018), and a characteristic physical appearance that typically develops in the second decade of life (Hersh & Saul, 2011). Autism disorders are seen in approximately one third of FXS patients, affecting males more frequently than females (Ormazabal et al., 2019). Between 55 and 90% of patients with autism and FXS report gaze aversion, hand flapping, repetitive behaviors, reduced social interaction, anxiety, speech preservations, and aggressive behaviors (Reisinger et al., 2020).

Any genetic alteration that results in a lack of functional FMRP can cause FXS symptoms. The most common type of mutation of *FMR1* is the expansion of a CGG trinucleotide repeat in the 5′ untranslated region of the gene (Jin & Warren, 2000). Normally, this ranges in size from seven to about 60 repeats, with 30 being most common (Peprah, 2012). The full mutation consists of expansions of over 200 repeats which become abnormally hypermethylated, silencing the *FMR1* gene and expression of FMRP (Maurin et al., 2014; Oberle et al., 1991). Molecular clinical correlations have shown that the resulting phenotype is related to the degree of methylation and mosaicism rather than the number of repeats (Hersh & Saul, 2011).

Alleles with 55 to 200 CGG repeats are generally unmethylated with normal transcript and FMRP level; however, they are extremely unstable during transmission to the next generation and are referred to as premutations (Zafarullah et al., 2020). Although premutation carriers produce normal levels of FMRP, mRNA levels are elevated, causing toxic effects such as protein sequestration and mitochondrial dysfunction (Garcia-Arocena & Hagerman, 2010; Tassone et al., 2000). As a consequence, RNA toxicity leads to neuronal toxicity and a spectrum of premutation associated disorders such as primary ovarian insufficiency (FXPOI) (Rosario & Anderson, 2020) and tremor ataxia syndrome (FXTAS) (Zafarullah et al., 2020). An increased frequency of neurological, psychological, endocrine, and immune-related characteristics has been documented in premutation carriers (Hagerman & Hagerman, 2013; Raspa et al., 2018). Those with the premutation have higher rates of anxiety, depression, autistic traits, and physical health symptoms such as chronic fatigue and pain, fibromyalgia, and sleep disorders (Johnson et al., 2020).

It has been found that AGG interruptions (when an error in DNA replication results in an AGG interrupting the CGG repeat tract with *FMR1*) may affect the stability of the fragile X triplet repeat in a positive manner. The presence of an AGG interruption has been found to substantially impact the risk of a full mutation expansion from a given repeat length. There is an emerging role for AGG genotyping to "clarify the course of fragile X genetic diagnosis, counseling, and patient management" (Latham et al., 2014).

Others have also noted that the risk of unstable transmissions should be based on the presence or absence of AGG interruptions, not on the classical cutoffs which define the risk categories of *FMR1* alleles (Villate et al., 2020).

Analytical Validity

While many fragile X testing methods have been developed, no single approach can characterize all aspects of *FMR1* mutations and expansions, especially when mosaicism is taken into consideration (Monaghan et al., 2013). In a diagnostic setting, it is important to not only detect presence of the CGG expansion, but to also determine its size and methylation status (Lim et al., 2017). Molecular diagnostic testing of *FMR1* currently relies on a combination of polymerase chain reaction (PCR) and Southern blot (the gold standard) for the CGG repeat expansion and methylation analyses (Cai et al., 2019; Rajan-Babu & Chong, 2016). Detection of rare point mutations and deletions requires sequence analysis (Sitzmann et al., 2018; Suhl & Warren, 2015). This has limited the ability to implement any type of population screening (Riley & Wheeler, 2017).

CGG repeat-primed PCR designed to detect the full range of fragile X expanded alleles followed by analysis via capillary electrophoresis (Chen et al., 2010; Lyon et al., 2010) and melt curve techniques (Rajan-Babu et al., 2015; Teo et al., 2012) minimizes the need for Southern blot analysis. The FastFraX *FMR1* test was evaluated in 198 archived clinical samples, yielding results of 100% sensitivity (95% CI, 91.03% to 100%) and 100% specificity (95% CI, 97.64% to 100%) in categorizing patient samples into the respective normal, intermediate, premutation, and full mutation genotypes (Lim et al., 2017).

The triplet-primed PCR method (dTP-PCR) has been validated by comparison to Southern blot analysis for use in determining mutations in the *FMR1* gene; clinical performance was confirmed with 40 samples resulting in 100% sensitivity and 90.48% specificity in the detection of CGG repeats greater than 30 (Skrlec et al., 2018). This testing method may be utilized to screen a general population by quickly determining specific allelic changes in the *FMR1* gene (Skrlec et al., 2018).

Immunohistochemical detection of FMRP has been validated in lymphocytes and chorionic villi samples as an alternative prenatal diagnostic method for detection of full mutations in male fetuses; however, staining is more complex in female fetuses due to X-inactivation and is insufficient for diagnostic use (Oostra & Willemsen, 2001; Willemsen et al., 2002). Clinical and analytical specificity and sensitivity of cytogenetic analysis for FXS are both insufficient (Monaghan et al., 2013).

In a retrospective design, Ramos et al. (2020) studied the performance of the commercial FragileEase PCR kit for FXS diagnosis and compared it to Southern blotting (SB), PCR, and AmplideX FMR1 PCR. Ninety DNA samples were analyzed using FragileEase from patients with a clinical suspicion of FXS or a family history and was compare with the results from the other methods. Overall, FragileEase PCR kit had high concordance with the results from PCR, SB, and AmplideX. FragileEase was able to detect normal, intermediate/gray zone, premutation, and full mutation alleles along with female homozygosity and mosaicism. The authors conclude that "FragileEase™ PCR, as well as other commercially available kits, efficiently detect *FMR1* mutations and simplify the workflow in laboratories that performing FXS diagnoses" (Ramos et al., 2020).

Clinical Utility and Validity

As the clinical phenotype of FMR-related diseases can be subtle, its detection, especially in the prepubertal period, can be difficult. Although phenotypic symptoms are not obvious at birth, both animal and neuroimaging studies suggest that the effects of FXS begin in the prenatal period (Riley & Wheeler, 2017). Families report significant delays in diagnosis of FXS with 24% of families reporting that they had seen a healthcare provider more than ten times before testing. On average, caregivers or other individuals first report concern in regard to the child's development by 13 months; however, professional confirmation of a developmental delay did not occur until an average age of 21 months, and the FXS diagnosis did not occur until an average age of nearly 32 months. Meanwhile, many families had additional children with FXS before becoming aware of the reproductive risk (Bailey et al., 2003). Establishing a diagnosis of FXS allows for an understanding of the disorder and education on appropriate management strategies. Psychopharmacologic intervention to modify behavioral problems, such as attentional deficits, impulse control, anxiety, and emotional lability in a child with FXS can be important in addition to speech therapy, occupational therapy, special educational services, and behavioral interventions (Hersh & Saul, 2011). A recent pilot of allopregnanolone in six males with FXTAS showed significant improvement in GABA metabolism, oxidative stress, and some of the mitochondria-related outcomes (Napoli et al., 2018).

Huang et al. (2019) utilized a GC-rich PCR method to detect *FMR1* gene mutations in 30 pregnant woman who were known carriers of *FMR1* mutations or who contained *FMR1* gene deletion mosaicism; samples utilized chorionic villus, amniotic fluid, or umbilical blood samples. Southern blotting was used as a confirmatory measure. PCR results showed that 18 fetuses were normal, while others presented with full *FMR1* gene mutations, premutations, and/or mosaicism. Even with successful results, the

authors state that the use of a single detention method may not be sufficient in determining *FMR1* genetic mutations (Huang et al., 2019).

Lee et al. (2020) utilized a customized PCR and software system to detect the *FMR1* gene expansions from dried blood spots (DBS) and performed analytical validity studies to determine its accuracy, specificity, sensitivity, and precision to be used for newborn screening. The study investigated 963 newborn dried blood spots, which were studied by DNA extraction, *FMR1* PCR amplification, and capillary electrophoresis for automated CGG repeat analysis. While previous *FMR1* newborn screening assays were unsuitable for a routine laboratory setting, this fit-for-purpose FMR1 screening method provides a reliable method for newborn screening that is both cost effective and compatible with simple DBS elution methods already used in newborn screening laboratories. From the 963 DBS samples tested, 957 samples (99.4%) samples were classified as normal and six samples (0.6%) had premutation alleles with 55-76 CGG repeat expansions. Five out of the six premutation samples had one normal allele in addition to the premutation allele, while one out of the six had only one allele. Accuracy testing results were 100% concordance with reference genotypes with no false positive or false negative test results found. CGG expansions were consistently within six CGG repeats for larger expansions up to 200, within three CGG repeats for expansions up to 137, and within a single repeat for CGG expansions less than 80. However, the authors wrote that "further studies are required to identify if early screening of Fragile X syndrome would lead to better outcomes for the children, families, and society" (Lee et al., 2020).

Approximately twenty individuals have been reported with rare missense or nonsense mutations in the *FMR1* gene; also reported were other coding disturbances of the same gene resulting in physical, cognitive, and behavioral features similarly seen in FXS (Sitzmann et al., 2018). Studies of other FMR mutations that can affect the level and function of the protein include analysis of SNPs showing that 31.66 % of the *FMR1* gene SNPs were disease-related and that 50% of SNPs from online databases had a pathogenic effect (Tekcan, 2016). Screening of 508 males with clinical signs of mental retardation and developmental delay, but without CGG and GCC repeat expansions in the *FMR1* gene, revealed two missense mutations in the *FMR1* gene that would have not been diagnosed with standard molecular testing for FXS (Handt et al., 2014).

Cao et al. (2021) studied the clinical utility of screening *FMR1* gene mutations during early and middle pregnancy for those carrying high-risk CGG trinucleotide expansions. DNA samples from, 316 pregnant women at 12-21 gestational weeks were collected and analyzed for CGG repeats using fluorescence PCR and capillary electrophoresis. The carrier rate of CGG repeats was one in 178 for the intermediate type and one in

772 for the premutation types. The highest frequency allele of CGG was 29 repeats, which accounted for 49.29%, followed by 30 repeats (28.56%) and 36 repeats (8.83%). In one case of a premutation type of CGG expansion, the couple chose to terminate the pregnancy. The authors conclude that "pregnant women should be screened for *FMR1* gene mutations during early and middle pregnancy, and those with high-risk CGG expansions should undergo prenatal diagnosis, genetic counseling and family study" (Cao et al., 2021). Ramos et al. (2021) conducted a cross-sectional study on *FMR1* gene mutations in 52 Brazilian women diagnosed with primary ovarian insufficiency. The authors extracted genomic DNA and used FragileEase PCR kits to analyze CGG trinucleotide repeat expansions in the *FMR1* gene. In total, 3.8% of participants had *FMR1* mutations. The authors further concluded that "the most frequent CGG-repeat sizes were 28 and 30" (Ramos et al., 2021).

Fisher et al. (2021) studied the predisposition of carriers to a neurodegenerative disease called Fragile X-associated tremor/ataxia syndrome (FXTAS). FXTAS is caused by "expansions of the CGG repeats in the 5' upstream region of the FMR1 gene from the normal range" (Fisher et al., 2021) The authors noted that individuals in the premutation group showed CGG expansion sizes with a peak in the 80-99 repeat size range. The two groups in the study included 33 participants who were controls and 41 participants who were FMR1 premutation carriers. The authors were interested in the role of mitochondrial dysfunction and associated cellular-stress signaling in carriers versus healthy control subjects. Results confirmed "the elevation of AMPK and mitochondrial respiratory activities and reduction in reactive O_2 species (ROS) levels in premutation cells and revealed for the first time that target of rapamycin complex I (TORC1) activities are reduced" (Fisher et al., 2021). This study also confirmed findings of a previous study in which they reported significant elevation of mitochondrial respiratory functions in FMR1 premutation carriers.

Lindstrand et al. (2022) conducted a study comparing diagnostic methods in patients with intellectual disability and neurodevelopmental disorders using genome sequencing or chromosomal microarray with or without *FMR1* analysis. From the genomic sequencing tests, when using it first, the diagnostic yield was 35%, 26% for when genomic sequencing was second, and 11% for CMA with or without FMR1 analysis. They also identified that costs were higher with CMA/FMR1, and that the majority (91%) of those with a negative result from the CMA/FMR1 analysis remain undiagnosed of definitive intellectual disability or neurodevelopmental disorder. This demonstrated that genome testing may be superior to traditional CMA and FMR1 analysis as a first-line test for those with neurocognitive difficulties, thus rendering it a faster and more cost effective method that is worth further investigating (Lindstrand et al., 2022).

VI. Guidelines and Recommendations

American College of Medical Genetics and Genomics (ACMG)

The American College of Medical Genetics and Genomics (ACMG) recommends FXS molecular genetic testing for:

"Fragile X syndrome:

- Individuals of either sex with mental retardation, developmental delay, or autism, especially if they have (a) any physical or behavioral characteristics of fragile X syndrome, (b) a family history of fragile X syndrome, or (c) male or female relatives with undiagnosed mental retardation.
- Individuals seeking reproductive counseling who have (a) a family history of fragile X syndrome or (b) a family history of undiagnosed mental retardation.
- Fetuses of known carrier mothers.
- Affected individuals or their relatives in the context of a positive cytogenetic fragile X test result who are seeking further counseling related to the risk of carrier status among themselves or their relatives. The cytogenetic test was used prior to the identification of the FMR1 gene and is significantly less accurate than the current DNA test. DNA testing on such individuals is warranted to accurately identify premutation carriers and to distinguish premutation from full mutation carrier women.

Ovarian dysfunction:

- Women who are experiencing reproductive or fertility problems associated with elevated follicle stimulating hormone (FSH) levels, especially if they have (a) a family history of premature ovarian failure, (b) a family history of fragile X syndrome, or (c) male or female relatives with undiagnosed mental retardation.

Tremor/ataxia syndrome:

- Men and women who are experiencing late onset intention tremor and cerebellar ataxia of unknown origin, especially if they have (a) a family history of movement disorders, (b) a family history of fragile X syndrome, or (c) male or female relatives with undiagnosed mental retardation" (Sherman et al., 2005).

The 2013 ACMG Fragile X testing standards and guidelines, with the American Congress of Obstetricians and Gynecologists, published the following indications for fragile X diagnostic testing and carrier detection:

- "The identification of a full mutation in a male is considered diagnostic rather than predictive, inasmuch as penetrance of fragile X syndrome is virtually 100% in males and the age of onset is not variable.
- The identification of a full mutation in a female may be diagnostic, but [over] 50% of females with full mutations have intellectual disability. They may, however, have some manifestations of the disease such as avoidance personality, mood, or stereotypic disorders. Nonrandom X inactivation may explain the milder phenotype in females, although the extent of symptoms cannot be determined by X-inactivation patterns from diagnostic tests that determine the expansion and methylation in blood.
- The identification of a premutation in an asymptomatic male or female undergoing carrier testing (e.g., due to a family history of intellectual disability) is predictive because FXPOI and FXTAS are not fully penetrant and are dependent on both age and allele size.
- All positive results should state that genetic counseling is recommended and testing is available for at-risk family members" (Monaghan et al., 2013).

In 2021, ACMG released an updated guideline for screening for autosomal recessive and X-linked conditions during pregnancy and pre-conception. Their practice resource aims to recommend "a consistent and equitable approach for offering carrier screening to all individuals during pregnancy and preconception" and replaces any earlier ACMG position statements on prenatal/preconception expanded carrier screening and provide the following recommendations:

- "Carrier screening enables those screened to consider their reproductive risks, reproductive options, and to make informed decisions."
- "The phrase "expanded carrier screening" be replaced by "carrier screening"."
- "Adopting a more precise tiered system based on carrier frequency:
  - Tier 4: <1/200 carrier frequency (includes Tier 3) genes/condition will vary by lab
  - Tier 3: ≥ 1/200 carrier frequency (includes Tier 2) includes X-linked conditions
  - Tier 2: ≥1/100 carrier frequency (includes Tier 1)
  - Tier 1: *CF* [Cystic Fibrosis] + *SMA* [spinal muscular atrophy] + Risk Based Screening
    - "Tier 1 screening conveys the recommendations previously adopted by ACMG and ACOG" and "adopts an ethnic and population neutral approach when screening for cystic fibrosis and spinal muscular atrophy. Beyond

these two conditions, additional carrier screening is determined after risk assessment, which incorporates personal medical and family history as well as laboratory and imaging information where appropriate".
    - "Tier 2 carrier screening stems from an ACOG recommendation for conditions that have a severe or moderate phenotype and a carrier frequency of at least 1/100." However, "data demonstrate that carrier screening for two common conditions using a carrier frequency threshold of 1/100 may not be equitable across diverse populations. Others have shown that limiting the carrier frequency to ≥1/100 creates missed opportunities to identify couples at risk for serious conditions."
    - "We define Tier 3 screening as carrier screening for conditions with a carrier frequency ≥1/200 . . . Tier 2 and Tier 3 screening prioritize carrier frequency as a way to think about conditions most appropriate for screening in the general population. However, when ACOG proposed this level, they did not specify whether it was thinking about carrier frequency in terms of the global population or subpopulations. We use "carrier frequency" to mean in any ethnic group with reasonable representation in the United States."
    - "Tier 4 includes genes less common than those in Tier 3 and can identify additional at-risk couples. Tier 4 has no lower limit carrier screening frequency and can greatly extend the number of conditions screened . . . the clinical validity at this level of carrier screening may be less compelling, therefore we suggest reserving this level of screening for consanguineous pregnancies (second cousins or closer) and in couples where family or medical history suggests Tier 4 screening might be beneficial . . . Importantly, patients should understand that their chance of being a carrier for one or more conditions increases as the number of conditions screened is increased."
- "All pregnant patients and those planning a pregnancy should be offered Tier 3 carrier screening."
- Tier 4 screening should be considered:
    - When a pregnancy stems from a known or possible consanguineous relationship (second cousins or closer);
    - When a family or personal medical history warrants.
- ACMG does NOT recommend:
    - Offering Tier 1 and/or Tier 2 screening, because these do not provide equitable evaluation of all racial/ethnic groups.
    - Routine offering of Tier 4 panels.
- "Carrier screening paradigms should be ethnic and population neutral and more inclusive of diverse populations to promote equity and inclusion."

- "All pregnant patients and those planning a pregnancy should be offered Tier 3 carrier screening for … X-linked (Table 6) conditions."
- "All XX patients should be offered screening for only those X-linked genes listed in Table 6 as part of Tier 3 screening."
- "When Tier 1 or Tier 2 carrier screening was performed in a prior pregnancy, Tier 3 screening should be offered" (Gregg et al., 2021).

Table 6. X-linked genes recommended for carrier screening.

OMIM gene	OMIM gene name	OMIM phenotype	Phenotype
300371	*ABCD1*	300100	Adrenoleukodystrophy (ALD)
300806	*AFF2*	309548	Mental retardation, X-linked, associated with fragile site FRAXE
300382	*ARX*	308350	Developmental and epileptic encephalopathy 1 (DEE1)
300377	*DMD*	300376	Muscular dystrophy, Becker type (BMD)
		310200	Muscular dystrophy, Duchenne type (DMD)
306700	*F8*	300841	Hemophilia A (HEMA)
300746	*F9*	306900	Hemophilia B (HEMB)
309550	*FMR1*	300624	Fragile X syndrome (FXS)
300644	*GLA*	301500	Fabry disease
308840	*L1CAM*	307000	Hydrocephalus due to congenital stenosis of aqueduct of Sylvius (HSAS)
300552	*MID1*	300000	Opitz GBBB syndrome, type I (GBBB1)
300473	*NR0B1*	300200	Adrenal hypoplasia, congenital (AHC)
300461	*OTC*	311250	Ornithine transcarbamylase deficiency
300401	*PLP1*	312920	Spastic paraplegia 2, X-linked (SPG2)
312610	*RPGR*	300029	Retinitis pigmentosa 3 (RP3; RP)
		300455	Retinitis pigmentosa, X-linked, and sinorespiratory
		300834	Infections, with or without deafness
			Macular degeneration, X-linked atrophic
300839	*RS1*	312700	Retinoschisis 1, X-linked, juvenile (RS1)
300036	*SLC6A8*	300352	Cerebral creatine deficiency syndrome 1 (CCDS1)

OMIM Online Mendelian Inheritance in Man.[55]

Table six from (Gregg et al., 2021)

The American College of Obstetricians and Gynecologists (ACOG)

The American College of Obstetricians and Gynecologists (ACOG) published committee opinion 691 in 2017, that was reaffirmed in 2023 which recommends Fragile X premutation carrier screening for women with a family history of fragile X-related disorders or intellectual disability suggestive of fragile X syndrome and who are considering pregnancy or are currently pregnant (ACOG, 2017).

If a woman has unexplained ovarian insufficiency or failure or an elevated follicle stimulating hormone level before age 40 years, fragile X carrier screening is recommended to determine whether she has an *FMR1* premutation.

All identified individuals with intermediate results and carriers of a fragile X premutation or full mutation should be provided follow-up genetic counseling to

discuss the risk to their offspring of inheriting an expanded full mutation fragile X allele and to discuss fragile X-associated disorders (premature ovarian insufficiency and fragile X tremor/ataxia syndrome).

Prenatal diagnostic testing for FXS should be offered to known carriers of the fragile X premutation or full mutation (ACOG, 2017).

Society of Obstetricians and Gynecologists of Canada (SOGC) and Canadian College of Medical Geneticists (CCMG) Guidelines

Guidelines for FXS genetic testing were given in a joint statement from the SOGC and CCMG. It is stated that "Any woman with a personal or family history of Fragile X- or Fragile X mental retardation 1–related disorders; unexplained intellectual disability or developmental delay; autism; ovarian insufficiency with elevated follicle stimulating hormone at age < 40 years of unknown etiology; or any woman with a family history of male relatives with developmental delay, autism, or isolated cerebellar ataxia and tremor should be offered screening for this condition (II-2A) (GRADE moderate/moderate)" (Wilson et al., 2016). It is also stated that "Population carrier screening for Fragile X syndrome in all women of reproductive age cannot be recommended at this time (II-2D) (GRADE moderate/moderate)" and "Fragile X carrier testing must only occur after detailed genetic counselling and informed consent from the woman to be tested has been obtained (III-A) (GRADE low/moderate)" (Wilson et al., 2016). This statement has since been retired as of 2023. The most updated guidelines regarding prenatal screening for fetal aneuploidy, fetal anomalies, and adverse pregnancy outcomes as well as guidelines for using chromosomal microarray analysis for prenatal diagnosis and assessment of fetal loss from SOGC and CCMG do not make a direct mention of FXS genetic testing (Armour et al., 2018; Audibert et al., 2017)..

National Society of Genetic Counselors (NSGC)

The National Society of Genetic Counselors published guidelines, which recommend: "Centers offering population screening should ensure that they have the resources available to provide pre- and post-test genetic counseling that supports the psychosocial and clinical needs of the patient and family. In light of widespread *FMR1* testing among women without known risk factors, genetic counselors should anticipate seeing patients who did not receive any pre-test information, have no prior knowledge of *FMR1*-associated disorders, and are unprepared to learn that they have an *FMR1* mutation. Prenatal diagnosis should be offered to women with pre- or full mutations. Males with premutation alleles should receive genetic counseling about potential

phenotypic risks to their daughters, all of whom will inherit premutations" (Finucane et al., 2012).

American Academy of Pediatrics Committee on Genetics (AAP)

The American Academy of Pediatrics recommends testing for FXS in children with any of the following, particularly when associated with physical and behavioral characteristics of FXS or a relative with undiagnosed intellectual disability: developmental delay, borderline intellectual abilities or intellectual disability, or diagnosis of autism without a specific etiology (Hersh & Saul, 2011).

European Molecular Genetics Quality Network (EMQN)

The EMQN published their best practice guidelines concerning FXS and fragile X-associated disorders in 2015. They state, "Prenatal testing is not indicated for the pregnant partner of a male with a premutation." but they do recommend offering prenatal diagnosis to any woman with 55 or more CGG repeats; "Prenatal testing may be considered for a female fetus of a full mutation father as a cautionary measure (full mutation or MoMP [mosaic premutation and full mutation] or MoMe [methylation mosaic])." Concerning molecular diagnostic analysis in FXS and fragile X-associated disorders, they state the following:

"It is best practice to use a method which detects the whole range of expansions when testing relatives (including prenatal diagnosis) in a family with any known fragile X disorder due to expansion. When testing the FMR1 gene in population screening, the report must specify that rare cases of point mutation or deletion cannot be detected, nor rare cases of CGG expansion mosaicism (MoMN) if the method used cannot detect the whole range of expansions. It could be useful to confirm results by an independent method when detecting an expansion in an index case depending on specific pitfalls of each method" (Biancalana et al., 2015).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81243	FMR1 (fragile X mental retardation 1) (e.g., fragile X mental retardation) gene analysis; evaluation to detect abnormal (e.g., expanded) alleles
81244	FMR1 (fragile X mental retardation 1) (e.g., fragile X mental retardation) gene analysis; characterization of alleles (e.g., expanded size and promoter methylation status)

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

ACOG. (2017). Committee Opinion No. 691: Carrier Screening for Genetic Conditions. *Obstet Gynecol*, *129*(3), e41-e55. https://doi.org/10.1097/aog.0000000000001952

Antar, L. N., Li, C., Zhang, H., Carroll, R. C., & Bassell, G. J. (2006). Local functions for FMRP in axon growth cone motility and activity-dependent regulation of filopodia and spine synapses. *Mol Cell Neurosci*, *32*(1-2), 37-48. https://doi.org/10.1016/j.mcn.2006.02.001

Armour, C. M., Dougan, S. D., Brock, J. A., Chari, R., Chodirker, B. N., DeBie, I., Evans, J. A., Gibson, W. T., Kolomietz, E., Nelson, T. N., Tihy, F., Thomas, M. A., & Stavropoulos, D. J. (2018). Practice guideline: joint CCMG-SOGC recommendations for the use of chromosomal microarray analysis for prenatal diagnosis and assessment of fetal loss in Canada. *J Med Genet*, *55*(4), 215-221. https://doi.org/10.1136/jmedgenet-2017-105013

Ascano, M., Jr., Mukherjee, N., Bandaru, P., Miller, J. B., Nusbaum, J. D., Corcoran, D. L., Langlois, C., Munschauer, M., Dewell, S., Hafner, M., Williams, Z., Ohler, U., & Tuschl, T. (2012). FMRP targets distinct mRNA sequence elements to regulate protein expression. *Nature*, *492*(7429), 382-386. https://doi.org/10.1038/nature11737

Audibert, F., De Bie, I., Johnson, J.-A., Okun, N., Wilson, R. D., Armour, C., Chitayat, D., & Kim, R. (2017). No. 348-Joint SOGC-CCMG Guideline: Update on Prenatal Screening

for Fetal Aneuploidy, Fetal Anomalies, and Adverse Pregnancy Outcomes. *Journal of Obstetrics and Gynaecology Canada*, *39*(9), 805-817. https://doi.org/10.1016/j.jogc.2017.01.032

Bailey, D. B., Jr., Skinner, D., & Sparkman, K. L. (2003). Discovering fragile X syndrome: family experiences and perceptions. *Pediatrics*, *111*(2), 407-416. https://doi.org/10.1542/peds.111.2.407

Bear, M. F., Huber, K. M., & Warren, S. T. (2004). The mGluR theory of fragile X mental retardation. *Trends Neurosci*, *27*(7), 370-377. https://doi.org/10.1016/j.tins.2004.04.009

Bechara, E. G., Didiot, M. C., Melko, M., Davidovic, L., Bensaid, M., Martin, P., Castets, M., Pognonec, P., Khandjian, E. W., Moine, H., & Bardoni, B. (2009). A novel function for fragile X mental retardation protein in translational activation. *PLoS Biol*, *7*(1), e16. https://doi.org/10.1371/journal.pbio.1000016

Biancalana, V., Glaeser, D., McQuaid, S., & Steinbach, P. (2015). EMQN best practice guidelines for the molecular genetic testing and reporting of fragile X syndrome and other fragile X-associated disorders. *Eur J Hum Genet*, *23*(4), 417-425. https://doi.org/10.1038/ejhg.2014.185

Cai, X., Arif, M., Wan, H., Kornreich, R., & Edelmann, L. J. (2019). Clinical Genetic Testing for Fragile X Syndrome by Polymerase Chain Reaction Amplification and Southern Blot Analyses. *Methods Mol Biol*, *1942*, 11-27. https://doi.org/10.1007/978-1-4939-9080-1_2

Cao, Q., Mu, W., Sun, D., Zhu, J., Ge, J., Peng, Y., & Zhang, J. (2021). [Significance and case analysis of FMR1 mutation screening during early and middle pregnancy]. *Zhonghua yi xue yi chuan xue za zhi = Zhonghua yixue yichuanxue zazhi = Chinese journal of medical genetics*, *38*(5), 450-453. https://doi.org/10.3760/cma.j.cn511374-20200319-00181

Castagnola, S., Delhaye, S., Folci, A., Paquet, A., Brau, F., Duprat, F., Jarjat, M., Grossi, M., Beal, M., Martin, S., Mantegazza, M., Bardoni, B., & Maurin, T. (2018). New Insights Into the Role of Cav2 Protein Family in Calcium Flux Deregulation in Fmr1-KO Neurons. *Front Mol Neurosci*, *11*, 342. https://doi.org/10.3389/fnmol.2018.00342

Chen, L., Hadd, A., Sah, S., Filipovic-Sadic, S., Krosting, J., Sekinger, E., Pan, R., Hagerman, P. J., Stenzel, T. T., Tassone, F., & Latham, G. J. (2010). An information-rich CGG repeat primed PCR that detects the full range of fragile X expanded alleles and minimizes the need for southern blot analysis. *J Mol Diagn*, *12*(5), 589-600. https://doi.org/10.2353/jmoldx.2010.090227

Coffee, B., Keith, K., Albizua, I., Malone, T., Mowrey, J., Sherman, S. L., & Warren, S. T. (2009). Incidence of fragile X syndrome by newborn screening for methylated FMR1 DNA. *Am J Hum Genet*, *85*(4), 503-514. https://doi.org/10.1016/j.ajhg.2009.09.007

Didiot, M. C., Tian, Z., Schaeffer, C., Subramanian, M., Mandel, J. L., & Moine, H. (2008). The G-quartet containing FMRP binding site in FMR1 mRNA is a potent exonic

splicing enhancer. *Nucleic Acids Res*, *36*(15), 4902-4912. https://doi.org/10.1093/nar/gkn472

Finucane, B., Abrams, L., Cronister, A., Archibald, A. D., Bennett, R. L., & McConkie-Rosell, A. (2012). Genetic counseling and testing for FMR1 gene mutations: practice guidelines of the national society of genetic counselors. *J Genet Couns*, *21*(6), 752-760. https://doi.org/10.1007/s10897-012-9524-8

Fisher, P. R., Allan, C. Y., Sanislav, O., Atkinson, A., Ngoei, K. R. W., Kemp, B. E., Storey, E., Loesch, D. Z., & Annesley, S. J. (2021). Relationships between Mitochondrial Function, AMPK, and TORC1 Signaling in Lymphoblasts with Premutation Alleles of the FMR1 Gene. *Int J Mol Sci*, *22*(19). https://doi.org/10.3390/ijms221910393

Garcia-Arocena, D., & Hagerman, P. J. (2010). Advances in understanding the molecular basis of FXTAS. *Hum Mol Genet*, *19*(R1), R83-89. https://doi.org/10.1093/hmg/ddq166

Gregg, A. R., Aarabi, M., Klugman, S., Leach, N. T., Bashford, M. T., Goldwaser, T., Chen, E., Sparks, T. N., Reddi, H. V., Rajkovic, A., Dungan, J. S., Practice, A. P., & Guidelines, C. (2021). Screening for autosomal recessive and X-linked conditions during pregnancy and preconception: a practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *23*(10), 1793-1806. https://doi.org/10.1038/s41436-021-01203-z

Hagerman, R., & Hagerman, P. (2013). Advances in clinical and molecular understanding of the FMR1 premutation and fragile X-associated tremor/ataxia syndrome. *Lancet Neurol*, *12*(8), 786-798. https://doi.org/10.1016/S1474-4422(13)70125-X

Handt, M., Epplen, A., Hoffjan, S., Mese, K., Epplen, J. T., & Dekomien, G. (2014). Point mutation frequency in the FMR1 gene as revealed by fragile X syndrome screening. *Mol Cell Probes*, *28*(5-6), 279-283. https://doi.org/10.1016/j.mcp.2014.08.003

Hersh, J. H., & Saul, R. A. (2011). Health supervision for children with fragile X syndrome. *Pediatrics*, *127*(5), 994-1006. https://doi.org/10.1542/peds.2010-3500

Huang, W., Xue, J., Kang, H., Guan, X., Teng, Y., Wu, L., & Duan, R. (2019). [Prenatal diagnosis for 30 women carrying a FMR1 mutation]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*, *36*(9), 866-869. https://www.ncbi.nlm.nih.gov/pubmed/31515777

Jin, P., & Warren, S. T. (2000). Understanding the molecular basis of fragile X syndrome. *Hum Mol Genet*, *9*(6), 901-908. https://doi.org/10.1093/hmg/9.6.901

Johnson, K., Herring, J., & Richstein, J. (2020). Fragile X Premutation Associated Conditions (FXPAC). *Front Pediatr*, *8*, 266. https://doi.org/10.3389/fped.2020.00266

Kenny, P. J., Zhou, H., Kim, M., Skariah, G., Khetani, R. S., Drnevich, J., Arcila, M. L., Kosik, K. S., & Ceman, S. (2014). MOV10 and FMRP regulate AGO2 association with microRNA recognition elements. *Cell Rep*, *9*(5), 1729-1741. https://doi.org/10.1016/j.celrep.2014.10.054

Latham, G. J., Coppinger, J., Hadd, A. G., & Nolin, S. L. (2014). The role of AGG interruptions in fragile X repeat expansions: a twenty-year perspective. *Front Genet*, *5*, 244. https://doi.org/10.3389/fgene.2014.00244

Lee, S., Taylor, J. L., Redmond, C., Hadd, A. G., Kemppainen, J. A., Haynes, B. C., Shone, S., Bailey, D. B., Jr., & Latham, G. J. (2020). Validation of Fragile X Screening in the Newborn Population Using a Fit-for-Purpose FMR1 PCR Assay System. *J Mol Diagn*, *22*(3), 346-354. https://doi.org/10.1016/j.jmoldx.2019.11.002

Lim, G. X., Yeo, M., Koh, Y. Y., Winarni, T. I., Rajan-Babu, I. S., Chong, S. S., Faradz, S. M., & Guan, M. (2017). Validation of a commercially available test that enables the quantification of the numbers of CGG trinucleotide repeat expansion in FMR1 gene. *PLoS One*, *12*(3), e0173279. https://doi.org/10.1371/journal.pone.0173279

Lindstrand, A., Ek, M., Kvarnung, M., Anderlid, B. M., Björck, E., Carlsten, J., Eisfeldt, J., Grigelioniene, G., Gustavsson, P., Hammarsjö, A., Helgadóttir, H. T., Hellström-Pigg, M., Kuchinskaya, E., Lagerstedt-Robinson, K., Levin, L., Lieden, A., Lindelöf, H., Malmgren, H., Nilsson, D., . . . Nordgren, A. (2022). Genome sequencing is a sensitive first-line test to diagnose individuals with intellectual disability. *Genet Med*, *24*(11), 2296-2307. https://doi.org/10.1016/j.gim.2022.07.022

Lyon, E., Laver, T., Yu, P., Jama, M., Young, K., Zoccoli, M., & Marlowe, N. (2010). A simple, high-throughput assay for Fragile X expanded alleles using triple repeat primed PCR and capillary electrophoresis. *J Mol Diagn*, *12*(4), 505-511. https://doi.org/10.2353/jmoldx.2010.090229

Maurin, T., Zongaro, S., & Bardoni, B. (2014). Fragile X Syndrome: from molecular pathology to therapy. *Neurosci Biobehav Rev*, *46 Pt 2*, 242-255. https://doi.org/10.1016/j.neubiorev.2014.01.006

Mila, M., Rodriguez-Revenga, L., & Matilla-Duenas, A. (2016). FMR1 Premutation: Basic Mechanisms and Clinical Involvement. *Cerebellum*, *15*(5), 543-545. https://doi.org/10.1007/s12311-016-0808-7

Monaghan, K. G., Lyon, E., Spector, E. B., & American College of Medical, G. a. G. (2013). ACMG Standards and Guidelines for fragile X testing: a revision to the disease-specific supplements to the Standards and Guidelines for Clinical Genetics Laboratories of the American College of Medical Genetics and Genomics. *Genet Med*, *15*(7), 575-586. https://doi.org/10.1038/gim.2013.61

Napoli, E., Schneider, A., Wang, J. Y., Trivedi, A., Carrillo, N. R., Tassone, F., Rogawski, M., Hagerman, R. J., & Giulivi, C. (2018). Allopregnanolone Treatment Improves Plasma Metabolomic Profile Associated with GABA Metabolism in Fragile X-Associated Tremor/Ataxia Syndrome: a Pilot Study. *Mol Neurobiol*. https://doi.org/10.1007/s12035-018-1330-3

NORD. (2022, April 18). *Fragile X Syndrome*. https://rarediseases.org/rare-diseases/fragile-x-syndrome/

Oberle, I., Rousseau, F., Heitz, D., Kretz, C., Devys, D., Hanauer, A., Boue, J., Bertheas, M. F., & Mandel, J. L. (1991). Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. *Science*, *252*(5009), 1097-1102. https://doi.org/10.1126/science.252.5009.1097

Oostra, B. A., & Willemsen, R. (2001). Diagnostic tests for fragile X syndrome. *Expert Rev Mol Diagn*, *1*(2), 226-232. https://doi.org/10.1586/14737159.1.2.226

Ormazabal, M., Solari, A., Espeche, L., Castro, T., & Buzzalino, N. (2019). [Fragile X syndrome and other entities associated with the FMR1 gene: Study of 28 affected families]. *Arch Argent Pediatr*, *117*(3), e257-e262. https://doi.org/10.5546/aap.2019.e257 (Fragilidad del X y otras entidades asociadas al gen FMR1: estudio de 28 familias afectadas.)

Parvin, S., Takeda, R., Sugiura, Y., Neyazaki, M., Nogi, T., & Sasaki, Y. (2018). Fragile X mental retardation protein regulates accumulation of the active zone protein Munc18-1 in presynapses via local translation in axons during synaptogenesis. *Neurosci Res*. https://doi.org/10.1016/j.neures.2018.09.013

Peprah, E. (2012). Fragile X syndrome: the FMR1 CGG repeat distribution among world populations. *Ann Hum Genet*, *76*(2), 178-191. https://doi.org/10.1111/j.1469-1809.2011.00694.x

Rais, M., Binder, D. K., Razak, K. A., & Ethell, I. M. (2018). Sensory Processing Phenotypes in Fragile X Syndrome. *ASN Neuro*, *10*, 1759091418801092. https://doi.org/10.1177/1759091418801092

Rajan-Babu, I. S., & Chong, S. S. (2016). Molecular Correlates and Recent Advancements in the Diagnosis and Screening of FMR1-Related Disorders. *Genes (Basel)*, *7*(10). https://doi.org/10.3390/genes7100087

Rajan-Babu, I. S., Teo, C. R., Lian, M., Lee, C. G., Law, H. Y., & Chong, S. S. (2015). Single-tube methylation-specific duplex-PCR assay for rapid and accurate diagnosis of Fragile X Mental Retardation 1-related disorders. *Expert Rev Mol Diagn*, *15*(3), 431-441. https://doi.org/10.1586/14737159.2015.1001749

Ramos, C., Ocampos, M., Barbato, I. T., Graça Bicalho, M. d., & Nisihara, R. (2020). Molecular analysis of FMR1 gene in a population in Southern Brazil: Comparison of four methods. *Practical Laboratory Medicine*, *21*, e00162. https://doi.org/10.1016/j.plabm.2020.e00162

Ramos, C., Ocampos, M., Barbato, I. T., Niehues, V. M. S., Bicalho, M. D. G., & Nisihara, R. (2021). Association between mutations in the FMR1 gene and ovarian dysfunction in Brazilian patients. *JBRA Assist Reprod*.

Raspa, M., Wylie, A., Wheeler, A. C., Kolacz, J., Edwards, A., Heilman, K., & Porges, S. W. (2018). Sensory Difficulties in Children With an FMR1 Premutation. *Front Genet*, *9*, 351. https://doi.org/10.3389/fgene.2018.00351

Reisinger, D. L., Shaffer, R. C., Tartaglia, N., Berry-Kravis, E., & Erickson, C. A. (2020). Delineating Repetitive Behavior Profiles across the Lifespan in Fragile X Syndrome. *Brain Sci*, *10*(4). https://doi.org/10.3390/brainsci10040239

Riley, C., & Wheeler, A. (2017). Assessing the Fragile X Syndrome Newborn Screening Landscape. *Pediatrics*, *139*(Suppl 3), S207-S215. https://doi.org/10.1542/peds.2016-1159G

Rosario, R., & Anderson, R. (2020). The molecular mechanisms that underlie fragile X-associated premature ovarian insufficiency: is it RNA or protein based? *Molecular Human Reproduction*. https://doi.org/10.1093/molehr/gaaa057

Santoro, M. R., Bray, S. M., & Warren, S. T. (2012). Molecular mechanisms of fragile X syndrome: a twenty-year perspective. *Annu Rev Pathol*, *7*, 219-245. https://doi.org/10.1146/annurev-pathol-011811-132457

Saul, R. A., & Tarleton, J. C. (1993). FMR1-Related Disorders. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews((R))*. https://www.ncbi.nlm.nih.gov/pubmed/20301558

Sherman, S., Pletcher, B. A., & Driscoll, D. A. (2005). Fragile X syndrome: diagnostic and carrier testing. *Genet Med*, *7*(8), 584-587. https://doi.org/10.1097%2F01.GIM.0000182468.22666.dd

Sitzmann, A. F., Hagelstrom, R. T., Tassone, F., Hagerman, R. J., & Butler, M. G. (2018). Rare FMR1 gene mutations causing fragile X syndrome: A review. *Am J Med Genet A*, *176*(1), 11-18. https://doi.org/10.1002/ajmg.a.38504

Skrlec, I., Barisic, K., & Wagner, J. (2018). Validation of a Screening Method for Dynamic Mutations in the FMR1 Gene. *Ann Clin Lab Sci*, *48*(6), 810-813. https://www.ncbi.nlm.nih.gov/pubmed/30610057

Suhl, J. A., & Warren, S. T. (2015). Single-Nucleotide Mutations in FMR1 Reveal Novel Functions and Regulatory Mechanisms of the Fragile X Syndrome Protein FMRP. *J Exp Neurosci*, *9*(Suppl 2), 35-41. https://doi.org/10.4137/JEN.S25524

Tassone, F., Hagerman, R. J., Taylor, A. K., Gane, L. W., Godfrey, T. E., & Hagerman, P. J. (2000). Elevated levels of FMR1 mRNA in carrier males: a new mechanism of involvement in the fragile-X syndrome. *Am J Hum Genet*, *66*(1), 6-15. https://doi.org/10.1086/302720

Tekcan, A. (2016). In Silico Analysis of FMR1 Gene Missense SNPs. *Cell Biochem Biophys*, *74*(2), 109-127. https://doi.org/10.1007/s12013-016-0722-0

Teo, C. R., Law, H. Y., Lee, C. G., & Chong, S. S. (2012). Screening for CGG repeat expansion in the FMR1 gene by melting curve analysis of combined 5' and 3' direct triplet-primed PCRs. *Clin Chem*, *58*(3), 568-579. https://doi.org/10.1373/clinchem.2011.174615

Villate, O., Ibarluzea, N., Maortua, H., de la Hoz, A. B., Rodriguez-Revenga, L., Izquierdo-Alvarez, S., & Tejada, M. I. (2020). Effect of AGG Interruptions on FMR1 Maternal Transmissions. *Front Mol Biosci*, *7*, 135. https://doi.org/10.3389/fmolb.2020.00135

Willemsen, R., Bontekoe, C. J., Severijnen, L. A., & Oostra, B. A. (2002). Timing of the absence of FMR1 expression in full mutation chorionic villi. *Hum Genet*, *110*(6), 601-605. https://doi.org/10.1007/s00439-002-0723-5

Wilson, Bie, Armour, Brown, Campagnolo, Carroll, R. C., Okun, Nelson, & Zwingerman. (2016). Joint SOGC–CCMG Opinion for Reproductive Genetic Carrier Screening: An Update for All Canadian Providers of Maternity and Reproductive Healthcare in the Era of Direct-to-Consumer Testing. *Journal of Obstetrics and Gynaecology Canada*, *38*(8), 742–762. https://www.jogc.com/article/S1701-2163(16)39347-1/fulltext

Yang, Y. M., Arsenault, J., Bah, A., Krzeminski, M., Fekete, A., Chao, O. Y., Pacey, L. K., Wang, A., Forman-Kay, J., Hampson, D. R., & Wang, L. Y. (2018). Identification of a molecular locus for normalizing dysregulated GABA release from interneurons in the Fragile X brain. *Mol Psychiatry*. https://doi.org/10.1038/s41380-018-0240-0

Yu, S., Pritchard, M., Kremer, E., Lynch, M., Nancarrow, J., Baker, E., Holman, K., Mulley, J. C., Warren, S. T., Schlessinger, D., & et al. (1991). Fragile X genotype characterized by an unstable region of DNA. *Science*, *252*(5009), 1179-1181. https://doi.org/10.1126/science.252.5009.1179

Zafarullah, M., Tang, H.-T., Durbin-Johnson, B., Fourie, E., Hessl, D., Rivera, S. M., & Tassone, F. (2020). FMR1 locus isoforms: potential biomarker candidates in fragile X-associated tremor/ataxia syndrome (FXTAS). *Scientific Reports*, *10*(1), 11099. https://doi.org/10.1038/s41598-020-67946-y

Genetic Testing for Germline Variants of the RET Proto-Oncogene

Policy Number: AHS – M2078 – Genetic Testing for Germline Variants of the RET Proto-Oncogene	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

The rearranged during transfection (Elisei et al.) proto-oncogene encodes a transmembrane receptor tyrosine kinase (Takahashi et al., 1985) that regulates a complex network of signal transduction pathways during development, survival, proliferation, differentiation, and migration of the enteric nervous system progenitor cells (Hedayati et al., 2016). Disruption of RET signaling by mutation, gene rearrangement, overexpression or transcriptional up-regulation of the *RET* gene is implicated in several human cancers (Plaza-Menacho et al., 2014), most commonly thyroid cancer, but also chronic myelomonocytic leukemia, acute myeloid leukemia, and lung, breast, pancreatic, and colon cancers (Gordon et al., 2018). Mutation of the *RET* gene in a germline cell results in an autosomal dominant hereditary cancer syndrome, multiple endocrine neoplasia type 2 (MEN2). characterized by medullary thyroid carcinoma (MTC), pheochromocytoma (PHEO), and primary parathyroid hyperplasia (PPTH) (Figlioli et al., 2013).

This policy covers genetic testing for germline variants in the *RET* gene. For information on testing of tumors for *RET* variants for guiding chemotherapy, see AHS-M2030 Testing for Targeted Therapy of Non-Small-Cell Lung Cancer, AHS-M2108 Molecular Markers in Fine Needle Aspirates of the Thyroid, and AHS-M2171 Esophageal Pathology Testing.

II. Related Policies

Policy Number	Policy Title
AHS-M2030	Testing for Targeted Therapy of Non-Small-Cell Lung Cancer
AHS-M2108	Molecular Markers in Fine Needle Aspirates of the Thyroid
AHS-M2171	Esophageal Pathology Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Genetic testing for *RET* proto-oncogene variants **MEETS COVERAGE CRITERIA** when any of the following conditions are met:
   a) For individuals who are a member of a family with defined *RET* gene mutations.
   b) For individuals who are a member of a family that is known to be affected by inherited medullary thyroid cancer but whose family has not previously been evaluated for *RET* mutations.
   c) For individuals who are a first-degree relative (see Note 1) of individuals with MTC.
   d) For individuals with a diagnosis of MTC, a clinical diagnosis of multiple endocrine neoplasia type 2 (MEN2), or primary C-cell hyperplasia.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

2) For all other situations not discussed above, genetic testing for germline variants in the *RET* gene **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: First-degree relatives include parents, full siblings, and children of the individual.

IV. Table of Terminology

Term	Definition
A750T	Alanine-750-threonine
A883F	Alanine-883-phenylalanine
AKT	*AKT serine/threonine kinase 1*
ALK	*ALK receptor tyrosine kinase*
AMP	Association for Molecular Pathology
ATA	American Thyroid Association
BTA	British Thyroid Association
C634R	Cysteine-634-arginine

C634Y	Cystine-634-tyrosine
CAP	College of American Pathologists
CDKN2A	*Cyclin dependent kinase inhibitor 2A*
CLA	Cutaneous lichen amyloidosis
CLIA'88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
DNA	Deoxyribonucleic acid
EGFR	*Epidermal growth factor receptor*
ESMO	European Society for Medical Oncology
ETA	European Thyroid Association
FDA	Food and Drug Administration
FFPE	Formalin-fixed, paraffin-embedded
FISH	Fluorescent in situ hybridization
FLT3	*Fms related receptor tyrosine kinase 3*
FMTC	Familial medullary thyroid carcinoma
HD	Hirschsprung disease
HPTH	Primary hyperparathyroidism
HSCR	Hirschsprung's disease
IASLC	International Association for the Study of Lung Cancer
IHC	Immunohistochemistry
L790F	Leucine-790-phenylalanine
LDTs	Laboratory developed tests
M918T	Methionine-918-threonine
MAPK	*Mitogen-activated protein kinase*
MEN	Multiple endocrine neoplasia
MEN2	Multiple endocrine neoplasia type 2
MEN2A	Multiple endocrine neoplasia type 2A
MEN2B	Multiple endocrine neoplasia type 2B
MTC	Medullary thyroid carcinoma
NAT2	*N-acetyltransferase 2*
NCCN	National Comprehensive Cancer Network
NGS	Next-generation sequencing
NHS	National Health Services
NSCLC	Non-small cell lung cancer
PTH	Parathyroid hyperplasia
PHEO	Pheochromocytoma
PPTH	Primary parathyroid hyperplasia
QOE	Quality of evidence
Q-PCR	Quantitative polymerase chain reaction

R387Q	Arginine-387-glutamine
R693H	Arginine-693-histidine
RET	Rearranged during transfection
ROS1	*ROS proto-oncogene 1, receptor tyrosine kinase*
RT-PCR	Real-time polymerase chain reaction
SNP	Single nucleotide polymorphism
SOR	Strength of recommendation
V292M	Valine-292-methionine
V804M	Valine-804-methionine
VUS	Variants of unknown significance

V. Scientific Background

The *RET* gene encodes a receptor tyrosine kinase that transduces growth and differentiation signals from the glial cell-derived neurotrophic factor family of ligands (Saarma, 2001). *RET* is expressed in the neuroendocrine parafollicular C-cells of the thyroid gland, adrenal medulla, neurons, and other tissues (Takaya et al., 1996). Unlike loss of function mutations that inactivate tumor suppressor proteins, oncogenic *RET* mutations result in a gain-of-function, inducing ligand-independent autophosphorylation of the RET receptor (Santoro et al., 1995), uncontrolled activation of MAPK and phosphoinositide 3-kinase pathways, and ultimately uncontrolled growth and cell dedifferentiation (Hansford & Mulligan, 2000; Raue & Frank-Raue, 2018).

Oncogenic activation of the *RET* gene can result from either somatic or germline alterations. Activating germline point mutations in *RET* with autosomal dominant heritability have been identified as the primary initiating events causative of malignancy in C-cells of the thyroid gland (MTC) and other clinical presentations of MEN2 (Hansford & Mulligan, 2000; Mulligan, 2014). These mutations are identified in 98-100% of MEN2 cases (Raue & Frank-Raue, 2018; Romei et al., 2016), which are responsible for 25% of MTC cases overall (Raue & Frank-Raue, 2015). An estimated 64,000 individuals are diagnosed with thyroid cancer in the United States annually, and 1-2% of these cases are due to MTC. The most common alterations in the *RET* proto-oncogene are missense gain-of-function mutations mainly located in the extracellular domain of the *RET* gene (exons 10 or 11) and in the *RET* tyrosine kinase domain (exons 13, 14, 15 and 16) (ATA, 2016).

Germline *RET* mutations are associated with clear genotype-phenotype correlations (Plaza-Menacho et al., 2014). These clinical phenotypes can be divided into two subclasses of MEN2: multiple endocrine neoplasia type 2A (MEN2A) including familial medullary thyroid carcinoma (FMTC) and MEN type 2B (MEN2B) (Jasim et al., 2011). Over 100 *RET* point mutations, duplications, insertions, deletions, and fusions have

been identified in individuals with MEN2A, with the C634R mutation in exon 11 being the most common mutation, whereas only two *RET* mutations have been identified in individuals with MEN2B (mainly M918T, and rarely A883F) (Giani et al., 2020; Romei et al., 2018). New variants continue to be reported (Paragliola et al., 2018; Qi et al., 2018). For example, in a case study of a 7-year-old girl in Italy, a "de novo" new germline *RET* deletion in exon 11 was found to cause features of both MEN2B without PHEO (pheochromocytoma), but "with a pelvic plexiform neurofibroma and with HPTH (primary hyperparathyroidism), which is typical of MEN2A" (Giani et al., 2020).

The MEN2A subclass is characterized by MTC and variable rates of PHEO, PPTH or both, with *RET* mutations in codons 609, 611, 618, or 620 of exon 10 and codon 634 of exon 11. Subtypes of classical MEN2A include development of cutaneous lichen amyloidosis and Hirschsprung disease. Absence of any clinical finding other than MTC in at least four family members is classified as FMTC (Wells et al., 2015).

The MEN2B subclass is characterized by highly aggressive MTC, usually PHEO, but not PPTH, and may exhibit musculoskeletal abnormalities and developmental defects with *RET* mutations in codons 918 and 883 of exon 15 (Wells et al., 2015).

Figure 1: *RET* point mutations in MEN2A, MEN2B, and FMTC (Wells et al., 2013).

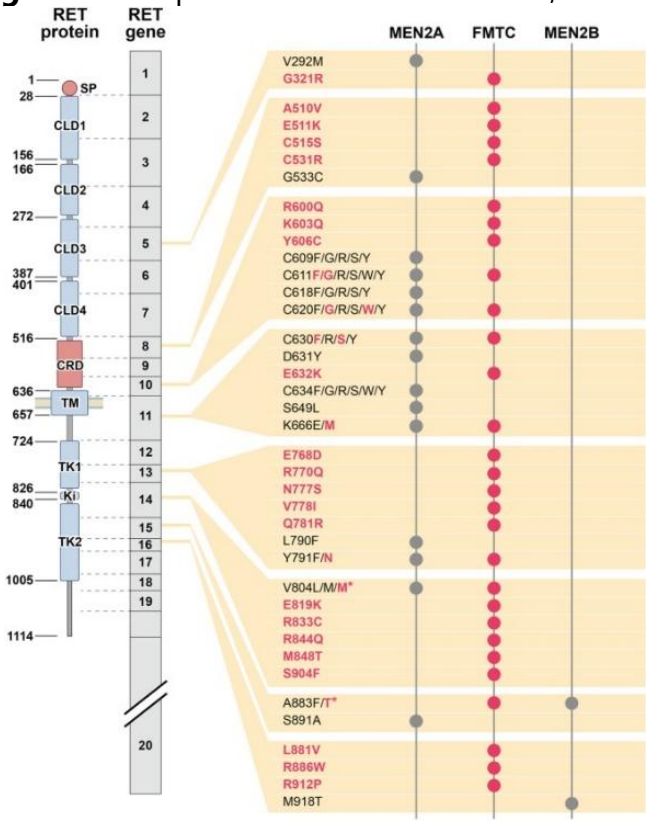


Table 1: Clinical expression of familial MTC-associated syndromes (Links et al., 2015).

	FMTC (%)	MEN2A (%)	MEN2B (%)
MTC	100	100	100
Pheochromocytoma	0	10-60	50
Hyperparathyroidism	0	10-30	0
Marfanoid habitus	0	0	100
Intestinal ganglioneuromatosis	0	0	60-90
Mucosal neuromas	0	0	70-100

Clinical Utility and Validity

The development of tyrosine kinase inhibitors that specifically target *RET* (Suyama & Iwase, 2018) has allowed for genetic analysis of *RET* germline mutations to become the standard of care in the initial workup for detecting germline mutations and familial risk and identifying targeted therapy in MTC (Ernani et al., 2016; Wells et al., 2015). Further, somatic *RET* rearrangements have recently been implicated in a variety of cancers, including chronic myelomonocytic leukemia; acute myeloid leukemia; and lung, breast, pancreatic, and colon cancers; an individual previously diagnosed with lung cancer underwent genomic profiling, and the identification of a *RET* point mutation associated with MTC allowed researchers to determine that this lung cancer diagnosis was incorrect (Gordon et al., 2018). A change in treatments proved to be very helpful for this individual. Other researchers have reported *RET* translocations in lung cancer cases, but they state that this is extremely rare (Zhao et al., 2016).

Guan et al. (2020) identified *RET* mutations in human epithelial ovarian cancer, providing another area of benefit from genetic testing of the *RET* gene for developing targeted therapies. Results showed that R693H and A750T mutants, in the juxtamembrane region and intracellular kinase domain, respectively, could promote the MAPK and AKT signaling pathway in ovarian cancer, and that the RET inhibitor vandetanib could decrease signal transduction and inhibit cancer growth (Guan et al., 2020).

Though activating variants in the receptor tyrosine kinase rearranged during transfection are known to be associated with MEN 2, not all variants are created equal. Hansen et al. (2021) explored the pathogenic role of the variant c.166C>A, p.Leu56Met in *RET*, which was identified in individuals presenting short segment HSCR and more recently in two individuals with MTC. Seven unrelated *RET* Leu56Met carriers from a Danish cohort were evaluated in the study, none of whom displayed evidence of MEN 2 or MTC based on the authors' predefined criteria for MEN2B ("(i) the individual demonstrates more than one MEN 2 manifestation, including histologically verified MTC, histologically verified pheochromocytoma, histologically verified gastrointestinal

or mucosal neuromas, histologically verified HSCR, biochemically verified PHPT, and clinically or histologically verified cutaneous lichen amyloidosis, or (Martins-Costa et al.) the individual has one MEN 2 manifestation and a relative with MTC and the RET Leu56Met variant"). Moreover, while known causative variants of MEN 2 such as p.Cys634Tyr and p.Met918Thr boast allele frequencies below 0.001%, the allele frequency for the Leu56Met variant computed from the in-house diagnostic cohort was much higher at 0.59%. These considerations, combined with the lack of family history for MEN 2, suggest that Leu56Met is "a common variant without clinical significance in the Danish population" and "is most likely a benign variant" (Hansen et al., 2021).

Researchers have also found in two *RET* L790F index individuals that somatic *RET* variants were not responsible for the early onset and aggressiveness of MTC in a *RET* germline mutation carrier. Normally, variations in MTC presentation could be attributed to *RET* germline variants (Mathiesen et al., 2020). However, Mathiesen et al. (2020) found an *FLT3* R387Q variant - FLT3 being a protein commonly found in hematopoietic malignancies - that could have been a genetic modifier instead.

The strong genotype-phenotype correlation of *RET* mutations makes genetic screening of significant value in diagnosis, prognosis, and management of MEN2 (Eng et al., 1996; Frank-Raue et al., 2010; Romei et al., 2015) and resultant MTC (Machens, Lorenz, et al., 2018), PHEO (Kimura et al., 2018), and PPTH. Each specific *RET* mutation correlates with MEN2 presentation, age at onset of MTC, and MTC aggressiveness (Brandi et al., 2001). Screening and early treatment of the manifestations of MEN2 can prevent metastasis of MTC and the morbidity and mortality caused by PHEO (Gagel et al., 1988; Makri et al., 2018). Moreover, screening has been associated with improved survivorship and outcomes (Raue et al., 2018). Based upon these genotype-phenotype correlations, *RET* mutations have been stratified into three risk levels based on the penetrance and aggressiveness of the MTC (Brandi et al., 2001; Wells et al., 2015). Consequently, mutation type should guide major management decisions, such as whether and when to perform thyroidectomy (Machens, Elwerr, et al., 2018; Machens, Lorenz, et al., 2018). Children in the highest risk category should undergo thyroidectomy in their first year of life, and perhaps even in their first months of life (Machens, Elwerr, et al., 2018; Machens, Lorenz, et al., 2018). Those with mutations in the high-risk category (codon 634 mutations) "should undergo thyroidectomy before reaching the age of 5 years" (Larouche et al., 2019). Annual biochemical screening in individuals with a family history of FMTC or MEN2 can also be stopped in those individuals who test negative for mutations (Wells et al., 2015).

Martins-Costa et al. (2018) performed *RET* genetic sequencing on exons 8, 10, 11, and 13-16 in 247 individuals with MTC or who are at risk of developing MTC due to family

history. Before genetic testing, 54 of the individuals were diagnosed with sporadic disease and six were diagnosed with hereditary disease; after genetic testing, 31 individuals were diagnosed with sporadic disease and 29 with hereditary disease (Martins-Costa et al., 2018). *RET* screening allowed several individuals to be classified as hereditary who were initially diagnosed with sporadic MTC; 73 at-risk relatives were identified as mutation carriers, which will assist in long-term life and reproductive decisions (Martins-Costa et al., 2018).

A meta-analysis consisting of 438 Indian individuals with MTC and 489 healthy controls of similar ages and genders was completed; all participants received molecular genetic testing including *RET* gene sequencing and SNP genotyping (Mishra et al., 2019). This study identified *RET* SNPs as a significant risk factor for developing hereditary MTC; *CDKN2A* and *NAT2* SNPs with a significant risk of developing sporadic MTC (Mishra et al., 2019).

Genetic screening for *RET* was also provided to a total of 2031 Italian subjects; this included 1264 individuals with sporadic MTC symptoms, 117 individuals with hereditary MTC symptoms, and 650 relatives (Elisei et al., 2019). The researchers state, "A *RET* germline mutation was found in 115/117 (98.3%) hereditary and in 78/1264 (6.2%) apparently sporadic cases: in total, 42 distinct germline variants were found" (Elisei et al., 2019). This thereby underscores the significance of genetic screening in unsuspected MEN2 families. Sporadic MTC cases were present most commonly with a V804M mutation, and all M918T mutations were *de novo* "and exclusively associated with MEN2B" (Elisei et al., 2019). These researchers also identified several variants of unknown significance (VUS).

A paper by Milićević et al. (2021) focused on examining the crude annual incidence rate of MTC and *RET* mutation frequency. The study involved Slovenian individuals at the Institute of Oncology Ljubljana from 1995 to 2015 and involved their family members who participated in genetic counseling and testing there. It was found that among 143 individuals with MTC, 37 (25.9%) harbored a germline *RET* mutation, and said mutations were uncovered in exons 10, 11, 13, 14, and 16. Also, the researchers noted that "*RET* germline mutations are quite commonly discovered even in the apparently sporadic form of the disease", such that 14.2% of individuals with a negative family history were presented with them. It was also reported that the most frequent *RET* germline mutations were found on codons 634 and 618 (30.0%) and exon 11 was the most frequently altered, though mutations of codons 790, 804, and 918 were observed in smaller but noticeable percentages (25.0%, 10.0%, and 5.0%, respectively). However, despite the low compliance of family members resulting in a smaller pool of participants, the authors extol the use of genetic counseling in this case, as "Annual incidence increase and nation-specific frequency of discovered *RET*

mutations justify the continuation of gene counseling and testing of MTC individuals in Slovenia" (Milićević et al., 2021).

A study by Fussey et al. (2021) aimed to lend their perspective on the diagnostic potential of *RET* genetic testing. Between 1997 and 2018, the Exeter Genomics Laboratory at the Royal Devon and Exeter NHS Foundation Trust collected information on 1058 index individuals with MTC and other MEN2-related clinical features. They found that in total, 92 of the 766 UK individuals with MTC were harboring a germline *RET* pathogenic variant, and that variants in 10, 11, and 14 comprised its bulk, with codons 634 in exon 11 and 804 in exon 14 being most often affected. As such, the researchers believe that "the use of somatic *RET* analysis to confirm the diagnosis of sporadic MTC in individuals with no identified germline RET variants may be a useful adjunct both in terms of reassuring family members about the lack of a heritable pathogenic germline variant, and risk-stratifying sporadic tumours based on somatic variants" (Fussey et al., 2021).

Genetic screening for *RET* could also disclose new variants with their respective phenotypes. Yang et al. (2020) described a compound C634Y/V292M transmutation in a northern Chinese family that was associated with a more aggressive clinical presentation. Carriers of this variant had bilateral MTC with PHEO or lymph node metastasis with faster cell growth (cell growth speed identified *in vitro*). On the other hand, carriers of the V292M variant were asymptomatic, and carriers of the C634Y mutation only had elevated calcitonin (Yang et al., 2020). This has demonstrated the striking variability in MTC clinical presentation based on *RET* gene variants, making it critical to aid in any future potential treatment regimen.

Though activating variants in the receptor tyrosine kinase rearranged during transfection are known to be associated with MEN 2, not all variants are created equal. Hansen et al. (2021) explored the pathogenic role of the variant c.166C>A, p.Leu56Met in *RET*, which was identified in individuals presenting short segment HSCR and more recently in two individuals with MTC. Seven unrelated *RET* Leu56Met carriers from a Danish cohort were evaluated in the study, none of whom displayed evidence of MEN 2 or MTC based on the authors' predefined criteria for MEN2B ("(i) the individual demonstrates more than one MEN 2 manifestation, including histologically verified MTC, histologically verified pheochromocytoma, histologically verified gastrointestinal or mucosal neuromas, histologically verified HSCR, biochemically verified PHPT, and clinically or histologically verified cutaneous lichen amyloidosis, or (Martins-Costa et al.) the individual has one MEN 2 manifestation and a relative with MTC and the RET Leu56Met variant"). Moreover, while known causative variants of MEN 2 such as p.Cys634Tyr and p.Met918Thr boast allele frequencies below 0.001%, the allele frequency for the Leu56Met variant computed from the in-house diagnostic cohort

was much higher at 0.59%. These considerations, combined with the lack of family history for MEN 2, suggest that Leu56Met is "a common variant without clinical significance in the Danish population" and "is most likely a benign variant" (Hansen et al., 2021).

Bhandari et al. (2023) studied the testing patterns for *RET* in the United States for people with advanced or metastatic medullary thyroid cancer. The authors completed a retrospective analysis of 203 individuals being cared for by 75 oncologists. Here, "A total of 59.6% (121 of 203) of patients underwent testing for RET, and 37.2% (45 of 121) had a RET mutation, of which 55.6% were identified as RET mutation-positive before initial diagnosis." The authors also note that "90 (44.3%) patients were tested for biomarkers on or after initial diagnosis, with RET being the most tested (95.6%) biomarker." The authors conclude that there is opportunity to improve testing rates (Bhandari et al., 2023).

VI. Guidelines and Recommendations

European Society for Medical Oncology (ESMO)

The ESMO has published clinical practice guidelines on diagnosis, treatment, and follow-up of thyroid cancer, stating that "All patients with MTC should be offered genetic counselling and screened for germline *RET* mutations" (Filetti et al., 2020). Filetti et al. (2020) also stated that "screening for somatic *RET* mutations is only recommended if *RET* inhibitor therapy is planned."

In 2021, ESMO Translational Research and Precision Medicine Working Group expanded upon the algorithm to identify individuals eligible for anti-RET therapy across three scenarios based on the malignancies. The recommendations are captured below:

"Scenario A: Patients affected by NSCLC, non-MTC or other solid tumours, with available formalin-fixed, paraffin-embedded (FFPE) specimen need to be screened for detection of RET fusion. If NGS is not available, FISH or RT-PCR is indicated in NSCLC and non-MTC, depending on local availability, cost and/or amount of tumour cells. In case of a negative test result, it is recommended to perform an NGS panel. It should be noted, however, that the recent ESMO recommendations suggest using multigene NGS to assess NSCLC level I alterations, including *RET* fusions.

In addition to *RET* fusion testing, patients affected by other solid tumours may need to be tested also for *RET* mutation according to the results of pending clinical trials. This

can be done preferably by NGS; if this is not available, Q-PCR can be used. In none of the above cases is RET IHC testing recommended.

Scenario B: For patients affected by NSCLC, non-MTC or other solid tumours whose FFPE specimens are not available or are exhausted, we suggest performing a liquid biopsy (cell-free nucleic acid NGS panel) to test for *RET* alteration.

It is important to notice that if an *RET* alteration is not detected by liquid biopsy, then tumour tissue testing is still required to definitively exclude the possibility of an RET fusion.

Scenario C: Patients affected by MTC need to be screened for detection of *RET* mutation. They should be referred to genetic counselling in order to study the presence of MEN syndrome or FMTC. Mutations in the RET gene, in fact, are canonical in hereditary MTC, but can be found also in sporadic MTC. A Q-PCR or NGS can be carried out on sputum or blood of the patient. In case of known familial *RET* mutation, a simple Sanger test can be carried out on blood leukocyte DNA. If a germline *RET* mutation is present, family counselling is indicated. In case of absence of germline *RET* mutations, if the patient with MTC becomes metastatic, a Q-PCR of NGS analysis on FFPE tissue specimens from metastatic site of disease should be carried out in order to confirm or reject the presence of this alteration.

In none of the above cases is RET IHC testing recommended" (Belli et al., 2021).

American Thyroid Association (ATA)

The ATA published revised guidelines (Wells et al., 2015) which state that:

- "Initial testing for patients with MEN2A phenotype is either a single or multi-tiered analysis to detect *RET* mutations in exon 10 (codons 609, 611, 618, and 620), exon 11 (codons 630 and 634), and exons 8, 13, 14, 15, and 16. Grade B Recommendation"
- Initial testing for patients with MEN2B phenotype should be tested for the *RET* codon M918T mutation (exon 16), and if negative, the *RET* codon A883F mutation (exon 15).
- "Sequencing of the entire coding region should be reserved for situations in which no *RET* mutation is identified or there is a discrepancy between the MEN2 phenotype and the expected genotype. Grade B Recommendation
- Patients with the MEN2B phenotype should be tested for the *RET* codon M918T mutation (exon 16), and if negative, the *RET* codon A883F mutation (exon 15). If

there are no mutations identified in these two exons the entire RET coding region should be sequenced. Grade B Recommendation

- Patients with presumed sporadic MTC should have genetic testing to detect a germline *RET* mutation. If a *RET* mutation is found the patient should have genetic testing. Grade B Recommendation
- In very rare families who meet the clinical criteria for MEN2A or 2B, despite negative sequencing of the entire *RET* coding region, the relatives at risk should be periodically screened by conventional methods for MTC, PHEO, and HPTH. After the initial evaluation, screening should continue at 1- to 3-year intervals. Grade C Recommendation
- Genetic counseling and genetic testing for *RET* germline mutations should be offered to
    - First-degree relatives of patients with proven hereditary MTC,
    - Parents whose infants or young children have the classic phenotype of MEN2B,
    - Patients with CLA [cutaneous lichen amyloidosis], and
    - Infants or young children with HD and exon 10 *RET* germline mutations, and adults with MEN2A and exon 10 mutations who have symptoms suggestive of HD" (Wells et al., 2015).

National Comprehensive Cancer Network (NCCN)

NCCN guidelines for neuroendocrine and adrenal tumors (NCCN, 2024b) recommends that for diagnosis of or clinical suspicion of MEN2, genetic counseling and *RET* genetic testing should be offered in the following cases:

- "Individuals with MTC or primary C-cell hyperplasia or a clinical diagnosis of MEN2."
- "At-risk relative of an individual with a known germline *RET* mutation at a very young age."
- "All patients with MTC should be tested for germline mutation of the *RET* oncogene even if the family history is not suggestive of a hereditary syndrome, because about 50% of patients with presumed sporadic MTC have a *de novo* germline mutation" (NCCN, 2024b).

NCCN Guidelines for Thyroid Carcinoma (NCCN, 2024c) stated that as part of the additional workup after medullary thyroid carcinoma is identified by initial thyroid surgery, a "Screen for germline *RET PV* (exons 10, 11, 13-16); genetic counseling may be indicated" and "Germline mutation should prompt specific mutation testing in subsequent family members and genetic counseling" (NCCN, 2024c).

The FDA has approved several targeted therapeutic agents for use in esophageal and EGJ cancers. NCCN guidelines for Esophageal and Esophagogastric Junction Cancers (NCCN, 2024a) lists *RET* gene fusion as a targeted biomarker for NGS. "Immunohistochemistry/in situ hybridization/targeted PCR should be considered first for the identification of biomarkers, followed by NGS testing" (NCCN, 2024a).

The College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP)

The 2013 guidelines from CAP, IASLC and AMP for molecular testing in lung cancer individuals have been updated in 2018; new recommendations state that *RET* testing is approved in lung cancer specimens "as part of larger testing panels performed either initially or when routine *EGFR*, *ALK*, and *ROS1* testing are negative" because "*RET* molecular testing is not recommended as a routine stand-alone assay outside the context of a clinical trial" (Lindeman et al., 2018).

The British Thyroid Association (BTA)

Regarding MTC, the BTA has stated that "In all confirmed cased of MTC, *RET* mutation analysis to establish the possible genetic basis for the disease within an individual or kindred, should be performed even in the absence of a positive family history." Other key recommendations to consider before genetic testing are listed below.

- "Because of the possibility of heritable disease, every case of MTC should be offered genetic testing"
- "Testing should always begin with the affected individual, if they are available"
- "If the affected individual is not available, the decision and strategy for testing should be discussed with the clinical genetics service"
- "Before blood is taken, a clear explanation must be given of the nature of the test, the possible outcomes, and of the implications of a positive or negative result for the individual and the family. This explanation should be recorded in the case notes for each individual" (Perros et al., 2014).

European Thyroid Association (Giani et al.)

The ETA Executive Committee in 2012 issued guidelines specifically devoted to *RET* genetic screening for individuals affected by MTC with reference to three different phenotypes: multiple endocrine neoplasia types 2A and 2B and familial MTC (FMTC) (Elisei et al., 2013). In their attempt to improve the quality of care for individuals and

families with MTC, they made recommendations with varying valences, grading their own recommendations by the quality of evidence (QOE), which is indicated by plus signs at three levels, and the strength of recommendation (SOR) score, indicated by a 1 (strong recommendation for or against) or a 2 (" weak recommendation or a suggestion that may not be appropriate for every patient, depending on the context, patient values, and preferences"). The relevant parts of the guidelines are

From Recommendation 4:

> "(a) Exons 5, 8, 10, 11, 13, 14, 15, and 16 should always be analyzed starting from the most likely involved in the presenting syndrome (i.e. exon 10 in MEN 2A, exon 16 in MEN 2B, etc.). DNA from MTC individuals with a strong suggestion of familial disease should also be analyzed for all other uncommonly mutated exons when those listed above are negative… (QOE = +++; SOR = score 1)."
> (b) Subjects belonging to the few families (2–5% of all MEN 2/FMTC families) with clinically evident MEN 2/FMTC features but lacking evidence of an associated germline *RET* mutation should be followed up annually by measurement of basal serum Ct, metanephrines, calcium, and PTH. Neck and abdomen ultrasound could also be useful although not absolutely required if biochemical tests are still negative (QOE = ++; SOR = score 1).
> (c) Once a germline *RET* mutation has been identified, all first-degree relatives and other relevant family members should be screened for the specific causative mutation (QOE = +++; SOR = score 1).
> (d) Once a germline *RET* mutation has been discovered, further sequencing adds little, although some cases of double or triple RET mutations have been reported. These peculiar cases would likely be missed if RET screening was stopped when the first mutation was identified. The completeness of RET gene analysis is particularly indicated when the first identified mutation is rare and with a low or null transforming ability. These rare cases should be referred to specialized tertiary centers for a complete characterization of the mutation and its relationship with the disease (QOE = ++; SOR = score 2).
> (e) Family members negative for the mutation are not at risk for the development of MTC and their children are not at risk either. Such individuals should be reassured and do not require further investigation or follow-up (QOE = +++; SOR = score 1)" (Elisei et al., 2013).

From Recommendation 5:

"(a) All patients with either apparently sporadic or familial MTC should be screened for germline *RET* mutations (QOE = +++; SOR = score 1)."

From Recommendation 6:

"(a) Patients with bilateral PHEO [pheochromocytoma] should be considered for RET genetic screening when other MEN 2A endocrinopathies (MTC and/or PHPT [primary hyperparathyroidism]) or CLA are present in the same subject or if a family history of MEN 2A or 2B is present. The screening becomes mandatory if the basal serum Ct is above the normal range, independently of the presence of other MEN 2A endocrinopathies (QOE = ++; SOR = score 1).
(b) Patients with multiple adenomatosis of the parathyroid glands and PHPT should be considered for RET genetic screening if other MEN 2A endocrinopathies (MTC and/or PHEO) or CLA are present in the same subject or if a family history of MEN 2A is present. Also in this case, RET genetic screening must be performed if basal serum Ct levels are above the normal range, even if no other MEN 2A endocrinopathies are diagnosed (QOE = ++; SOR = score 1).
(c) Subjects with CLA should be investigated clinically and should undergo genetic testing for MEN 2A. In particular RET codon 634 in exon 11 should be analyzed (QOE = +++; SOR = score 1).
(d) Patients with Hirschsprung disease should have RET genetic screening for mutations involved in Hirschsprung disease, but analyses performed specifically to investigate an association with MEN 2 should be limited to exon 10 (QOE = +++; SOR = score 1)" (Elisei et al., 2013).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and

Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81404	Molecular pathology procedure, Level 5 Gene: ***RET*** (ret proto-oncogene) (eg, multiple endocrine neoplasia, type 2B and familial medullary thyroid carcinoma), common variants (eg, M918T, 2647_2648delinsTT, A883F)
81405	Molecular pathology procedure, Level 6 Gene: ***RET*** (ret proto-oncogene) (eg, multiple endocrine neoplasia, type 2A and familial medullary thyroid carcinoma), targeted sequence analysis (eg, exons 10, 11, 13-16)
81406	Molecular pathology procedure, Level 7 Gene: ***MUTYH*** (mutY homolog [E.coli]) (eg, MYH-associated polyposis), full gene sequence
S3840	DNA analysis for germline mutations of the ret proto-oncogene for susceptibility to multiple endocrine neoplasia type 2

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

ATA. (2016). *Cancer of the Thyroid*. https://www.thyroid.org/wp-content/uploads/patients/brochures/medullary-thyroid-cancer-brochure.pdf

Belli, C., Penault-Llorca, F., Ladanyi, M., Normanno, N., Scoazec, J. Y., Lacroix, L., Reis-Filho, J. S., Subbiah, V., Gainor, J. F., Endris, V., Repetto, M., Drilon, A., Scarpa, A., André, F., Douillard, J. Y., & Curigliano, G. (2021). ESMO recommendations on the standard methods to detect RET fusions and mutations in daily practice and clinical research. *Ann Oncol*, *32*(3), 337-350. https://doi.org/10.1016/j.annonc.2020.11.021

Bhandari, N. R., Hess, L. M., Parikh, R. C., Sireci, A. N., Krein, P. M., & Kaye, J. A. (2023). Biomarker testing in patients diagnosed with advanced/metastatic medullary thyroid cancer in the USA. *Per Med*, *20*(2), 131-142. https://doi.org/10.2217/pme-2022-0050

Brandi, M. L., Gagel, R. F., Angeli, A., Bilezikian, J. P., Beck-Peccoz, P., Bordi, C., Conte-Devolx, B., Falchetti, A., Gheri, R. G., Libroia, A., Lips, C. J., Lombardi, G., Mannelli, M., Pacini, F., Ponder, B. A., Raue, F., Skogseid, B., Tamburrano, G., Thakker, R. V., . . . Marx, S. J. (2001). Guidelines for diagnosis and therapy of MEN type 1 and type 2. *J Clin Endocrinol Metab*, *86*(12), 5658-5671. https://doi.org/10.1210/jcem.86.12.8070

Elisei, R., Alevizaki, M., Conte-Devolx, B., Frank-Raue, K., Leite, V., & Williams, G. R. (2013). 2012 European thyroid association guidelines for genetic testing and its clinical consequences in medullary thyroid cancer. *Eur Thyroid J*, *1*(4), 216-231. https://doi.org/10.1159/000346174

Elisei, R., Tacito, A., Ramone, T., Ciampi, R., Bottici, V., Cappagli, V., Viola, D., Matrone, A., Lorusso, L., Valerio, L., Giani, C., Campopiano, C., Prete, A., Agate, L., Molinaro, E., & Romei, C. (2019). Twenty-Five Years Experience on RET Genetic Screening on Hereditary MTC: An Update on The Prevalence of Germline RET Mutations. *Genes (Basel)*, *10*(9). https://doi.org/10.3390/genes10090698

Eng, C., Clayton, D., Schuffenecker, I., Lenoir, G., Cote, G., Gagel, R. F., van Amstel, H. K., Lips, C. J., Nishisho, I., Takai, S. I., Marsh, D. J., Robinson, B. G., Frank-Raue, K., Raue, F., Xue, F., Noll, W. W., Romei, C., Pacini, F., Fink, M., . . . et al. (1996). The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET mutation consortium analysis. *JAMA*, *276*(19), 1575-1579. https://www.ncbi.nlm.nih.gov/pubmed/8918855

Ernani, V., Kumar, M., Chen, A. Y., & Owonikoko, T. K. (2016). Systemic treatment and management approaches for medullary thyroid cancer. *Cancer Treat Rev*, *50*, 89-98. https://doi.org/10.1016/j.ctrv.2016.09.006

Figlioli, G., Landi, S., Romei, C., Elisei, R., & Gemignani, F. (2013). Medullary thyroid carcinoma (MTC) and RET proto-oncogene: mutation spectrum in the familial cases and a meta-analysis of studies on the sporadic form. *Mutat Res*, *752*(1), 36-44. https://doi.org/10.1016/j.mrrev.2012.09.002

Filetti, Durante, Hartl, Leboulleux, Locati, Newbold, Papotti, & Berruti. (2020, April 16 2020). *Thyroid Cancer - ESMO Clinical Practice Guidelines for diagnosis, treatment, and follow-up*. European Society for Medical Oncology (ESMO). Retrieved October 5 from https://www.esmo.org/content/download/286312/5688706/1/Clinical-Practice-Guidelines-Slide-Set-Thyroid-Cancer.pdf

Frank-Raue, K., Rondot, S., & Raue, F. (2010). Molecular genetics and phenomics of RET mutations: Impact on prognosis of MTC. *Mol Cell Endocrinol*, *322*(1-2), 2-7. https://doi.org/10.1016/j.mce.2010.01.012

Fussey, J. M., Smith, J. A., Cleaver, R., Bowles, C., Ellard, S., Vaidya, B., & Owens, M. (2021). Diagnostic RET genetic testing in 1,058 index patients: A UK centre perspective. *Clin Endocrinol (Oxf)*, *95*(2), 295-302. https://doi.org/10.1111/cen.14395

Gagel, R. F., Tashjian, A. H., Jr., Cummings, T., Papathanasopoulos, N., Kaplan, M. M., DeLellis, R. A., Wolfe, H. J., & Reichlin, S. (1988). The clinical outcome of prospective screening for multiple endocrine neoplasia type 2a. An 18-year experience. *N Engl J Med*, *318*(8), 478-484. https://doi.org/10.1056/NEJM198802253180804

Giani, C., Ramone, T., Romei, C., Ciampi, R., Tacito, A., Valerio, L., Agate, L., Ugolini, C., Marinò, M., Basolo, F., Franchi, A., Borsari, S., Michelucci, A., Selli, C., Materazzi, G., Cetani, F., & Elisei, R. (2020). A New MEN2 Syndrome with Clinical Features of Both MEN2A and MEN2B Associated with a New RET Germline Deletion. *Case Rep Endocrinol*, *2020*, 4147097. https://doi.org/10.1155/2020/4147097

Gordon, E. J., Parker, D., Barth, K., Pena, J., Elvin, J. A., DeLeon, T., & Karlin, N. J. (2018). Genomic Profiling Reveals Medullary Thyroid Cancer Misdiagnosed as Lung Cancer. *Case Rep Oncol*, *11*(2), 399-403. https://doi.org/10.1159/000490238

Guan, L., Li, Z., Xie, F., Pang, Y., Zhang, C., Tang, H., Zhang, H., Chen, C., Zhan, Y., Zhao, T., Jiang, H., Jia, X., Wang, Y., & Lu, Y. (2020). Oncogenic and drug-sensitive RET mutations in human epithelial ovarian cancer. *J Exp Clin Cancer Res*, *39*(1), 53. https://doi.org/10.1186/s13046-020-01557-3

Hansen, A. R., Borgwardt, L., Rasmussen Å, K., Godballe, C., Poulsen, M. M., Vieira, F. G., Mathiesen, J. S., & Rossing, M. (2021). Germline RET Leu56Met Variant Is Likely Not Causative of Multiple Endocrine Neoplasia Type 2. *Front Endocrinol (Lausanne)*, *12*, 764512. https://doi.org/10.3389/fendo.2021.764512

Hansford, J. R., & Mulligan, L. M. (2000). Multiple endocrine neoplasia type 2 and RET: from neoplasia to neurogenesis. *J Med Genet*, *37*(11), 817-827. https://www.ncbi.nlm.nih.gov/pubmed/11073534

Hedayati, M., Zarif Yeganeh, M., Sheikholeslami, S., & Afsari, F. (2016). Diversity of mutations in the RET proto-oncogene and its oncogenic mechanism in medullary thyroid cancer. *Crit Rev Clin Lab Sci*, *53*(4), 217-227. https://doi.org/10.3109/10408363.2015.1129529

Jasim, S., Ying, A. K., Waguespack, S. G., Rich, T. A., Grubbs, E. G., Jimenez, C., Hu, M. I., Cote, G., & Habra, M. A. (2011). Multiple endocrine neoplasia type 2B with a RET proto-oncogene A883F mutation displays a more indolent form of medullary thyroid carcinoma compared with a RET M918T mutation. *Thyroid*, *21*(2), 189-192. https://doi.org/10.1089/thy.2010.0328

Kimura, N., Takekoshi, K., & Naruse, M. (2018). Risk Stratification on Pheochromocytoma and Paraganglioma from Laboratory and Clinical Medicine. *J Clin Med*, *7*(9). https://doi.org/10.3390/jcm7090242

Larouche, V., Akirov, A., Thomas, C. M., Krzyzanowska, M. K., & Ezzat, S. (2019). A primer on the genetics of medullary thyroid cancer. *Curr Oncol*, *26*(6), 389-394. https://doi.org/10.3747/co.26.5553

Lindeman, N. I., Cagle, P. T., Aisner, D. L., Arcila, M. E., Beasley, M. B., Bernicker, E. H., Colasacco, C., Dacic, S., Hirsch, F. R., Kerr, K., Kwiatkowski, D. J., Ladanyi, M., Nowak, J. A., Sholl, L., Temple-Smolkin, R., Solomon, B., Souter, L. H., Thunnissen, E., Tsao, M. S., . . . Yatabe, Y. (2018). Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors: Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. *Arch Pathol Lab Med*, *142*(3), 321-346. https://doi.org/10.5858/arpa.2017-0388-CP

Links, T. P., Verbeek, H. H., Hofstra, R. M., & Plukker, J. T. (2015). Endocrine tumours: progressive metastatic medullary thyroid carcinoma: first- and second-line strategies. *Eur J Endocrinol*, *172*(6), R241-251. https://doi.org/10.1530/EJE-14-0726

Machens, A., Elwerr, M., Lorenz, K., Weber, F., & Dralle, H. (2018). Long-term outcome of prophylactic thyroidectomy in children carrying RET germline mutations. *Br J Surg*, *105*(2), e150-e157. https://doi.org/10.1002/bjs.10746

Machens, A., Lorenz, K., Weber, F., & Dralle, H. (2018). Genotype-specific progression of hereditary medullary thyroid cancer. *Hum Mutat*, *39*(6), 860-869. https://doi.org/10.1002/humu.23430

Makri, A., Akshintala, S., Derse-Anthony, C., Del Rivero, J., Widemann, B., Stratakis, C. A., Glod, J., & Lodish, M. (2018). Pheochromocytoma in children and adolescents with multiple endocrine neoplasia type 2B. *J Clin Endocrinol Metab*. https://doi.org/10.1210/jc.2018-00705

Martins-Costa, M. C., Lindsey, S. C., Cunha, L. L., Carreiro-Filho, F. P., Cortez, A. P., Holanda, M. E., Farias, J. W. M., Lima, S. B., Ferreira, L. A. A., Maia Filho, P. C., Camacho, C. P., Furuzawa, G. K., Kunii, I. S., Dias-da-Silva, M. R., Martins, J. R. M., & Maciel, R. M. B. (2018). A pioneering RET genetic screening study in the State of Ceara, Brazil, evaluating patients with medullary thyroid cancer and at-risk relatives: experience with 247 individuals. *Arch Endocrinol Metab*, *62*(6), 623-635. https://doi.org/10.20945/2359-3997000000088

Mathiesen, J. S., Nielsen, S. G., Rasmussen Å, K., Kiss, K., Wadt, K., Hermann, A. P., Nielsen, M. F., Larsen, S. R., Brusgaard, K., Frederiksen, A. L., Godballe, C., & Rossing, M. (2020). Variability in Medullary Thyroid Carcinoma in RET L790F Carriers: A Case Comparison Study of Index Patients. *Front Endocrinol (Lausanne)*, *11*, 251. https://doi.org/10.3389/fendo.2020.00251

Milićević, S., Bergant, D., Žagar, T., & Perić, B. (2021). Crude annual incidence rate of medullary thyroid cancer and RET mutation frequency. *Croat Med J*, *62*(2), 110-119. https://doi.org/10.3325/cmj.2021.62.110

Mishra, V., Kowtal, P., Rane, P., & Sarin, R. (2019). Genetic risk association of CDKN1A and RET gene SNPs with medullary thyroid carcinoma: Results from the largest MTC cohort and meta-analysis. *Cancer Med*, *8*(13), 6151-6161. https://doi.org/10.1002/cam4.2443

Mulligan, L. M. (2014). RET revisited: expanding the oncogenic portfolio. *Nat Rev Cancer*, *14*(3), 173-186. https://doi.org/10.1038/nrc3680

NCCN. (2024a, July 30, 2024). *Esophageal and Esophagogastric Junction Cancers Version 4.2024*. Retrieved November 7, 2024 from https://www.nccn.org/professionals/physician_gls/pdf/esophageal.pdf

NCCN. (2024b, May 23, 2022). *Neuroendocrine and Adrenal Tumors Version 2.2024 — August 1, 2024*. Retrieved October 26 from https://www.nccn.org/professionals/physician_gls/pdf/neuroendocrine.pdf

NCCN. (2024c, May 5, 2022). *Thyroid Carcinoma Version 4.2024 — August 19, 2024*. Retrieved October 26, 2022 from https://www.nccn.org/professionals/physician_gls/pdf/thyroid.pdf

Paragliola, R. M., Lovicu, R. M., Papi, G., Capoluongo, E., Minucci, A., Canu, G., Pontecorvi, A., & Corsello, S. M. (2018). Medullary Thyroid Carcinoma With Exon 2 p.L56M RET Variant: Clinical Particular Features in Two Patients. *Front Endocrinol (Lausanne)*, *9*, 398. https://doi.org/10.3389/fendo.2018.00398

Perros, P., Colley, S., Boelaert, K., Evans, C., Evans, R., Gerrard, G., Gilbert, J., Harrison, B., Johnson, S., Giles, T., Moss, L., Lewington, V., Newbold, K., Taylor, J., Thakker, R., Watkinson, J., & Williams, G. (2014). *British Thyroid Association Guidelines for the Management of Thyroid Cancer*. https://onlinelibrary.wiley.com/doi/pdf/10.1111/cen.12515

Plaza-Menacho, I., Mologni, L., & McDonald, N. Q. (2014). Mechanisms of RET signaling in cancer: current and future implications for targeted therapy. *Cell Signal*, *26*(8), 1743-1752. https://doi.org/10.1016/j.cellsig.2014.03.032

Qi, X. P., Peng, J. Z., Yang, X. W., Zao, Z. L., Yu, X. H., Fang, X. D., Zhang, D. H., & Zhao, J. Q. (2018). The RET C611Y mutation causes MEN 2A and associated cutaneous. *Endocr Connect*, *7*(9), 998-1005. https://doi.org/10.1530/EC-18-0220

Raue, F., Dralle, H., Machens, A., Bruckner, T., & Frank-Raue, K. (2018). Long-Term Survivorship in Multiple Endocrine Neoplasia Type 2B Diagnosed Before and in the New Millennium. *J Clin Endocrinol Metab*, *103*(1), 235-243. https://doi.org/10.1210/jc.2017-01884

Raue, F., & Frank-Raue, K. (2015). Epidemiology and Clinical Presentation of Medullary Thyroid Carcinoma. *Recent Results Cancer Res*, *204*, 61-90. https://doi.org/10.1007/978-3-319-22542-5_3

Raue, F., & Frank-Raue, K. (2018). Update on Multiple Endocrine Neoplasia Type 2: Focus on Medullary Thyroid Carcinoma. *J Endocr Soc*, *2*(8), 933-943. https://doi.org/10.1210/js.2018-00178

Romei, C., Ciampi, R., Casella, F., Tacito, A., Torregrossa, L., Ugolini, C., Basolo, F., Materazzi, G., Vitti, P., & Elisei, R. (2018). RET mutation heterogeneity in primary advanced medullary thyroid cancers and their metastases. *Oncotarget*, *9*(11), 9875-9884. https://doi.org/10.18632/oncotarget.23986

Romei, C., Ciampi, R., & Elisei, R. (2016). A comprehensive overview of the role of the RET proto-oncogene in thyroid carcinoma. *Nat Rev Endocrinol*, *12*(4), 192-202. https://doi.org/10.1038/nrendo.2016.11

Romei, C., Tacito, A., Molinaro, E., Agate, L., Bottici, V., Viola, D., Matrone, A., Biagini, A., Casella, F., Ciampi, R., Materazzi, G., Miccoli, P., Torregrossa, L., Ugolini, C., Basolo, F., Vitti, P., & Elisei, R. (2015). Twenty years of lesson learning: how does the RET genetic screening test impact the clinical management of medullary thyroid cancer? *Clin Endocrinol (Oxf)*, *82*(6), 892-899. https://doi.org/10.1111/cen.12686

Saarma, M. (2001). GDNF recruits the signaling crew into lipid rafts. *Trends Neurosci*, *24*(8), 427-429. https://www.ncbi.nlm.nih.gov/pubmed/11476867

Santoro, M., Carlomagno, F., Romano, A., Bottaro, D. P., Dathan, N. A., Grieco, M., Fusco, A., Vecchio, G., Matoskova, B., Kraus, M. H., & et al. (1995). Activation of RET as a dominant transforming gene by germline mutations of MEN2A and MEN2B. *Science*, *267*(5196), 381-383. https://www.ncbi.nlm.nih.gov/pubmed/7824936

Suyama, K., & Iwase, H. (2018). Lenvatinib: A Promising Molecular Targeted Agent for Multiple Cancers. *Cancer Control*, *25*(1), 1073274818789361. https://doi.org/10.1177/1073274818789361

Takahashi, M., Ritz, J., & Cooper, G. M. (1985). Activation of a novel human transforming gene, ret, by DNA rearrangement. *Cell*, *42*(2), 581-588. https://www.ncbi.nlm.nih.gov/pubmed/2992805

Takaya, K., Yoshimasa, T., Arai, H., Tamura, N., Miyamoto, Y., Itoh, H., & Nakao, K. (1996). Expression of the RET proto-oncogene in normal human tissues, pheochromocytomas, and other tumors of neural crest origin. *J Mol Med (Berl)*, *74*(10), 617-621. https://www.ncbi.nlm.nih.gov/pubmed/8912182

Wells, S. A., Jr., Asa, S. L., Dralle, H., Elisei, R., Evans, D. B., Gagel, R. F., Lee, N., Machens, A., Moley, J. F., Pacini, F., Raue, F., Frank-Raue, K., Robinson, B., Rosenthal, M. S., Santoro, M., Schlumberger, M., Shah, M., Waguespack, S. G., & American Thyroid Association Guidelines Task Force on Medullary Thyroid, C. (2015). Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. *Thyroid*, *25*(6), 567-610. https://doi.org/10.1089/thy.2014.0335

Wells, S. A., Jr., Pacini, F., Robinson, B. G., & Santoro, M. (2013). Multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma: an update. *J Clin Endocrinol Metab*, *98*(8), 3149-3164. https://doi.org/10.1210/jc.2013-1204

Yang, Z., Qi, X., Gross, N., Kou, X., Bai, Y., Feng, Y., Wang, B., Zafereo, M. E., Li, G., Sun, C., Li, H., Chen, X., & Huang, Z. (2020). The synergy of germline C634Y and V292M RET

mutations in a northern Chinese family with multiple endocrine neoplasia type 2A. *J Cell Mol Med*. https://doi.org/10.1111/jcmm.15922

Zhao, W., Choi, Y. L., Song, J. Y., Zhu, Y., Xu, Q., Zhang, F., Jiang, L., Cheng, J., Zheng, G., & Mao, M. (2016). ALK, ROS1 and RET rearrangements in lung squamous cell carcinoma are very rare. *Lung Cancer*, *94*, 22-27. https://doi.org/10.1016/j.lungcan.2016.01.011

Genetic Testing for Hereditary Hearing Loss

Policy Number: AHS - G2148 – Genetic Testing for Hereditary Hearing Loss	Prior Policy Name and Number, as applicable: • AHS-G2148 Genetic Testing for Nonsyndromic Hereditary Hearing Loss
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Hearing loss is among the most etiologically heterogeneous disorders. More than 400 genetic syndromes include hearing loss as a feature; additionally, more than 100 genes are associated with nonsyndromic genetic hearing loss, and several non-genetic causes can also result in hearing loss. Genes associated with syndromic and nonsyndromic genetic hearing loss encode a variety of proteins involved in the development and function of the auditory system, including transcription factors, structural proteins, gap junction proteins, and ion channel (Alford et al., 2014). The genes may be associated with an autosomal dominant, autosomal recessive, X-linked, or mitochondrial inheritance pattern (A Eliot Shearer, 2017). Genetic counseling is strongly recommended for individuals pursuing genetic testing for nonsyndromic hereditary hearing loss.

For guidance on prenatal screening and preconception screening for hereditary hearing loss, please see AHS-M2179-Prenatal Screening (Genetic).

II. Related Policies

Policy Number	Policy Title
AHS-M2179	Prenatal Screening (Genetic)

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals who are in a family with a deleterious familial hearing loss gene mutation, the following genetic testing **MEETS COVERAGE CRITERIA**:
   a) Testing restricted to the known familial mutation.
   b) Comprehensive genetic testing using multi-gene panel testing when the specific familial mutation is unknown.

2) For individuals diagnosed with hearing loss (when hearing loss due to nonhereditary causes [e.g., infection, injury, age-related] has been excluded), multi-gene panel testing (panel must include *GJB2* and *GJB6*) **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

3) Genetic testing for hereditary hearing loss-related mutations more than once per lifetime **DOES NOT MEET COVERAGE CRITERIA**.

4) For all other situations not described above, genetic testing for hearing loss **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
AAO	American Academy of Otolaryngology
AAP	American Academy of Pediatrics
ACMG	American College of Medical Genetics and Genomics
ACTG1	*Actin gamma 1*
ANSD	Auditory neuropathy spectrum disorder

ASHA	American Speech-Language-Hearing Association
BSND	*Barttin CLCNK type accessory subunit beta*
CDH23	*Cadherin related 23*
CLDN14	*Claudin 14*
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CLRN1	*Clarin 1*
CMS	Centers For Medicare and Medicaid
CMV	Cytomegalovirus
CNV	Contingent negative variation
COCH	*Cochlin*
COL11A2	*Collagen type Xl alpha 2 chain*
DFN	Different gene loci for nonsyndromic deafness
DFNA	Autosomal dominant inherited deafness
DFNA5	*Nonsyndromic hearing impairment protein 5*
DFNB	Autosomal recessive inherited deafness
DFNB1	*Nonsyndromic hearing loss and deafness*
DFNB31	*Deafness autosomal recessive 31 - Whirlin*
DFNB59	*Deafness autosomal recessive 59*
DFNX	X-linked Inheritance
DNA	*Deoxyribonucleic acid*
EKG	Electrocardiogram
ESPN	*Espin*
ESRRB	*Estrogen related receptor beta*
EYA4	*Transcriptional coactivator and phosphatase 4*
FDA	Food And Drug Administration
GJB2	Gap junction protein, beta 2
GJB6	Gap junction protein, beta 6
GPR98	*G-protein coupled receptor 98*
GRXCR1	*Glutaredoxin and cysteine rich domain containing 1*
HGF	*Hepatocyte growth factor*
IPOG	International Pediatric Otolaryngology Group
JCIH	Joint Commission on Infant Hearing
KCNQ4	*Potassium voltage-gated channel subfamily Q member 4*
LDTs	Laboratory-developed tests
LHFPL5	*LHFPL tetraspan subfamily member 5*

MARVELD2	*MARVEL domain containing 2*
MT-RNR1	*Mitochondrially encoded 12S ribosomal ribonucleic acid*
MT-TS1	*Mitochondrially encoded transfer ribonucleic acid-SER*
MYO15A	*Myosin XVA*
MYO6	*Myosin VI*
MYO7A	*Myosin VIIA*
NGS	Next-generation sequencing
NSHL	Nonsyndromic hearing loss
OMIM	Online Mendelian Inheritance in Man
OTOA	*Otoancorin*
OTOF	*Otoferlin*
PCDH15	*Protocadherin related 15*
PCR	Polymerase chain reaction
POU3F4	*POU class 3 homeobox 4*
PTPRQ	*Protein tyrosine phosphatase receptor type Q*
RDX	*Radixin*
SLC26A4	*Solute carrier family 26 member 4*
SNL	Sensorineural hearing loss
STRC	*Stereocilin*
TECTA	*Tectorin alpha*
TMC1	*Transmembrane channel like 1*
TMIE	*Transmembrane inner ear*
TMPRSS3	*Transmembrane serine protease 3*
TRIOBP	*TRIO and F-actin binding protein*
USH1C	*Usher syndrome type 1 protein component harmonin*
WES	Whole exome sequencing
WFS1	*Wolframin ER transmembrane glycoprotein*
WGS	Whole genome sequencing
WHRN	*Whirlin*

V. Scientific Background

Approximately one in every 500 children born in the United States is deaf or has a hearing loss significant enough to affect speech and language development. Ninety-five percent of newborns with hearing loss identified by newborn hearing screening programs are born to hearing parents, obscuring the fact that most newborns have a hereditary cause for their hearing loss (Alford et al., 2014). Approximately 80 percent of

cases of hereditary hearing loss are inherited in an autosomal recessive pattern, 19 percent are autosomal dominant, and the remaining cases X-linked (mainly recessive) or mitochondrial (A Eliot Shearer, 2017).

Hearing loss is typically described in terms related to its clinical presentation. In general, it is categorized as either syndromic or nonsyndromic. Syndromic hearing loss is associated with other medical or physical findings, including malformations of the external ear or other organs, or with medical problems involving other organ systems. An estimated 30% of hereditary hearing loss is syndromic. Nonsyndromic hearing loss (NSHL) is defined as hearing loss that is not associated with visible abnormalities of the external ear or any related medical problems. For NSHL, it is more difficult to determine whether the etiology is hereditary or acquired because there are no other clinical manifestations at the time of the hearing loss presentation. NSHL accounts for an estimated 70% of genetically determined hearing loss (Angeli et al., 2012), and it is frequently congenital and sensorineural (Sloan-Heggen et al., 2016).

The genetic loci where mutations associated with nonsyndromic hereditary hearing loss are commonly found are termed DFN. DFN loci are named based on their mode of inheritance: DFNA associated with autosomal dominant inheritance; DFNB with autosomal recessive inheritance; and DFNX with x-linked inheritance (A Eliot Shearer, 2017). The DFNB1 locus, which includes the *GJB2* gene encoding the gap junction protein connexin 26 and the *GJB6* gene encoding the gap junction protein connexin 30, accounts for an estimated 50% of all autosomal recessive nonsyndromic hearing loss and 15–40% of all deaf individuals in a variety of populations (Alford et al., 2014).

GJB2 is a small gene with a single coding exon, which codes for the Cx26 connexin protein (OMIM, 2016). At least 83 deafness-causing variants have been identified in *GJB2*, but a few common mutations account for a large percentage of alleles in several populations. Probands with this mutation generally have congenital hearing loss (A Eliot Shearer, 2017). Mutations in the *GJB6* gene lead to similar effects on abnormal expression of connexin protein Cx30 (OMIM, 2014). *GJB6* deletions have been observed in multiple populations, although they appear to be a relatively uncommon explanation for hearing loss in the United States (Alford et al., 2014).

In addition to mutations in the *GJB6* and *GJB2* genes, many less common pathologic mutations are found in other genes. Some of these are: *ACTG1, BSND, CDH23, CLDN14, COCH, COL11A2, DFNA5, DFNB31, DFNB59, ESPN, ESRRB, EYA4, GRXCR1, HGF, KCNQ4, LHFPL5, MARVELD2, MT-TS1, MYO15A, MYO6, MYO7A, OTOA, OTOF, PCDH15, POU3F4, PTPRQ, RDX, SLC26A4, STRC, TECTA, TMC1, TMIE, TMPRSS3, TRIOBP, USH1C, WFS1*, and *WHRN* genes (A Eliot Shearer, 2017). Several gene panels exist for assessment of hereditary hearing loss. For example, Shang et al evaluated the

"MiamiOtoGenes" panel, which consists of 180 genes. The investigators examined five unrelated probands with varying degrees of hearing loss onset and severity and found seven different genetic variants (Shang et al., 2018). Other entities offering proprietary genetic panels include BluePrint (239 genes), GeneDx (146 genes), OtoSCOPE by the University of Iowa (152 genes), Otogenetics Gx (167 genes), OtoGenome™ Test (84 genes), Hearing Loss Advanced Sequencing and CNV Evaluation by Athena Diagnostics (183 genes), Invitae Comprehensive Deaf Panel (203 genes), and AudioloGene Hereditary Hearing Loss Panel by Mayo Clinic Laboratories (160 genes) (BluePrint, 2023; GeneDx, 2024; Invitae, 2024; Iowa, 2020; Mayo Clinic, 2024; Otogenetics, 2021; Partners Healthcare, 2021).

Clinical Utility and Validity

Shearer and Smith (2015) performed a meta-analysis focusing on the current genetic tests used to evaluate hearing loss. A total of 20 studies were included, containing 426 controls and 603 patients with idiopathic hearing loss. Several genetic panels such as OtoGenetics Deafness Test and OtoGenome™ were used. Overall, the controls showed good sensitivity and specificity (over 99%), and the diagnostic rate was found to be 41% (with a range of 10%-83%). The authors concluded that "comprehensive genetic testing should form the cornerstone of a tiered approach to clinical evaluation of patients with hearing loss along with history, physical exam, and audiometry and can determine further testing that may be required, if any" (Shearer & Smith, 2015).

Sloan-Heggen et al. (2016) performed parallel sequencing on 1119 "sequentially accrued" patients. A total of 440 (39%) of these patients were found to have a genetic etiology for hearing loss. Pathogenic variants were found in 49 genes, and various alterations such as missense variants (49% of the alterations), copy number variants (18%), insertions or deletions (13%), and nonsense variants (8%) were found. The authors noted the wide variety of the genetic spectrum of hearing loss (Sloan-Heggen et al., 2016).

D'Aguillo et al. (2019) examined the role of genetic screening as an adjunct to universal newborn hearing screening. The authors evaluated 16 studies and identified the rate of children that passed the universal newborn hearing screening but who also tested positive on a genetic screening. Of the 137895 infants included in the studies, pathogenic mutations were detected in 8.66% of them. Of this cohort, 545 infants passed the universal screening, but also tested positive on a genetic screening (1.4%) (D'Aguillo et al., 2019).

Costales et al. (2020) studied the application of Otogenetics, a Next Generation Sequencing panel, in 27 patients diagnosed with sensorineural hearing loss (SNL) within a childhood hearing loss unit. A genetic diagnosis of SNL was made in 56%

(15/27) of the patients whereas 44% (12/27) had pathogenic variants in genes associated with isolated SNL, syndromic SNL, and non-syndromic SNL. According to the authors, this study demonstrated that "it is possible to implement genetic diagnosis in the daily routine" (Costales et al., 2020).

Yang et al. (2021) developed a multiplex PCR sequencing assay to sequence the *GJB2*, *SLC26A4*, and *MT-RNR1* genes and demonstrated that genetic screening can play an important role in newborn hearing screening. To validate the assay, 103 samples with known genotypes were analyzed using the multiplex PCR, which accurately identified all the variants with a 100% sensitivity and specificity. In the pilot study, 300 samples were analyzed and 12.3% were found to carry at least one pathogenic variant in the *GJB2, SLC26A4*, and *MT-RNR1* genes. The study also revealed that pathogenic variants in the *GJB2* gene had an 8% carrier rate in the newborn population. The authors concluded that "the assay is an accurate and reliable test and can be used to screen genetic hearing loss in newborns" (Yang et al., 2021).

Liao et al. (2022) put together a retrospective cohort study of 2075 patients who were seen at the Children's Communications Clinic; 517 patients completed a hearing loss testing with a GeneDx panel. Researchers gathered sociodemographic characteristics, hearing loss characteristics, and medical variables of hearing loss. On univariable analysis, those who received a genetic diagnosis were younger than those who did not receive a genetic diagnosis (OR, 0.92; 95% CI, 0.88-0.96). Twenty-four diagnostic genes were found in the study cohort, the most common diagnostic gene being *GJB2*, which encodes for connexin 26, a major protein found in gap junction protein in the cochlea. Additionally, the most prevalent diagnostic variant was different across races and ethnicities, that is, Asian patients were more likely to have the C.109 G>A variant and white patients were more likely to have the C.35del, and Hispanic patients were "almost equally" likely to have the c.35del variant as any other variant (aside from c.109 G>A) (Liao et al., 2022).

VI. Guidelines and Recommendations

The American College of Medical Genetics and Genomics (ACMG)

In 2014, the ACMG issued the following guidelines for the clinical evaluation and diagnosis of hearing loss. For individuals lacking physical findings suggestive of a known syndrome and having medical and birth histories that do not suggest an environmental cause of hearing loss, ACMG recommends that a tiered diagnostic approach should be implemented.

- "Pretest genetic counseling should be provided, and, with patient's informed consent, genetic testing should be ordered."

- "Single-gene testing may be warranted in cases in which the medical or family history, or presentation of the hearing loss, suggests a specific etiology. For example, testing for mitochondrial DNA mutations associated with aminoglycoside ototoxicity may be considered for individuals with a history of use of aminoglycoside antibiotics."
- "In the absence of any specific clinical indications and for singleton cases and cases with apparent autosomal recessive inheritance, the next step should be testing for DFNB1-related hearing loss (due to mutations in *GJB2* and adjacent deletions in *GJB6*)."
- "If initial genetic testing is negative, genetic testing using gene panel tests, NGS technologies such as large sequencing panels targeted toward hearing loss–related genes, WES, or WGS may be considered. Because several tests are clinically available, the clinician must be aware of the genes included in the test (panel) chosen and the performance characteristics of the platform chosen, including coverage, analytic sensitivity, and what types of mutations will be detected. It should be noted that the cost of these new genetic sequencing technologies is decreasing so rapidly that a tiered approach to testing may soon no longer be cost effective. In particular, for large sequencing panels targeted toward hearing loss– related genes, it may, in some cases, already be more cost effective to use NGS technologies as the initial test in the evaluation of hearing loss. However, issues related to genomic testing, such as the likelihood of incidental findings, will have to be addressed."
- "If genetic testing reveals mutation(s) in a hearing loss–related gene, mutation-specific genetic counseling should be provided, followed by appropriate medical evaluations and referrals."
- "If genetic testing fails to identify an etiology for a patient's hearing loss, the possibility of a genetic or acquired etiology remains. This point must be emphasized because it can be misunderstood by clinicians and by patients and their families. For interested patients and families, further genetic testing may be pursued on a research basis."
- "CMV testing should be done at the same time as genetic testing for infants with congenital hearing loss. For later-onset or progressive hearing loss, CMV testing can be obtained, but the likelihood that a positive test is due to postnatal exposure increases with age."

For individuals with findings that suggest a syndromic genetic etiology for their hearing loss:

- "Pretest genetic counseling should be provided, and, with patient's informed consent, genetic testing, if available, should be ordered to confirm the diagnosis—this testing may include single-gene tests, hearing loss sequencing

panels, WES, WGS, chromosome analysis, or microarray-based copy-number analysis, depending on clinical findings."
- "Appropriate studies should be undertaken to determine whether other organs are involved; and
- "Appropriate near-term and long-term screening and management should be arranged, including referrals to specialists, as indicated by the associated manifestations of the particular syndrome" (Alford et al., 2014).

The ACMG also published an algorithm stating to "consider" *GJB2, GJB6* or other gene specific testing if familial or nonsyndromic hearing loss was suspected. If nonsyndromic and mitochondrial inheritance was suspected, the ACMG recommended testing for the A1555G mutation (ACMG, 2018).

In 2021, ACMG released an updated guideline for screening for autosomal recessive and X-linked conditions during pregnancy and preconception. Their practice resource aims to recommend "a consistent and equitable approach for offering carrier screening to all individuals during pregnancy and preconception" and replaces any earlier ACMG position statements on prenatal/preconception expanded carrier screening and provide the following recommendations:

- "Carrier screening enables those screened to consider their reproductive risks, reproductive options, and to make informed decisions."
- "The phrase "expanded carrier screening" be replaced by "carrier screening"."
- "Adopting a more precise tiered system based on carrier frequency:
  - Tier 4: <1/200 carrier frequency (includes Tier 3) genes/condition will vary by lab
  - Tier 3: ≥ 1/200 carrier frequency (includes Tier 2) includes X-linked conditions
  - Tier 2: ≥1/100 carrier frequency (includes Tier 1)
  - Tier 1: *CF* [Cystic Fibrosis] + *SMA* [spinal muscular atrophy] + Risk Based Screening
    - "Tier 1 screening conveys the recommendations previously adopted by ACMG and ACOG" and "adopts an ethnic and population neutral approach when screening for cystic fibrosis and spinal muscular atrophy. Beyond these two conditions, additional carrier screening is determined after risk assessment, which incorporates personal medical and family history as well as laboratory and imaging information where appropriate"
    - "Tier 2 carrier screening stems from an ACOG recommendation for conditions that have a severe or moderate phenotype and a carrier frequency of at least 1/100." However, "data demonstrate that carrier screening for two common conditions using a carrier frequency threshold of 1/100 may not be equitable across diverse populations. Others have

shown that limiting the carrier frequency to ≥1/100 creates missed opportunities to identify couples at risk for serious conditions."

    - "We define Tier 3 screening as carrier screening for conditions with a carrier frequency ≥1/200 . . . Tier 2 and Tier 3 screening prioritize carrier frequency as a way to think about conditions most appropriate for screening in the general population. However, when ACOG proposed this level, they did not specify whether it was thinking about carrier frequency in terms of the global population or subpopulations. We use "carrier frequency" to mean in any ethnic group with reasonable representation in the United States."
    - "Tier 4 includes genes less common than those in Tier 3 and can identify additional at-risk couples. Tier 4 has no lower limit carrier screening frequency and can greatly extend the number of conditions screened . . . the clinical validity at this level of carrier screening may be less compelling, therefore we suggest reserving this level of screening for consanguineous pregnancies (second cousins or closer) and in couples where family or medical history suggests Tier 4 screening might be beneficial . . . Importantly, patients should understand that their chance of being a carrier for one or more conditions increases as the number of conditions screened is increased."

- "All pregnant patients and those planning a pregnancy should be offered Tier 3 carrier screening.
- Tier 4 screening should be considered:
    - When a pregnancy stems from a known or possible consanguineous relationship (second cousins or closer);
    - When a family or personal medical history warrants.
- ACMG does NOT recommend:
    - Offering Tier 1 and/or Tier 2 screening, because these do not provide equitable evaluation of all racial/ethnic groups.
    - Routine offering of Tier 4 panels.
- "Carrier screening paradigms should be ethnic and population neutral and more inclusive of diverse populations to promote equity and inclusion."
- "All pregnant patients and those planning a pregnancy should be offered Tier 3 carrier screening for autosomal recessive [Table 1] . . . conditions."
- "Reproductive partners of pregnant patients and those planning a pregnancy may be offered Tier 3 carrier screening for autosomal recessive conditions [Table 1] when carrier screening is performed simultaneously with their partner."
- "When Tier 1 or Tier 2 carrier screening was performed in a prior pregnancy, Tier 3 screening should be offered" (Gregg et al., 2021).

Table 1. Autosomal recessive genes for screening with carrier frequency ≥1/50.

OMIM gene	OMIM gene name	Maximum carrier frequency[a]	OMIM phenotype	Conditions
141900	*HBB*	0.119837	603903	Sickle cell anemia β-thalassemia
			613985	
613208	*XPC*	0.050885	278720	Xeroderma pigmentosum
606933	*TYR*	0.049337	203100	Oculocutaneous albinism type 1A and 1B
			606952	
613815	*CYP21A2*	0.048459	201910	Congenital adrenal hyperplasia due to 21-hydroxylase deficiency
612349	*PAH*	0.046068	261600	Phenylketonuria
602421	*CFTR*	0.040972	219700	Cystic fibrosis
600985	*TNXB*	0.035134	606408	Ehlers–Danlos-like syndrome due to tenascin-X deficiency
606869	*HEXA*	0.033146	272800	Tay–Sachs disease
121011	*GJB2*	0.026200	220290	Nonsyndromic hearing loss recessive 1A
			601544	Nonsyndromic hearing loss dominant 3A
602858	*DHCR7*	0.023709	270400	Smith–Lemli–Opitz syndrome
277900	*ATP7B*	0.021983	606882	Wilson disease
608034	*ASPA*	0.019856	271900	Canavan disease
607008	*ACADM*	0.016583	201450	Medium-chain acyl-coenzyme A dehydrogenase deficiency
602716	*NPHS1*	0.015994	256300	Finnish congenital nephrotic syndrome
601785	*PMM2*	0.015877	212065	Carbohydrate-deficient glycoprotein syndrome type Ia
607440	*FKTN*	0.015660	611615	Cardiomyopathy, dilated, 1X
			253800	Walker–Warburg congenital muscular dystrophy
605646	*SLC26A4*	0.015422	600791	Deafness autosomal recessive 4
			274600	Pendred syndrome
126340	*ERCC2*	0.015255	610756	Cerebrooculofacioskeletal syndrome 2
			601675	Trichothiodystrophy 1, photosensitive
603297	*DYNC2H1*	0.014817	613091	Short-rib thoracic dysplasia 3 with or without polydactyly

OMIM Online Mendelian Inheritance in Man.[55]
[a]Values round to ≥ 0.02 (two decimal places).

(Gregg et al., 2021)

In 2022, the ACMG released updated guidelines for clinical evaluation and etiologic diagnosis of hearing loss. The updated guideline suggests the following recommendations:

1. "All newborns and infants with confirmed HL should undergo a comprehensive evaluation in which patient-focused medical and birth histories, a 3-generation pedigree, and family medical history are obtained, and a physical examination that focuses on dysmorphic physical findings is performed. Evaluation of children and young adults with HL should follow a similar approach. Evaluation of deaf or hard-of-hearing adults should be customized based on the age of onset and other characteristics of HL."
    - "The medical and birth histories may be helpful in differentiating between acquired vs inherited causes of HL."
2. "For individuals with findings that suggest a syndromic genetic etiology for their HL:

- "Pretest genetic counseling should be provided, and, with patient's or caregiver's informed consent, genetic testing should be ordered to confirm the diagnosis. This testing may include single-gene tests, HL multigene panels, ES, GS, chromosome analysis, or microarray- or NGS-based copy number analysis, depending on clinical findings."
- "Appropriate studies should be undertaken to determine whether other organs are involved."
- "Appropriate near-term and long-term screening and management should be arranged, including referrals to specialists, as indicated by the associated manifestations of the syndrome."

3. "For individuals lacking physical findings suggestive of a known syndrome, a tiered diagnostic approach should be implemented."
   - "Unless clinical and/or family history suggests a specific genetic etiology, comprehensive HL gene panel testing should be initiated. If panel testing is negative, genome-wide testing, such as ES or GS, may be considered.
     - The HL panel should include the genes recommended by the HL Gene Curation Expert Panel [https://search.clinicalgenome.org/kb/affiliate/10007?page=1&size=25&search=].
     - If genetic testing reveals variant(s) in an HL-related gene, gene-specific genetic counseling should be provided, followed by appropriate medical evaluations and referrals.
     - If genetic testing fails to identify an etiology fora patient's HL, the possibility of a genetic etiology remains."
   - "Temporal bone imaging by computed tomography or magnetic resonance imaging should be considered as a complement to genetic testing, particularly if the diagnosis remains unclear; if cochlear im-plantation is being considered; if auditory neuropathy is noted, in cases of progressive HL; or if other clinical concerns exist. The anticipated clinical utility of imaging studies should be balanced against the risks associated with radiation exposure and sedation."
   - "CMV testing should be done as soon as possible after birth but within the first 3 weeks of life for infants with congenital HL. For later-onset or progressive HL, CMV testing can be obtained, but the likelihood that a positive test is caused by postnatal exposure increases with age."
4. "Referral to a multidisciplinary care center, when available, is recommended."
   - "A team approach that includes otolaryngologists, clinical geneticists, genetic counselors, audiologists, speech and language specialists, early hearing intervention and family support specialists and other appropriate specialists offers optimal opportunity to provide ongoing management and support of

deaf and hard-of-hearing individuals and their families as their needs change over time."

- "For cases in which the genetic evaluation failed to identify an underlying cause, periodic follow-up care every 3 years with a geneticist may be appropriate for several reasons. First, subtle features of syndromic forms of HL may not be apparent at birth or early in childhood but may appear as deaf or hard-of-hearing individuals grow into adulthood. These may prompt additional medical tests or referrals for specialty care. Second, follow-up visits offer the opportunity to inform individuals about new genetic tests that may have become available or changes in the interpretation of previous test results as medical knowledge advances. Finally, follow-up visits may also help identify clinical concerns unrelated to HL, for which referral for specialty care may be appropriate."

"Regardless of whether genetic test results are positive, negative, or inconclusive, results should be communicated through the process of genetic counseling and potential risks to other family members should be conveyed" (Li et al., 2022).

The Joint Committee on Infant Hearing (JCIH)

In 2007, the JCIH recommended that evaluation of infants with confirmed hearing loss should include a review of family history of specific genetic disorders or syndromes, including genetic testing for gene mutations such as GJB2 (connexin-26), and syndromes commonly associated with early-onset childhood sensorineural hearing loss (JCIH, 2007). In 2013, a supplement by the ASHA was added to the JCIH. The 2013 supplement also stated that medical providers must "understand atypical development etiologies and diagnoses, and refer for medical-genetic evaluation" and that families must be educated on the "importance of medical, genetic, ophthalmologic, and cardiac (EKG) evaluations on children with any type and degree of hearing loss" (ASHA, 2013).

In 2019, the JCIH published an updated position statement. They note that the American College of Medical Genetics and Genomics recommends offering genetic counseling and testing to all infants who are deaf or hard of hearing and their families. A geneticist's evaluation should include "a review of family history of specific genetic disorders or syndromes, genetic testing for gene mutations such as *GJB2* (connexin-26), and syndromes commonly associated with early-onset hearing loss" (JCIH, 2019).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state

coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81252	GJB2 (gap junction protein, beta 2, 26kDa, connexin 26) (eg, nonsyndromic hearing loss) gene analysis; full gene sequence
81253	GJB2 (gap junction protein, beta 2, 26kDa, connexin 26) (eg, nonsyndromic hearing loss) gene analysis; known familial variants
81254	GJB6 (gap junction protein, beta 6, 30kDa, connexin 30) (eg, nonsyndromic hearing loss) gene analysis, common variants (eg, 309kb [del(GJB6-D13S1830)] and 232kb [del(GJB6-D13S1854)])
81430	Hearing loss (eg, nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); genomic sequence analysis panel, must include sequencing of at least 60 genes, including CDH23, CLRN1, GJB2, GPR98, MTRNR1, MYO7A, MYO15A, PCDH15, OTOF, SLC26A4, TMC1, TMPRSS3, USH1C, USH1G, USH2A, and WFS1
81431	Hearing loss (eg, nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); duplication/deletion analysis panel, must include copy number analyses for STRC and DFNB1 deletions in GJB2 and GJB6 genes
S3844	DNA analysis of the connexin 26 gene (GJB2) for susceptibility to congenital, profound deafness

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

A Eliot Shearer, M. S. H., and Richard JH Smith. (2017). Hereditary Hearing Loss and Deafness Overview. In. https://www.ncbi.nlm.nih.gov/books/NBK1434/

ACMG. (2018). *Hearing Loss* https://www.acmg.net/PDFLibrary/Hearing-Loss-Algorithm.pdf

Alford, R. L., Arnos, K. S., Fox, M., Lin, J. W., Palmer, C. G., Pandya, A., Rehm, H. L., Robin, N. H., Scott, D. A., & Yoshinaga-Itano, C. (2014). American College of Medical Genetics and Genomics guideline for the clinical evaluation and etiologic diagnosis of hearing loss. *Genet Med*, *16*(4), 347-355. https://doi.org/10.1038/gim.2014.2

Angeli, S., Lin, X., & Liu, X. Z. (2012). Genetics of hearing and deafness. *Anat Rec (Hoboken)*, *295*(11), 1812-1829. https://doi.org/10.1002/ar.22579

ASHA. (2013). *Supplement to the JCIH 2007 position statement: principles and guidelines for early intervention following confirmation that a child is deaf or hard of hearing [Position Statement]*. American Speech-Language-Hearing Association. https://www.asha.org/policy/ps2013-00339/

BluePrint. (2023). *Comprehensive Hearing Loss and Deafness Panel*. Retrieved 03/29/2021 from https://blueprintgenetics.com/tests/panels/ear-nose-throat/comprehensive-hearing-loss-and-deafness-panel/

Costales, M., Diñeiro, M., Cifuentes, G. Á., Capín, R., Otero, A., Viejo-Díaz, M., Plasencia, A., Núñez, F., Gómez, J. R., Llorente, J. L., Cadiñanos, J., & Cabanillas, R. (2020). Clinical Utility of Next-generation Sequencing in the Aetiological Diagnosis of Sensorineural Hearing Loss in a Childhood Hearing Loss Unit. *Acta Otorrinolaringologica (English Edition)*, *71*(3), 166-174. https://doi.org/10.1016/j.otoeng.2019.05.005

D'Aguillo, C., Bressler, S., Yan, D., Mittal, R. A.-O. X., Fifer, R., Blanton, S. H., & Liu, X. (2019). Genetic screening as an adjunct to universal newborn hearing screening: literature review and implications for non-congenital pre-lingual hearing loss. (1708-8186 (Electronic)).

GeneDx. (2024). Hearing Loss Panel. https://providers.genedx.com/tests/detail/hearing-loss-panel-925

Gregg, A. R., Aarabi, M., Klugman, S., Leach, N. T., Bashford, M. T., Goldwaser, T., Chen, E., Sparks, T. N., Reddi, H. V., Rajkovic, A., & Dungan, J. S. (2021). Screening for autosomal recessive and X-linked conditions during pregnancy and preconception: a practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *23*(10), 1793-1806. https://doi.org/10.1038/s41436-021-01203-z

Invitae. (2024). Invitae Comprehensive Deafness Panel. https://www.invitae.com/us/providers/test-catalog/test-55009

Iowa, U. o. (2020). OtoSCOPE Genetic Testing. https://morl.lab.uiowa.edu/clinical-diagnostics/deafness-otoscope/otoscope-genetic-testing

JCIH. (2007). Year 2007 Position Statement: Principles and Guidelines for Early Hearing Detection and Intervention Programs. *Pediatrics*, *120*(4), 898. https://doi.org/10.1542/peds.2007-2333

JCIH. (2019). Year 2019 Position Statement: Principles and Guidelines for Early Hearing Detection and Intervention Programs. *Journal of Early Hearing Detection and Intervention*, *4*(2), 1-44. https://doi.org/https://doi.org/10.15142/fptk-b748

Li, M. M., Tayoun, A. A., Distefano, M., Pandya, A., Rehm, H. L., Robin, N. H., Schaefer, A. M., & Yoshinaga-Itano, C. (2022). Clinical evaluation and etiologic diagnosis of hearing loss: A clinical practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genetics in Medicine*, *24*(7), 1392-1406. https://doi.org/10.1016/j.gim.2022.03.018

Liao, E. N., Taketa, E., Mohamad, N. I., & Chan, D. K. (2022). Outcomes of Gene Panel Testing for Sensorineural Hearing Loss in a Diverse Patient Cohort. *JAMA Network Open*, *5*(9), e2233441-e2233441. https://doi.org/10.1001/jamanetworkopen.2022.33441

Mayo Clinic. (2024). Hearing Loss Panel. https://www.mayocliniclabs.com/test-catalog/overview/619372

OMIM. (2014). GAP JUNCTION PROTEIN, BETA-6; GJB6. https://www.omim.org/entry/604418

OMIM. (2016). GAP JUNCTION PROTEIN, BETA-2; GJB2. https://www.omim.org/entry/121011

Otogenetics. (2021). Hearing Loss & Molecular Screening of Congenital Hearing Loss. https://www.otogenetics.com/products/clinical-genetic-testing/hearing-loss/

Partners Healthcare. (2021). OtoGenome™ Test for Hearing Loss and Related Syndromes (110 Genes) Details. https://personalizedmedicine.partners.org/Laboratory-For-Molecular-Medicine/Tests/Hearing-Loss/OtoGenome.aspx

Shang, H., Yan, D., Tayebi, N., Saeidi, K., Sahebalzamani, A., Feng, Y., Blanton, S., & Liu, X. (2018). Targeted Next-Generation Sequencing of a Deafness Gene Panel (MiamiOtoGenes) Analysis in Families Unsuitable for Linkage Analysis. *Biomed Res Int*, *2018*, 3103986. https://doi.org/10.1155/2018/3103986

Shearer, A. E., & Smith, R. J. (2015). Massively Parallel Sequencing for Genetic Diagnosis of Hearing Loss: The New Standard of Care. *Otolaryngol Head Neck Surg*, *153*(2), 175-182. https://doi.org/10.1177/0194599815591156

Sloan-Heggen, C. M., Bierer, A. O., Shearer, A. E., Kolbe, D. L., Nishimura, C. J., Frees, K. L., Ephraim, S. S., Shibata, S. B., Booth, K. T., Campbell, C. A., Ranum, P. T., Weaver, A. E., Black-Ziegelbein, E. A., Wang, D., Azaiez, H., & Smith, R. J. (2016). Comprehensive genetic testing in the clinical evaluation of 1119 patients with hearing loss. *Hum Genet*, *135*(4), 441-450. https://doi.org/10.1007/s00439-016-1648-8

Yang, H., Luo, H., Zhang, G., Zhang, J., Peng, Z., & Xiang, J. (2021). A multiplex PCR amplicon sequencing assay to screen genetic hearing loss variants in newborns. *BMC Medical Genomics*, *14*(1), 61. https://doi.org/10.1186/s12920-021-00906-1

Genetic Testing for Hereditary Hemochromatosis

Policy Number: AHS – M2012 – Genetic Testing for Hereditary Hemochromatosis	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Hereditary hemochromatosis (HH) is a genetic disease that causes excessive absorption of dietary iron and storage in the skin, heart, liver, pancreas, and joints due to mutations of genes involved in iron metabolism and homeostasis. The genes include the *HFE* gene, and those encoding for hepcidin, hemojuvelin, transferrin receptor, ferritin, ferroportin, and ceruloplasmin (Bacon & Camaschella, 2024; Bacon & Phatak, 2024).

For policy regarding diagnostic testing of ferritin, transferrin, and hepcidin, please see AHS-G2011.

Terms such as male and female are used when necessary to refer to sex assigned at birth.

II. Related Policies

Policy Number	Policy Title
AHS-G2011	Diagnostic Testing of Iron Homeostasis & Metabolism

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with serum transferrin saturation ≥45% or elevated serum ferritin (see Note 1) **or** who have a first-degree relative (see Note 2) with confirmed hereditary hemochromatosis (HH), *homeostatic iron regulator* (*HFE)* variant genotyping (C282Y, H63D, or S65C) **MEETS COVERAGE CRITERIA**.

2) Multi-gene panel testing (see Note 3) for additional HH-related genes **MEETS COVERAGE CRITERIA** only when **all** of the following conditions are met:

   a) Individual has tested negative for common likely pathogenic or pathogenic variants in *HFE* (C282Y, H63D, and S65C);

   b) Individual presents with atypical symptoms of iron overload, such as endocrine or cardiac involvement;

   c) All other potential causes of elevated serum iron have been ruled out.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

3) For the general population, screening for HH via genetic testing **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Elevated serum ferritin is defined as >300 ng/mL in males or postmenopausal females; >200 ng/mL in premenopausal females (Bacon & Phatak, 2024).

Note 2: First-degree relatives include parents, full siblings, and children of the individual.

Note 3: For two or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
1ATD	Alpha 1-antitrypsin deficiency
AASLD	American Association for the Study of Liver Diseases
AUD	Alcohol use disorder
ACG	American College of Gastroenterology
BSH	British Society for Haematology
CASL	Canadian Association for the Study of the Liver
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CWC	Choosing Wisely Canada
DNA	Deoxyribonucleic acid
EASL	European Association for the Study of the Liver
FBC	Full blood count
FPN1	Ferroportin

FTH1	Ferritin heavy chain 1
GH	Genetic hemochromatosis
HAMP	*Hepcidin antimicrobial peptide*
HC	Hemochromatosis
HFE	*Homeostatic iron regulator*
HJV	Hemojuvelin
HH	Hereditary hemochromatosis
IPF	Idiopathic pulmonary fibrosis
LDTs	Laboratory-developed tests
LFT	Liver function test
NAFLD	Nonalcoholic fatty liver disease
NGS	Next-generation sequencing
PCR	Polymerase chain reaction
SF	Serum ferritin
SLC11A3	Previous gene name of *SLC40A1*
SLC40A1	*Solute carrier family 40-member 1 gene*
TfR/TFR	Transferrin receptor
Tsat	Transferrin saturation
USPSTF	U.S. Preventive Services Task Force

V. Scientific Background

Iron homeostasis is a complex process where the small peptide hormone hepcidin plays a major role by binding the sole mammalian iron exporter, ferroportin (FPN1), leading to FPN1 degradation by lysosomes. Hepcidin production is sensitive to extracellular iron concentrations by way of *HFE* and transferrin receptors (TfR). The HFE protein has been shown to interact with both TfR1 and TfR2 and initiate the BMP-SMAD signaling pathway upon transferrin binding. This signaling cascade ultimately increases expression of the *HAMP* gene that encodes for hepcidin (Pietrangelo, 2015; Vujić, 2014).

Hereditary hemochromatosis (HH) is an iron-storage disease caused by genetic mutations, most often in the *HFE* gene, resulting in chronic hyperabsorption of dietary iron and iron accumulation primarily in the liver, pancreas, and heart. This may cause impaired organ structure and function, and can ultimately lead to liver cirrhosis, liver cancer, diabetes, cardiac hypertrophy, congestive heart failure, and osteoarthritis, as well as other serious conditions (Adris et al., 2019; Milman et al., 2019). A diagnosis of *HFE*-associated HH is often difficult without the presence of physical features (Adris et al., 2019). Non-*HFE* hemochromatosis, which accounts for about 20% of HH diagnoses, includes genetic mutations to hepcidin, ferroportin, ferritin, TfR2, and ceruloplasmin. If

left untreated, iron overload can result in death (Bacon & Camaschella, 2024; Bacon & Phatak, 2024; Fleming & Ponka, 2012; Santos et al., 2012).

Three point mutations in *HFE* have been identified in HH: C282Y, H63D, and S65C. Homozygous C282Y mutation, according to one study in the U.S., was present in 83% of HH cases (Bacon & Camaschella, 2024; Feder et al., 1996). C282Y *HFE* mutations are relatively common, especially in Caucasians of northern European origin, particularly Nordic or Celtic ancestry; homozygosity among Caucasians was reported at 1:200-300. The frequency of the C282Y allele ranges from as high as 12.5% in Ireland to 0% in southern Europe (Pietrangelo, 2015). The prevalence of HH Type one (HFE C282Y mutation) varies by ethnicity; "0.000039% in Asian individuals, 0.012% in black, 0.027 in Hispanic to 0.44% in non-Hispanic individuals with a peak of 1.2% in Ireland" (Piperno et al., 2020). Despite the common nature of this mutation, only a proportion of these patients with the homozygous C282Y mutation will develop symptoms of HH (Piperno et al., 2020). This mutation disrupts disulfide bridge formation, preventing association with TfR1 (Vujić, 2014). The H63D mutation stabilizes the HFE-TfR1 complex and has a higher prevalence with a mean allele frequency of approximately 14%; however, phenotypically, H63D homozygosity rarely leads to HH (Pietrangelo, 2015; Vujić, 2014). A recent study by Joly et al. (2017) identified a link between the H63D mutation and patients with severe complications from alpha 1-antitrypsin deficiency (1ATD). 1ATD patients who also have the H63D *HFE* mutation have an increased risk of "developing significant chronic hepatic injuries (hepatomegaly, chronic cholestasis, elevated liver enzymes)" and are at risk of developing liver cirrhosis (Joly et al., 2017). An earlier study reported that 7.8% of HH genes, rather than containing neither the C282Y nor H63D mutations, actually had the S65C mutation, suggesting that it is associated with a more mild form of HH (Mura et al., 1999). Moreover, *HFE* gene variants are found more frequently in patients suffering from idiopathic pulmonary fibrosis (IPF), such that "The frequency of C282Y, S65C and H63D *HFE* allelic variants was markedly higher in IPF compared with controls (40.4% versus 22.4%, OR 2.35, p=0.008) and was associated with higher iron-dependent oxygen radical generation… [suggesting] iron dysregulation associated with *HFE* allelic variants may play an important role in increasing susceptibility to environmental exposures, leading to recurring injury and fibrosis in IPF" (Sangiuolo et al., 2015).

Juvenile hemochromatosis (JH) or type II hemochromatosis is caused by mutations in the gene encoding the protein hemojuvelin (*HJV/HFE2* causes Type IIA HH). This recessive, rare form of hemochromatosis is suspected of inhibiting hepcidin production by a decrease in the bone morphogenetic protein signaling pathway (Bacon & Camaschella, 2024). This condition is fully penetrant, but with varying degrees of onset depending on the mutation; *HFE2* HH can present in adult or older age instead of in juvenile years (Piperno et al., 2020). Unlike classical HH, JH typically develops before

the age of 30, progresses at a greater rate, and is associated with iron overload, leading to severe clinical complications, such as liver damage, cardiomyopathy, and hypogonadotrophic hypogonadism (Santos et al., 2012). An Italian study identified at least 17 different mutations that can cause JH (Lanzara et al., 2004). Takami et al. (2020) discovered that JH develops "in females and males at a ratio of 3:2 with no age difference in the two groups." Earlier JH onset was also seen among patients with a L101P/L101P or R385X/R385X *HJV* gene mutation and later in those with I281T/I281T *HJV* gene mutations when compared to the most common G320V/G320V mutation (Takami et al., 2020).

A second form of JH (type IIB), also autosomal recessive, is caused by mutations to the *HAMP* gene that encodes for hepcidin (Radford-Smith et al., 2018). This form also typically presents before the age of 30 with both hepatic and extrahepatic symptoms, including hypogonadism, cardiac abnormalities, and endocrine dysfunctions; however, with early treatment, symptoms can improve and iron levels can normalize (Fonseca et al., 2016; Lescano et al., 2017).

Mutations in the transferrin receptor two (*TFR2*) gene are rare; however, either homozygosity or compound heterozygosity can result in a phenotypic Type three HH (Bacon & Camaschella, 2024; Santos et al., 2012). TfR2 is typically a sensor for iron levels in the body and is involved in hepcidin synthesis (Santos et al., 2012). Typically, the first biochemical abnormality is evident when transferrin saturation (TSAT) elevation occurs in the second or third decade of life; *TFR2* HH onset is normally earlier than *HFE* HH (De Gobbi & Roetto, 2023). This form of hemochromatosis can result in liver disease due to hepatocellular iron accumulation and fibrosis and can vary in severity "depending on the phenotypic impact of the mutation" (Bardou-Jacquet et al., 2013; Pietrangelo, 2004). This phenotype can include "abnormal liver function, diabetes, hypogonadism, cardiomyopathy and arthritis" (Santos et al., 2012).

Mutations in the iron exporter ferroportin, FPN1, encoded by the gene *SLC40A1* (classified as *SLC11A3* in older literature), can result in an autosomal dominant form of HH (Moreno-Carralero et al., 2014; Politou et al., 2004). Ferroportin is normally responsible for iron transport across enterocytes and iron recycling by the reticuloendothelial system (Santos et al., 2012). This type of ferroportin disease is dictated by the nature of the mutation. For example, loss of function mutations, such as in classical ferroportin disease, result in excess iron accumulation in macrophages, ferritinemia, and mild anemia, whereas gain of function mutations, as seen in non-classical ferroportin disease or Type four HH, result in hepcidin-resistant ferroportin, leading to iron accumulation in the hepatic parenchyma (Bacon & Camaschella, 2024). These patients "hyperabsorb iron and present with high TSAT, high serum ferritin, ad tissue iron overload with evidence of toxic damage that may develop into adult age" (Piperno et al., 2020). Typically, the earliest biochemical abnormalities can begin to

appear within the first decade of life, but the clinical onset of liver disease may not appear until adulthood (patients in their 40s); unlike *HFE*-derived HH, ferroportin diseases, although rare, are pan-ethnic (Pietrangelo, 2004; Zhang et al., 2017).

Proprietary Testing

Several proprietary gene panels are available for hereditary hemochromatosis, such as Invitae's panel (six genes, *FTH1, HAMP, HFE, HJV, SLC40A1, TFR2*) (Invitae, 2024) and Blueprint Genetics' panel (five genes, *HAMP, HFE, HFE2, SLC40A1, TFR2*) (Blueprint, 2023). These panels often encompass the primary gene, *HFE*, as well as the rarer pathogenic variants that may also cause HH.

The standard of care for all forms of HH is reduction of iron via therapeutic, life-long phlebotomy with early initiation of treatment; iron chelation and modifications to diet, such as avoidance of iron, discontinuance of iron-containing supplements, and avoidance of alcohol can also be recommended (Rombout-Sestrienkova et al., 2016). Monitoring of serum ferritin and TSAT are required to manage treatment and assess disease progression where "Improvements in overall wellbeing, including fatigue, liver function (pre-cirrhosis) and skin pigmentations, are most noticeable. On the other hand, if cirrhosis is already well established, it is generally considered irreversible" (Radford-Smith et al., 2018).

Analytical Validity

Pietrangelo (2015) states that "*HFE* gene testing can be used to diagnose hemochromatosis in symptomatic patients, but analyses of liver histology and full gene sequencing are required to identify patients with rare, non-*HFE* forms of the disease. Due to the central pathogenic role of hepcidin, it is anticipated that nongenetic causes of hepcidin loss (e.g., end-stage liver disease) can cause acquired forms of hemochromatosis."

Specifically for the C282Y *HFE* mutation, Palomaki et al. (2003) found that the "analytic sensitivity for C282Y homozygosity is 98.4% (95% CI 95.9%–99.5%). The analytic specificity is 99.8% (99.4%–99.9%). At a frequency of 40 per 10,000 for the homozygous genotype, the analytic positive predictive value is 66%" after analyzing results from the Molecular Genetic Survey collected by the American College of Medical Genetics/College of American Pathologists between 1998 and 2002. The authors noted these results as a critical part of any confirmatory testing for identifying false-positive C282Y mutation test results in any potential population-based HH screening program.

A comprehensive German study researching the technical performance and clinical relevance of *HFE* C282Y testing found that 1.7% of the patients tested for this specific point mutation were homozygous for C282Y; although, it should be noted that 42.6%

of these patients had already been clinically diagnosed with hemochromatosis. Regarding the technical performance of the genetic test, it had an accuracy of 99.6% with an overall error rate of 0.24%. The analytic specificity "with respect to the detection of homozygosity for C282Y was 100% (7726 of 7726 nonhomozygous test challenges, 95% CI: 99.95-100%), while the analytic sensitivity was 97% (130 of 134 homozygous test challenges, 95% CI: 92.5-99.2%)...We conclude that the test methods for C282Y are robust, highly sensitive and specific, and that a DNA-based HH-screening program can be performed at reasonable laboratory costs" (Stuhrmann et al., 2005).

The College of American Pathologists provided blinded proficiency testing (PT) for any interested laboratories completing common *HFE* genetic testing methods; researchers used ten years of data provided from these PT studies to determine overall *HFE* testing laboratory performance. A total of 257 different labs participated and several different genotyping methods were used including "differential restriction enzyme fragment lengths (51%), melting curve-based methods (15%), probe-specific real-time PCR (TaqMan) methods (eight percent), direct sequencing (seven percent), and allele-specific PCR (seven percent)" (Press et al., 2016). A very low error rate was found (0.73% in 7,663 results), with more errors found in specific variants (C282Y heterozygous, H63D homozygous, and C282Y homozygous); very high sensitivity and specificity values were identified at >98.5% and >99.5% respectively (Press et al., 2016).

Clinical Utility and Validity

A study by Bulaj et al. (1996) published in the New England Journal of Medicine found that of the ten percent of Caucasians heterozygous for classical HH, 20% of males and eight percent of females had higher than normal mean serum ferritin concentrations than the control group and that four percent of males and eight percent of females had elevated TSAT levels as compared to the control, wildtype group. "The clinical and biochemical expression of hemochromatosis was more marked in heterozygotes with paternally transmitted mutations than in those with maternally transmitted mutations. Liver-biopsy abnormalities were generally associated with alcohol abuse, hepatitis, or porphyria cutanea tarda. The phenotype of persons heterozygous for hemochromatosis differs from that of normal subjects, but complications due to iron overload alone in these heterozygotes are extremely rare" (Bulaj et al., 1996).

Another systematic review in 2008 of eleven different studies for classical HH testing in at-risk populations show that the "clinical sensitivity of C282Y homozygosity for hereditary haemochromatosis ranged from 28.4% to 100%; when considering studies that used strict criteria to classify hereditary haemochromatosis, the clinical sensitivity ranged from 91.3% to 92.4%" (Bryant et al., 2008). Another study investigating the accuracy of self-reporting family history of hemochromatosis showed that 81.4% of patients reporting a family history for hemochromatosis correlated positively. The

authors then concluded: "Self-reported family history of hemochromatosis or iron overload can be used to identify individuals whose risk of hemochromatosis or iron overload and associated conditions is increased. These individuals could benefit from further evaluation with iron phenotyping and *HFE* mutation analysis" (Acton et al., 2008).

Lanktree et al. (2017) utilized next-generation sequencing (NGS) of an iron metabolism gene panel to provide patients with a non-*HFE* hemochromatosis diagnosis; the panel was constructed of 15 genes related to iron metabolism. A total of 190 patients with a potential iron overload were screened, and six were diagnosed with non-*HFE* iron overload based on homozygous hemojuvelin (*HFE2*) mutations (Lanktree et al., 2017). Additional pathogenic mutations were found from molecular sequencing results.

Rabideau et al. (2014) also used NGS in a panel with *HFE, HAMP, HFE2, SLC40A1,* and *TFR2* genes to detect rare, other HH-causing mutations not typically assayed. They found that these particular genes "resulted in an additional diagnostic yield compared to *HFE* C282Y and H63D testing alone," and that in patients with such a genetic attribution, management of care "can be personalized based on genotype-phenotype correlation (e.g. N144Y *SLC40A1* mutations may lead to reduced phlebotomy tolerance) and at-risk family members can be screened" (Rabideau et al., 2014).

A 2018 study by Sandhu et al. (2018) conducted a phenotypic analysis across both *HFE* and non-*HFE* variants of hemochromatosis to identify differences in severity of iron overload and disease presentation. Data from 156 patients with genetically confirmed autosomal recessive non-*HFE* HH were compared against 984 patients with *HFE*-p.C282Y homozygous HH, and were found to have both "an earlier age of onset and a more severe clinical course than *HFE* HH, with the most severe presentations found among those with *HJV* and *HAMP* HH (Sandhu et al., 2018). These two juvenile forms had a greater association with all clinical outcomes as well, including cardiomyopathy and hypogonadism, but not arthritis and arthropathy, which was found more in the *HFE* HH population. Higher proportions of those with non-*HFE* HH were derived from Italy (30%) and Greece (ten percent), and most of those with non-*HFE* HH were homozygous for their respective mutations. This study conveys how the type of HH mutation would influence the level of urgency for treatment of a patient's presentation (Sandhu et al., 2018).

The *HFE* gene mutations, specifically C282Y and H63D, have also been tested in association with cancer. From a meta-analysis performed by Lv et al. (2016), "C282Y was found to increase the risk of cancer twofold in the recessive model and 1.1-fold in the allele mode…The results suggest that the C282Y/C282Y genotype is associated with a twofold elevated risk for breast cancer, a 1.7-fold elevated risk of colorectal, and a 3.6-fold increased risk of hepatocellular cancer." This study demonstrated the utility of *HFE* gene mutations beyond HH to cancer risk, and how "living environment,

genetic background and dietary habits are candidate factors that influence the risk of cancer because of HFE mutations" (Lv et al., 2016).

The finding with hepatocellular cancer was conferred by Atkins et al. (2020), who, in a cohort of 451,186 UK Biobank participants who had the HFE p.282Y and p.H63D phenotypes, found that among 1294 male p.282Y homozygotes, there were 21 incident hepatic malignancies. A significant finding was not found to be statistically significant in women. For projections of the male p.282Y homozygote lifetime risk of primary hepatic malignancy up to age 75, the risk was "7.2% (95% CI, 3.9%-13.1%), compared with 0.6% (95% CI, 0.4%-0.7%) for men with neither variant, and the risk of death was 19.5% (95% CI, 15.8%-24.0%), compared with 15.1% (95% CI, 14.7%-15.5%) among men with neither variant" (Atkins et al., 2020).

Moreover, Lim et al. (2020) performed a retrospective analysis on individuals in Newfoundland and Labrador, Canada, homozygous for the C282Y mutation between 1999 and 2009. Of the 306 individuals with C282Y/C282Y and with adequate follow-up, 56 (18.3%) were observed to have end-organ damage, with 5.8% developing liver disease and that such end organ damage was observed more frequently in men (24.3%) than in women (10.5%); however, when separated across pre- vs post-menopausal groups for women, iron overload-related disease was met by 4.1% and 18.3%, respectively. As such, the authors concluded that given the relatively low clinical penetrance of HH mutations, C282Y homozygosity uncommonly causes end-organ damage and liver disease (Lim et al., 2020).

Hereditary hemochromatosis, with mutations in the *HFE* gene, has been associated with osteoarthritis (Milman et al., 2019). A recent prospective cross-sectional study analyzed data from 940 patients younger than 70 years old previously diagnosed with end-stage osteoarthritis of the hip; all participants were compared to a healthy control of similar age and sex (Oppl et al., 2018). Results did not show a relationship between *HFE* mutations and osteoarthritis; the authors stated that "No greater prevalence of C282Y homozygosity mutation or elevated serum ferritin or transferrin saturation levels was found in the study cohort with severe osteoarthritis of the hip than in controls from the general population" (Oppl et al., 2018).

Tamosauskaite et al. (2019) also conducted a study on musculoskeletal effects of HH C282 homozygosity among 200,975 UK Biobank participants aged 60-70 years. They tested the association with Fried frailty, sarcopenia, and chronic pain, and found that "C282Y homozygote men had increased likelihood of reporting chronic pain (odds ratio [OR] 1.23: 95% confidence interval [CI] 1.05-1.45, p = .01) and diagnoses of polymyalgia rheumatica… They were also more likely to have sarcopenia (OR 2.38: 1.80-3.13, p = 9.70 × 10-10) and frailty (OR 2.01: 1.45-2.80, p = 3.41 × 10-05). C282Y homozygote women (n = 312, 0.7%) aged 65-70 were more likely to be frail (OR 1.73: 1.05-2.84, p = .032) and have chronic knee, hip, and back pain" (Tamosauskaite et al.,

2019). This continues to solidify the association between musculoskeletal comorbidities and HH and could ultimately contribute to precision medicine approaches to improving quality of life among the elderly.

Hereditary hemochromatosis patients with *HFE* mutations typically present with elevated erythrocyte levels; until recently, this data has not been used in clinical practice. Adris et al. (2019) analyzed data from a total of 2,688 participants (144 with HH, 1844 healthy controls, and 700 with chronic diseases). Results showed that the "mean cell volume (MCV) and mean cell haemoglobin (MCH) were always significantly higher" in HH subjects when compared to other participants; thus, the use of erythrocyte parameters "demonstrated excellent diagnostic utility for detection of HH in men and women (AUROC 0.83-0.9; maximal sensitivity and specificity 82% and 78%)" (Adris et al., 2019).

VI. Guidelines and Recommendations

United States Preventive Services Task Force (USPSTF)

The USPSTF recommends against genetic screening for HH in the general, asymptomatic population, due to the low penetrance of the disease among those with causative mutations (USPSTF, 2014; Whitlock et al., 2006). This 2006 guideline is now listed as an "Inactive Topic" as of 09/17/2018. USPSTF (2006) guidelines state the following:

The U.S. Preventive Services Task Force (USPSTF) has decided not to review the evidence and update its recommendations for this topic. The previous evidence review, and recommendation may contain information that is outdated.

The USPSTF bases its recommendations on current evidence about preventive services. The USPSTF decides not to update some topics (or "inactivate" them) for several reasons. Topics may be inactivated because they are no longer relevant to clinical practice. This may be the result of changes in technology, a new understanding of the etiology or natural history of the disease, or the evolving natural history of the disease. Topics may also be inactivated because they involve services that cannot be implemented in a primary care setting or are not referable by a primary care clinician. In addition, topics that have a low public health burden or that otherwise fall outside the scope of the USPSTF may be inactivated.

The USPSTF encourages primary care clinicians to consult other sources for current evidence regarding this topic. If new evidence becomes available, the USPSTF may elect to update this topic" (USPSTF, 2006).

Canadian Association for the Study of the Liver (CASL) and Choosing Wisely Canada (CWC) Choosing Wisely Canada-Top Five List in Hepatology

The CASL developed a list of the top five recommendations in hepatology based on data provided by the CWC. One of these recommendations mention *HFE* genotyping:

- "Don't order *HFE* genotyping based on serum ferritin values alone to diagnose hereditary hemochromatosis" (Brahmania et al., 2019).

American Association for the Study of Liver Diseases (AASLD)

The AASLD has published the following recommendations in Bacon et al. (2011):

1. "We recommend that patients with abnormal iron studies should be evaluated as patients with hemochromatosis, even in the absence of symptoms. (A)
2. All patients with evidence of liver disease should be evaluated for hemochromatosis. (1B)
3. In a patient with suggestive symptoms, physical findings, or family history, a combination of TS and ferritin should be obtained rather than relying on a single test. (1B) If either is abnormal (TS ≥45% or ferritin above the upper limit of normal), then *HFE* mutation analysis should be performed. (1B)
4. Diagnostic strategies using serum iron markers should target high-risk groups such as those with a family history of HH or those with suspected organ involvement. (1B)
5. We recommend screening (iron studies and HFE mutation analysis) of first-degree relatives of patients with *HFE*-related HH to detect early disease and prevent complications. (1A)"

European Association for the Study of the Liver (EASL)

The EASL published clinical practice guidelines for *HFE* hemochromatosis. In general and patient populations, EASL (2010) states:

- "General population:
  - Genetic screening for HFE-HC is not recommended, because disease penetrance is low and only in few C282Y homozygotes will iron overload progress (1B).
- Patient populations:
  - HFE testing should be considered in patients with unexplained chronic liver disease pre-selected for increased transferrin saturation (1C).
  - HFE testing could be considered in patients with:
    - Porphyria cutanea tarda (1B).

- Well-defined chondrocalcinosis (2C).
- Hepatocellular carcinoma (2C).
- Type 1 diabetes (2C).

- HFE testing is not recommended in patients with
  - Unexplained arthritis or arthralgia (1C)
  - Type 2 diabetes (1B)."

Guidelines from 2022 recommend that "Individuals with clinical and biochemical signs of haemochromatosis, elevated transferrin saturation and high serum ferritin concentrations, or otherwise unexplained persistently elevated transferrin saturation should be genetically tested for haemochromatosis after informed consent for genetic testing has been obtained (LoE 2, strong recommendation, strong consensus). Patients with increased liver iron evident on liver biopsy or MRI should be clinically assessed and biochemically tested for haemochromatosis (serum ferritin and transferrin saturation) (LoE 2, strong recommendation, strong consensus)" (EASL, 2023).

"Genotyping for p.C282Y in HFE should be carried out in individuals of European origin with biochemical evidence of iron overload (females with transferrin saturation >45% and serum ferritin >200 lg/L and males with transferrin saturation >50% and ferritin >300 lg/L, or otherwise unexplained persistently elevated transferrin saturation) with or without clinical signs or symptoms suggestive of haemochromatosis (LoE 2, strong recommendation, strong consensus). Adult (>18 years of age) first-degree relatives of patients with p.C282Y homozygous haemochromatosis should be tested for the p.C282Y variant in HFE (LoE 4, strong recommendation, strong consensus)" (EASL, 2023).

Regarding family screening, the EASL states "Siblings of patients with HFE-related HC must undergo screening, since they have a 25% chance of being susceptible. Serum ferritin, and transferrin saturation should be assessed. Ideally HFE mutation analysis should be encouraged after appropriate counseling with regard to the pros and cons of testing (mortgage, insurance issues)" (EASL, 2010).

In the 2022 guidelines EASL (2023) recommends "Adult individuals with a positive family history of first-degree relatives with haemochromatosis should be genetically tested for haemochromatosis after informed consent for genetic testing has been obtained (LoE 4, strong recommendation, strong consensus)."

In using genetic testing to diagnose HH, the EASL adds:

- "Patients from liver clinics should be screened for fasting transferrin saturation and serum ferritin (1C) and offered genetic HFE testing if transferrin saturation is increased (1B)

- Diagnosis of HFE hemochromatosis should not be based on C282Y homozygosity alone but requires evidence of increased iron stores (1B).
- Genetic testing of 'other hemochromatosis genes' (*TFR2, SLC40A1, HAMP, HJV*) could be considered in patients with increased iron stores after exclusion of C282Y homozygosity if (i) iron excess has been proven by direct assessment, i.e. by MRI or liver biopsy, and (ii) other hepatic and haematological disorders have been ruled out (2C)."

European Molecular Quality Network (EMQN)

The EMQN has published the following recommendations for diagnostic and predictive testing:

1. "Population screening for the p.C282Y variant is not currently recommended (1B).
2. It is considered to be good practice to confirm elevated TS before *HFE* genetic diagnosis testing (1B).
3. Testing adult siblings (brothers and sisters) of p.C282Y homozygotes is recommended owing to the increased risk of p.C282Y homozygosity and related increased morbidity (1B).
4. Testing adult offspring of p.C282Y homozygotes is recommended owing to increased risk of p.C282Y homozygosity and related increased morbidity (1C).
5. Testing asymptomatic parents of p.C282Y homozygotes is not recommended systematically but rather as a clinical decision depending on their age, sex and ferritin, all three influencing the probability to develop severe iron overload (1C).
6. Systematic testing of adult first-degree relatives of p.C282Y heterozygotes is not currently recommended, in the absence of evidence of benefit (2C).
7. *HFE* testing of minors is not recommended (1B).
8. Prenatal diagnosis is not appropriate in *HFE*-related HH because it is a treatable, adult-onset condition (1C)" (Porto et al., 2016).

American College of Gastroenterology (ACG)

Hereditary hemochromatosis is one of the most common causes of inherited liver disorders which causes abnormal liver chemistries. In the cases where patients have abnormal liver chemistries without acute hepatitis, ACG recommends ("strong recommendation, very low level of evidence") that those patients should undergo testing for hereditary hemochromatosis with an iron level, transferrin saturation, and serum ferritin; further, "*HFE* gene mutation analysis should be performed in patients with transferrin saturation ≥45% and/or elevated serum ferritin" (Kwo et al., 2017).

In regards to family members, the ACG has stated that "We recommend that family members, particularly first-degree relatives, of patients diagnosed with HH should be

screened for HH (strong recommendation, moderate quality of evidence)" (Kowdley et al., 2019).

The ACG also recommends that "individuals with the H63D or S65C mutation in the absence of C282Y mutation should be counseled that they are not at increased risk of iron overload (conditional recommendation, very low quality of evidence) … We suggest against further genetic testing among patients with iron overload testing negative for the C282Y and H63D alleles (conditional recommendation, very low quality of evidence)" (Kowdley et al., 2019).

Regarding non-*HFE* HH, the ACG writes that "Before pursuing testing for non-*HFE* hemochromatosis, alternative explanations for elevated serum iron tests should be excluded, because abnormal iron studies due to conditions such as AUD or NAFLD are far more common than non-*HFE* hemochromatosis. Contrarily, sequencing of non-*HFE* genes may be considered in atypical cases of iron overload, such as a younger patient presenting with endocrine or cardiac involvement" (Kowdley et al., 2019).

American College of Medical Genetics and Genomics (ACMG)

The ACMG has listed *HFE* genetic testing on its Choosing Wisely list. The ACMG recommends that physicians "Don't order HFE genetic testing for a patient without iron overload or a family history of HFE-associated hereditary hemochromatosis." They expound upon this with the following: "*HFE* genotyping should only be performed among individuals with iron overload (e.g., elevated fasting transferrin saturation >45%) or a known family history of *HFE*-associated hereditary hemochromatosis. In the setting of genome or exome sequencing, it is now recommended that patients who are homozygous for the pathogenic variant C282Y in HFE should receive this result and consider evaluation" (ACMG, 2023).

British Society for Haematology (BSH)

The BSH recommendations include the following as published by Fitzsimons et al. (2018):

1. "Unselected population screening for *HFE* gene mutation is not recommended. (1B)
2. Genetic haemochromatosis (GH) patients who present with serum ferritin (SF) >1000 µg/l and any with raised transaminases should be referred to a hepatologist for fibrosis assessment and exclusion of cirrhosis. (1B)
3. Patients of north European ancestry with clinical features suggestive of GH should have the following laboratory investigations; full blood count (FBC), liver function tests (LFTs), SF and transferrin saturation (Tsat). Molecular testing for

HFE GH should follow if results fulfil the criteria of recommendation 5 (see below). (1B)

4. All adult patients of north European ancestry with unexplained raised SF and random Tsat (>300 µg/l and >50% males; >200 µg/l and >40% females) and normal FBC should have molecular testing for *HFE* GH. (1B)
5. Laboratory screening to include FBC, LFTs, SF, Tsat and *HFE* should be offered to family members after the diagnosis of *HFE* GH. Family screening should include parents (if available), siblings, partner and children (over the age of consent). Extended family screening is not recommended if an individual is identified as a C282Y/H63D compound heterozygote. (1B)
6. Investigation of all confirmed C282Y homozygotes should include FBC, LFTs, SF and Tsat. Thereafter further investigation may be required as follows:
   i. SF <1000 µg/l, normal LFTs, normal clinical examination; no further investigation required. Follow recommendation [7]. (1C)
   ii. SF >1000 µg/l and or abnormal LFTs. All such patients require referral to Hepatology for fibrosis assessment to exclude the presence of cirrhosis. A minimum would be elastography. For patients with confirmed cirrhosis monitor with ▪ -fetoprotein (AFP) and hepatic ultrasound every six months. (2C)
7. Non C282Y homozygotes with significant iron loading as confirmed by magnetic resonance imaging and or liver biopsy should be investigated for rare iron loading genotypes or digenic inheritance. (1C)
8. At diagnosis, all fit GH patients with biochemical iron loading should undergo weekly venesection until SF ~20–30 µg/l and Tsat <50%. During this phase of venesection FBC should be monitored weekly and SF Tsat monitored monthly. Homozygotes with normal iron indices and compound heterozygotes with minimal elevation of iron indices may be suitable for blood donation and annual monitoring of SF and Tsat. (1B)
9. During maintenance, venesect as required, preferably at a blood donation centre to maintain normal FBC, SF <50 µg/l and Tsat <50%. (1C)"

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Recently, the U.S. Food and Drug Administration (FDA) has authorized a direct-to-consumer Genetic Health Risk Hereditary Hemochromatosis test developed by 23andMe. This test provides information on an individual's genetic predisposition from European descent for Hereditary Hemochromatosis by testing two variants (C282Y; H63D) in the *HFE* gene in genomic DNA obtained from a human saliva. However, this test cannot determine an individual's overall risk of developing a disease (FDA, 2017).

LabCorp (2024) has developed a hereditary hemochromatosis test which utilizes PCR for DNA analysis; this testing method utilizes a whole blood or swab kit and analyzes the C282Y, H63D, and S65C mutations of the *HFE* gene. This test has not been approved by the FDA.

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81256	*HFE* (hemochromatosis) (e.g., hereditary hemochromatosis) gene analysis, common variants (e.g., C282Y, H63D)
81479	Unlisted molecular pathology procedure

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

ACMG. (2023, July 1). *American College of Medical Genetics and Genomics*. http://www.choosingwisely.org/clinician-lists/american-college-medical-genetics-genomics-hfe-genetic-testing/

Acton, R. T., Barton, J. C., Passmore, L. V., Adams, P. C., McLaren, G. D., Leiendecker-Foster, C., Speechley, M. R., Harris, E. L., Castro, O., Reiss, J. A., Snively, B. M., Harrison, B. W., & McLaren, C. E. (2008). Accuracy of family history of hemochromatosis or iron

overload: the hemochromatosis and iron overload screening study. *Clin Gastroenterol Hepatol*, *6*(8), 934-938. https://doi.org/10.1016/j.cgh.2008.04.003

Adris, N., Hazeldine, S., Bentley, P., Trinder, D., Chua, A. C. G., Powell, L. W., Ramm, L. E., Ramm, G. A., & Olynyk, J. K. (2019). Detection of HFE Haemochromatosis in the clinic and community using standard erythrocyte tests. *Blood Cells Mol Dis*, *74*, 18-24. https://doi.org/10.1016/j.bcmd.2018.10.003

Atkins, J. L., Pilling, L. C., Masoli, J. A. H., Kuo, C. L., Shearman, J. D., Adams, P. C., & Melzer, D. (2020). Association of Hemochromatosis HFE p.C282Y Homozygosity With Hepatic Malignancy. *Jama*, *324*(20), 2048-2057. https://doi.org/10.1001/jama.2020.21566

Bacon, B. R., Adams, P. C., Kowdley, K. V., Powell, L. W., & Tavill, A. S. (2011). Diagnosis and management of hemochromatosis: 2011 practice guideline by the American Association for the Study of Liver Diseases. *Hepatology*, *54*(1), 328-343. https://doi.org/10.1002/hep.24330

Bacon, B. R., & Camaschella, C. (2024, 05/19/2022). *HFE and other hemochromatosis genes*. https://www.uptodate.com/contents/hfe-and-other-hemochromatosis-genes

Bacon, B. R., & Phatak, P. (2024, 10/13/2023). *Clinical manifestations and diagnosis of hereditary hemochromatosis*. https://www.uptodate.com/contents/clinical-manifestations-and-diagnosis-of-hereditary-hemochromatosis

Bardou-Jacquet, E., Cunat, S., Beaumont-Epinette, M. P., Kannengiesser, C., Causse, X., Sauvion, S., Pouliquen, B., Deugnier, Y., David, V., Loreal, O., Aguilar-Martinez, P., Brissot, P., & Jouanolle, A. M. (2013). Variable age of onset and clinical severity in transferrin receptor 2 related haemochromatosis: novel observations. *Br J Haematol*, *162*(2), 278-281. https://doi.org/10.1111/bjh.12350

Blueprint. (2023). *Hereditary Hemochromatosis Panel*. https://blueprintgenetics.com/tests/panels/metabolic-disorders/hereditary-hemochromatosis-panel/

Brahmania, M., Renner, E. L., Coffin, C. S., Yoshida, E. M., Wong, P., Zeman, M., & Shah, H. (2019). Choosing Wisely Canada-Top Five List in Hepatology: Official Position Statement of the Canadian Association for the Study of the Liver (CASL) and Choosing Wisely Canada (CWC). *Ann Hepatol*, *18*(1), 165-171. https://doi.org/10.5604/01.3001.0012.7908

Bryant, J., Cooper, K., Picot, J., Clegg, A., Roderick, P., Rosenberg, W., & Patch, C. (2008). A systematic review of the clinical validity and clinical utility of DNA testing for hereditary haemochromatosis type 1 in at-risk populations. *J Med Genet*, *45*(8), 513-518. https://doi.org/10.1136/jmg.2007.055806

Bulaj, Z. J., Griffen, L. M., Jorde, L. B., Edwards, C. Q., & Kushner, J. P. (1996). Clinical and biochemical abnormalities in people heterozygous for hemochromatosis. *N Engl J Med*, *335*(24), 1799-1805. https://doi.org/10.1056/nejm199612123352403

De Gobbi, M., & Roetto, A. (2023). TFR2-Related Hereditary Hemochromatosis. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews((R))*. University of Washington, Seattle. https://www.ncbi.nlm.nih.gov/pubmed/20301523

EASL. (2010). EASL clinical practice guidelines for HFE hemochromatosis. *J Hepatol*, *53*(1), 3-22. https://doi.org/10.1016/j.jhep.2010.03.001

EASL. (2023). EASL Clinical Practice Guidelines on haemochromatosis. *Journal of Hepatology*, *77*(2), 479-502. https://doi.org/10.1016/j.jhep.2022.03.033

FDA. (2017, 05/02/2017). *EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR The 23andMe Personal Genome Service (PGS) Genetic Health Risk Test for Hereditary Thrombophilia, Alpha-1 Antitrypsin Deficiency, Alzheimer's Disease, Parkinson's Disease, Gaucher Disease Type 1, Factor XI Deficiency, Celiac Disease, G6PD Deficiency, Hereditary Hemochromatosis and Early-Onset Primary Dystonia DECISION SUMMARY* Food and Drug Administration. Retrieved 09/17/2018 from https://www.accessdata.fda.gov/cdrh_docs/reviews/den160026.pdf

Feder, J. N., Gnirke, A., Thomas, W., Tsuchihashi, Z., Ruddy, D. A., Basava, A., Dormishian, F., Domingo, R., Jr., Ellis, M. C., Fullan, A., Hinton, L. M., Jones, N. L., Kimmel, B. E., Kronmal, G. S., Lauer, P., Lee, V. K., Loeb, D. B., Mapa, F. A., McClelland, E., . . . Wolff, R. K. (1996). A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet*, *13*(4), 399-408. https://doi.org/10.1038/ng0896-399

Fitzsimons, E. J., Cullis, J. O., Thomas, D. W., Tsochatzis, E., & Griffiths, W. J. H. (2018). Diagnosis and therapy of genetic haemochromatosis (review and 2017 update). *Br J Haematol*, *181*(3), 293-303. https://doi.org/10.1111/bjh.15164

Fleming, R. E., & Ponka, P. (2012). Iron overload in human disease. *N Engl J Med*, *366*(4), 348-359. https://doi.org/10.1056/NEJMra1004967

Fonseca, P. F., Cancado, R. D., Uellendahl Lopes, M. M., Correia, E., Lescano, M. A., & Santos, P. C. (2016). HAMP Gene Mutation Associated with Juvenile Hemochromatosis in Brazilian Patients. *Acta Haematol*, *135*(4), 228-231. https://doi.org/10.1159/000444119

Invitae. (2024). *Invitae Hereditary Hemochromatosis Panel*. https://www.invitae.com/en/physician/tests/05201/

Joly, P., Vignaud, H., Di Martino, J., Ruiz, M., Garin, R., Restier, L., Belmalih, A., Marchal, C., Cullin, C., Arveiler, B., Fergelot, P., Gitler, A. D., Lachaux, A., Couthouis, J., & Bouchecareilh, M. (2017). ERAD defects and the HFE-H63D variant are associated with increased risk of liver damages in Alpha 1-Antitrypsin Deficiency. *PLoS One*, *12*(6), e0179369. https://doi.org/10.1371/journal.pone.0179369

Kowdley, K. V., Brown, K. E., Ahn, J., & Sundaram, V. (2019). ACG Clinical Guideline: Hereditary Hemochromatosis. *Am J Gastroenterol*, *114*(8), 1202-1218. https://doi.org/10.14309/ajg.0000000000000315

Kwo, P. Y., Cohen, S. M., & Lim, J. K. (2017). ACG Clinical Guideline: Evaluation of Abnormal Liver Chemistries. *Am J Gastroenterol*, *112*(1), 18-35. https://doi.org/10.1038/ajg.2016.517

LabCorp. (2024). *Hereditary Hemochromatosis, DNA Analysis*. https://www.labcorp.com/test-menu/27711/hereditary-hemochromatosis-dna-analysis#

Lanktree, M. B., Sadikovic, B., Waye, J. S., Levstik, A., Lanktree, B. B., Yudin, J., Crowther, M. A., Pare, G., & Adams, P. C. (2017). Clinical evaluation of a hemochromatosis next-generation sequencing gene panel. *Eur J Haematol*, *98*(3), 228-234. https://doi.org/10.1111/ejh.12820

Lanzara, C., Roetto, A., Daraio, F., Rivard, S., Ficarella, R., Simard, H., Cox, T. M., Cazzola, M., Piperno, A., Gimenez-Roqueplo, A. P., Grammatico, P., Volinia, S., Gasparini, P., & Camaschella, C. (2004). Spectrum of hemojuvelin gene mutations in 1q-linked juvenile hemochromatosis. *Blood*, *103*(11), 4317-4321. https://doi.org/10.1182/blood-2004-01-0192

Lescano, M. A., Tavares, L. C., & Santos, P. (2017). Juvenile hemochromatosis: HAMP mutation and severe iron overload treated with phlebotomies and deferasirox. *World J Clin Cases*, *5*(10), 381-383. https://doi.org/10.12998/wjcc.v5.i10.381

Lim, D. R., Vidyasankar, G., Phua, C., & Borgaonkar, M. (2020). Clinical Penetrance of Hereditary Hemochromatosis-Related End-Organ Damage of C282Y Homozygosity, A Newfoundland Experience. *Clin Transl Gastroenterol*, *11*(11), e00258. https://doi.org/10.14309/ctg.0000000000000258

Lv, Y. F., Chang, X., Hua, R. X., Yan, G. N., Meng, G., Liao, X. Y., Zhang, X., & Guo, Q. N. (2016). The risk of new-onset cancer associated with HFE C282Y and H63D mutations: evidence from 87,028 participants. *J Cell Mol Med*, *20*(7), 1219-1233. https://doi.org/10.1111/jcmm.12764

Milman, N. T., Schioedt, F. V., Junker, A. E., & Magnussen, K. (2019). Diagnosis and Treatment of Genetic HFE-Hemochromatosis: The Danish Aspect. *Gastroenterology Res*, *12*(5), 221-232. https://doi.org/10.14740/gr1206

Moreno-Carralero, M. I., Munoz-Munoz, J. A., Cuadrado-Grande, N., Lopez-Rodriguez, R., Jose Hernandez-Alfaro, M., del-Castillo-Rueda, A., Enriquez-de-Salamanca, R., Mendez, M., & Moran-Jimenez, M. J. (2014). A novel mutation in the SLC40A1 gene associated with reduced iron export in vitro. *Am J Hematol*, *89*(7), 689-694. https://doi.org/10.1002/ajh.23714

Mura, C., Raguenes, O., & Ferec, C. (1999). HFE mutations analysis in 711 hemochromatosis probands: evidence for S65C implication in mild form of hemochromatosis. *Blood*, *93*(8), 2502-2505.

Oppl, B., Husar-Memmer, E., Pfefferkorn, S., Blank, M., Zenz, P., Gollob, E., Wurnig, C., Engel, A., Stadlmayr, A., Uyanik, G., Brozek, W., Klaushofer, K., Zwerina, J., & Datz, C. (2018). HFE hemochromatosis screening in patients with severe hip osteoarthritis: A

prospective cross-sectional study. *PLoS One*, *13*(11), e0207415. https://doi.org/10.1371/journal.pone.0207415

Palomaki, G. E., Haddow, J. E., Bradley, L. A., Richards, C. S., Stenzel, T. T., & Grody, W. W. (2003). Estimated analytic validity of HFE C282Y mutation testing in population screening: the potential value of confirmatory testing. *Genet Med*, *5*(6), 440-443. https://doi.org/10.1097/01.gim.0000096500.66084.85

Pietrangelo, A. (2004). Non-HFE hemochromatosis. *Hepatology*, *39*(1), 21-29. https://doi.org/10.1002/hep.20007

Pietrangelo, A. (2015). Genetics, Genetic Testing, and Management of Hemochromatosis: 15 Years Since Hepcidin. *Gastroenterology*, *149*(5), 1240-1251.e1244. https://doi.org/10.1053/j.gastro.2015.06.045

Piperno, A., Pelucchi, S., & Mariani, R. (2020). Inherited iron overload disorders. *Transl Gastroenterol Hepatol*, *5*, 25. https://doi.org/10.21037/tgh.2019.11.15

Politou, M., Kalotychou, V., Pissia, M., Rombos, Y., Sakellaropoulos, N., & Papanikolaou, G. (2004). The impact of the mutations of the HFE gene and of the SLC11A3 gene on iron overload in Greek thalassemia intermedia and beta(s)/beta(thal) anemia patients. *Haematologica*, *89*(4), 490-492. https://www.ncbi.nlm.nih.gov/pubmed/15075083

Porto, G., Brissot, P., Swinkels, D. W., Zoller, H., Kamarainen, O., Patton, S., Alonso, I., Morris, M., & Keeney, S. (2016). EMQN best practice guidelines for the molecular genetic diagnosis of hereditary hemochromatosis (HH). *Eur J Hum Genet*, *24*(4), 479-495. https://doi.org/10.1038/ejhg.2015.128

Press, R. D., Eickelberg, G., McDonald, T. J., Halley, J., Long, T., Tafe, L. J., & Weck, K. E. (2016). Highly accurate molecular genetic testing for HFE hereditary hemochromatosis: results from 10 years of blinded proficiency surveys by the College of American Pathologists. *Genet Med*, *18*(12), 1206-1213. https://doi.org/10.1038/gim.2016.34

Rabideau, M. M., White, H. J., Anderson, M., & Deucher, A. (2014). Clinical Testing of Five Hereditary Hemochromatosis-Related Genes: Preliminary Evidence for the Benefit of Next Generation Sequencing. *Blood*, *124*(21), 1355-1355. https://doi.org/10.1182/blood.V124.21.1355.1355

Radford-Smith, D. E., Powell, E. E., & Powell, L. W. (2018). Haemochromatosis: a clinical update for the practising physician. *Internal Medicine Journal $V 48*(5), 509-516.

Rombout-Sestrienkova, E., Winkens, B., Essers, B. A., Nieman, F. H., Noord, P. A., Janssen, M. C., van Deursen, C. T., Boonen, A., Reuser-Kaasenbrood, E. P., Heeremans, J., van Kraaij, M., Masclee, A., & Koek, G. H. (2016). Erythrocytapheresis versus phlebotomy in the maintenance treatment of HFE hemochromatosis patients: results from a randomized crossover trial. *Transfusion*, *56*(1), 261-270. https://doi.org/10.1111/trf.13328

Sandhu, K., Flintoff, K., Chatfield, M. D., Dixon, J. L., Ramm, L. E., Ramm, G. A., Powell, L. W., Subramaniam, V. N., & Wallace, D. F. (2018). Phenotypic analysis of hemochromatosis subtypes reveals variations in severity of iron overload and clinical disease. *Blood*, *132*(1), 101-110. https://doi.org/10.1182/blood-2018-02-830562

Sangiuolo, F., Puxeddu, E., Pezzuto, G., Cavalli, F., Longo, G., Comandini, A., Di Pierro, D., Pallante, M., Sergiacomi, G., Simonetti, G., Zompatori, M., Orlandi, A., Magrini, A., Amicosante, M., Mariani, F., Losi, M., Fraboni, D., Bisetti, A., & Saltini, C. (2015). HFE gene variants and iron-induced oxygen radical generation in idiopathic pulmonary fibrosis. *Eur Respir J*, *45*(2), 483-490. https://doi.org/10.1183/09031936.00104814

Santos, P. C., Dinardo, C. L., Cançado, R. D., Schettert, I. T., Krieger, J. E., & Pereira, A. C. (2012). Non-HFE hemochromatosis. *Rev Bras Hematol Hemoter*, *34*(4), 311-316. https://doi.org/10.5581/1516-8484.20120079

Stuhrmann, M., Strassburg, C., & Schmidtke, J. (2005). Genotype-based screening for hereditary haemochromatosis. I: Technical performance, costs and clinical relevance of a German pilot study. *Eur J Hum Genet*, *13*(1), 69-78. https://doi.org/10.1038/sj.ejhg.5201287

Takami, A., Tatsumi, Y., Sakai, K., Toki, Y., Ikuta, K., Oohigashi, Y., Takagi, J., Kato, K., & Takami, K. (2020). Juvenile Hemochromatosis: A Case Report and Review of the Literature. *Pharmaceuticals (Basel)*, *13*(8). https://doi.org/10.3390/ph13080195

Tamosauskaite, J., Atkins, J. L., Pilling, L. C., Kuo, C. L., Kuchel, G. A., Ferrucci, L., & Melzer, D. (2019). Hereditary Hemochromatosis Associations with Frailty, Sarcopenia and Chronic Pain: Evidence from 200,975 Older UK Biobank Participants. *J Gerontol A Biol Sci Med Sci*, *74*(3), 337-342. https://doi.org/10.1093/gerona/gly270

USPSTF. (2006, 08/15/2006). *Hemochromatosis: Screening*. U. S. Preventive Services Task Force. Retrieved 09/17/2018 from https://www.uspreventiveservicestaskforce.org/BrowseRec/InactiveTopic/219

USPSTF. (2014, 06/2014). *Guide to Clinical Preventive Services*. Agency for Healthcare Research and Quality (AHRQ). Retrieved 09/17/2018 from https://www.ahrq.gov/professionals/clinicians-providers/guidelines-recommendations/guide/section2b.html#hemo

Vujić, M. (2014). Molecular basis of HFE-hemochromatosis. *Front Pharmacol*, *5*. https://doi.org/10.3389/fphar.2014.00042

Whitlock, E. P., Garlitz, B. A., Harris, E. L., Beil, T. L., & Smith, P. R. (2006). Screening for hereditary hemochromatosis: a systematic review for the U.S. Preventive Services Task Force. *Ann Intern Med*, *145*(3), 209-223.

Zhang, W., Lv, T., Huang, J., & Ou, X. (2017). Type 4B hereditary hemochromatosis associated with a novel mutation in the SLC40A1 gene: A case report and a review of the literature. *Medicine (Baltimore)*, *96*(38), e8064. https://doi.org/10.1097/md.0000000000008064

Genetic Testing for Hereditary Pancreatitis

Policy Number: AHS – M2079 – Genetic Testing for Hereditary Pancreatitis	Prior Policy Name and Number, as applicable:
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Pancreatitis is defined as inflammation of the pancreas that progresses from acute (AP) (sudden onset; duration <6 months) to recurrent acute (RAP) (>1 episode of acute pancreatitis) to chronic (CP) (duration >6 months) (LaRusch et al., 2014). This recurrent inflammation can lead to total destruction of the pancreas with subsequent pancreatic insufficiency, secondary diabetes, increased risk for pancreatic cancer, and severe unrelenting pain (Ravi Kanth & Nageshwar Reddy, 2014). Hereditary pancreatitis is the early onset form of chronic pancreatitis that is carried in an autosomal dominant pattern with variable penetrance (LaRusch et al., 2012).

II. Related Policies

Policy Number	Policy Title
AHS-G2153	Pancreatic Enzyme Testing for Acute Pancreatitis
AHS-M2114	Pancreatic Cancer Risk Testing Using Pancreatic Cyst Fluid

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals under 20 years of age, genetic testing for hereditary pancreatitis (see Note 1) **MEETS COVERAGE CRITERIA** when at least **one** of the following conditions is met:

a) For individuals with recurrent (two separate, documented episodes with hyperlipasemia) attacks of acute pancreatitis for which there is no identifiable cause.

b) For individuals with unexplained chronic pancreatitis.

c) For individuals with a first- or second-degree relative (see Note 2) with a history of recurrent acute pancreatitis, idiopathic chronic pancreatitis, **or** childhood pancreatitis without a known cause.

d) For individuals with an unexplained episode of pancreatitis that required hospitalization.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

2) For all other situations not described above, genetic testing for hereditary pancreatitis **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162-Reimbursement Policy.

Note 2: First-degree relatives include parents, full siblings, and children of the individual. Second-degree relatives include grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings of the individual.

IV. Table of Terminology

Term	Definition
ACG	American College of Gastroenterology
AP	Acute pancreatitis
ARP	Acute recurrent pancreatitis
ARUP	Associated Regional and University Pathologists, Inc.
ASCO	American Society of Clinical Oncology
CASR	*Calcium sensing receptor*
CEL	Carboxyl ester lipase
CF	Cystic fibrosis
CFTR	*Cystic fibrosis transmembrane conductance regulator*
CLDN2	*Claudin-2*

CP	Chronic pancreatitis
CPA1	Carboxypeptidase A1
CTRC	*Chymotrypsin C*
DNA	Deoxyribonucleic acid
DNA2	*Deoxyribonucleic acid replication helicase/nuclease 2*
DNAJC21	*Deoxyribonucleic acid J heat shock protein family (Hsp40) member C21*
EFL1	*Elongation factor like-1*
EPC	European Pancreatic Club
HP	Hereditary pancreatitis
HPSG	Hungarian Pancreatic Study Group
IAP	Idiopathic acute pancreatitis
INSPPIRE	International Study Group of Pediatric Pancreatitis
KSS	Kearns-Sayre syndrome
MAGI2	*Membrane-associated Guanylate Kinase Inverted-2*
MODY	Maturity-onset diabetes of the young
MT	Mitochondrial
MYO9B	Myosin IXB
NAPS2	North American Pancreatitis Study 2
NCCN	National Comprehensive Cancer Network
NGS	Next generation sequencing
OPA1	*Mitochondrial dynamin*
OR	Odds ratio
PARD3	Partitioning defective 3
PEO	Progressive external ophthalmoplegia
POLG	*DNA polymerase subunit gamma*
PRSS1	*Cationic* trypsinogen
RAP	Recurrent acute pancreatitis
RRM2B	*Ribonucleotide-diphosphate reductase subunit M2*
SBDS	Shwachman-Bodian-Diamond syndrome
SCP	Smoking-associated chronic pancreatitis
SLC25A4	*Solute carrier family 25 member 4*
SPINK1	Serine protease inhibitor
SRP54	*Pancreatic secretory trypsin inhibitor Kazal type 1*
TIGAR-O	Toxic-Metabolic; Idiopathic; Genetic; Autoimmune; Recurrent and Severe Acute Pancreatitis; Obstructive
TWNK	Twinkle mitochondrial deoxyribonucleic acid helicase
TYMP	Thymidine phosphorylase

UEG	United European Gastroenterology
WES	Whole exome sequencing

V. Scientific Background

Pancreatitis is caused by unregulated trypsin activity within the pancreatic acinar cell or pancreatic duct that leads to pancreatic autodigestion and pancreatic inflammation (Lerch & Gorelick, 2000; Whitcomb, 1999). Under acinar cell stress (e.g., hyperstimulation, intracellular hypercalcemia), intracellular trypsinogen is likely converted to trypsin, which activates other digestive enzymes causing injury. Injury releases immune system-activating molecules that cause an initial acute inflammatory response, followed by recruitment of tissue macrophages and activated pancreatic stellate cells. Recurrent injury leads to chronic pancreatitis and fibrosis, mediated by pancreatic stellate cells (Werlin et al., 2015).

Chronic pancreatitis (CP) is a progressive inflammatory disease in which the pancreatic tissue is destroyed over time and replaced by fibrous tissue. The process of fibrosis usually leads to progressive worsening in the structural integrity of the pancreas, changes in arrangement, and composition of the islets, and deformation of the large ducts, eventually resulting in the impairment of both exocrine and endocrine functions (Brock et al., 2013). The annual incidence of the disease has been estimated to be 5-10 per 100,000 persons (Molven et al., 2015). The main symptom of CP is pain; however, it is highly variable in character, frequency, and severity (Mullady et al., 2011; Whitcomb et al., 2008). Therapeutic efforts are mostly aimed at extracting stones and decompressing pancreatic ducts to achieve ideal drainage of the pancreatic duct (Li et al., 2010; Tandan & Nageshwar Reddy, 2013). Genetic risk factors play a larger role in early-onset CP as opposed to late-onset CP. Older adults have a host of environmental and genetic factors that contribute to CP development while hereditary pancreatitis is postulated to make up less than 4% of CP in late-onset groups (Wertheim-Tysarowska et al., 2021).

The etiologies of chronic pancreatitis are classified by the TIGAR-O system into alcoholism, hyperlipidemia, obstructive damage caused by trauma or congenital anomalies, hereditary pancreatitis, autoimmune pancreatitis, and idiopathic (Etemad & Whitcomb, 2001; Sun et al., 2015). The genetic factors listed in TIGAR-O are *PRSS1* (listed as "cationic trypsinogen"), *CFTR*, *SPINK1*, and alpha-1-antitrypsin (listed as "possible") (Etemad & Whitcomb, 2001). TIGAR-O Version 2 was published in 2019, and lists *PRSS1, CFTR, SPINK1, CTRC, CASR*, and *CEL* as genetic factors, as well as some modifier genes such as *CLDN2* (Whitcomb, 2019).

Hereditary pancreatitis (HP) presents as an autosomal dominant chronic pancreatitis with variable penetrance. It mainly develops in childhood (Wertheim-Tysarowska et al., 2021). This variability has been attributed to a genetic predisposition to chronic pancreatitis with the additive effects of environmental and inherited factors. Most genes associated with HP either directly encode components of the trypsin system of the exocrine pancreas or are likely to perturb this system indirectly. HP is recognized when pathogenic gene variants of the *PRSS1* gene are found or when acute or chronic pancreatitis develops with a distinct family history (Wertheim-Tysarowska et al., 2021). The phenotype of HP is increased susceptibility to acute pancreatitis, resulting in chronic pancreatitis (including pancreatic fibrosis, chronic pain, maldigestion, and diabetes mellitus) occurring in at least 50%. The risk of pancreatic cancer is also increased (Schwarzenberg, 2023).

Genes Linked to Hereditary Pancreatitis

PRSS1 encodes trypsin-1 (cationic trypsinogen), a major pancreatic digestive enzyme. Mutations in *PRSS1* typically result in a trypsin protein that is either prematurely activated or resistant to degradation (LaRusch et al., 2012; Masson et al., 2008), causing autosomal dominant pancreatitis in 60%-100% of families with hereditary pancreatitis (LaRusch & Whitcomb, 2011). "The age of onset for *PRSS-1* related HP ranges from 10 to 12 years" (Hasan et al., 2018).

SPINK1 encodes serine protease inhibitor, Kazel-type 1, a trypsin inhibitor that is upregulated by inflammation (Grendell, 2003). It is not a typical susceptibility gene for acute pancreatitis, but rather a susceptibility gene for the chronic pancreatitis that follows acute pancreatitis.

CTRC encodes chymotrypsin C. Prematurely activated trypsin is destroyed by *CTRC* by acting on the molecule within the calcium-binding loop in the absence of calcium and, therefore, is a crucial candidate gene in the pathogenesis of pancreatitis (Szmola & Sahin-Toth, 2007).

CASR encodes calcium sensing receptor, mutations of which can cause increased calcium ion levels increasing trypsin activation and failed trypsin degradation (Whitcomb, 2004).

CFTR encodes the cystic fibrosis transmembrane conductance protein. Mutations are associated with recurrent acute and chronic pancreatitis since dysfunctional *CFTR* can result in retention of zymogens that can become active and result in pancreatitis (LaRusch & Whitcomb, 2011).

CLDN2 encodes claudin-2, a tight-junction protein that seals the space between epithelial cells. Normally expressed in the proximal pancreatic duct, CLDN2 is thought to facilitate the transport of water and sodium into the duct to match the chloride and bicarbonate that are actively secreted by pancreatic duct cells through *CFTR*. It is strongly associated with alcohol-related chronic pancreatitis rather than recurrent acute pancreatitis (Ravi Kanth & Nageshwar Reddy, 2014).

CPA1 encodes carboxypeptidase A1; mutated CPA1 is associated with nonalcoholic chronic pancreatitis, especially with an early age of onset (Witt et al., 2013). Risk for chronic pancreatitis unrelated to trypsin activation appears to be related to endoplasmic reticulum stress from pathogenic CPA1 variants that alter protein folding, triggering the unfolded protein response.

MYO9B gene and the two tight-junction adaptor genes, **PARD3** and **MAGI2**, have been linked to gastrointestinal permeability. Impairment of the mucosal barrier plays an important role in the pathophysiology of acute pancreatitis (Nijmeijer et al., 2013).

CEL encodes carboxyl-ester lipase, and CEL mutations can cause an autosomal dominant syndrome of maturity-onset diabetes of the young (MODY) and exocrine pancreatic dysfunction (Molven et al., 2015).

TRPV6 is a potential novel susceptibility gene for CP that plays a role in epithelial calcium absorption and reabsorption. Variants of this gene also co-occur with pathogenic variants of genes such as *SPINK1* and *CFTR* (Wertheim-Tysarowska et al., 2021).

Disorder(s)	Genetic Cause(s)	Consequence(s)	Source Citation
Shwachman-Diamond syndrome	*SBDS, DNAJC21, EFL1,* and *SRP54*	affect RNA function	(Nelson & Myers, 2008)
Mitochondrial (mt)DNA deletion syndromes, including Kearns-Sayre syndrome (KSS), Pearson syndrome, and progressive external	Multiple possible mitochondrial genetic etiologies, including *SLC25A4, TWNK, POLG, TYMP, OPA1, RRM2B,*	defective oxidative phosphorylation	(Goldstein & Falk, 2003)

ophthalmoplegia (PEO)	*DNA2,* and *MT-TL1,*		
Carboxyl ester lipase (CEL-MODY)	*CEL*	pancreatic exocrine, endocrine dysfunction, and chronic pancreatitis	(O'Neill et al., 2013)
Johanson-Blizzard syndrome	*UBD1*	protein synthesis	(Kniffin & McKusick, 2012)

Syndromes that Include Pancreatitis or Pancreatic Insufficiency

Several genes are associated with rare disorders in which pancreatitis or pancreatic insufficiency is part of their phenotype (Durie, 1996; Lerch et al., 2006).

As the number of genes and mutations involved in the onset and progression of pancreatitis becomes higher (Ooi et al., 2010; Walker et al., 2013), the time and cost of screening and sequencing specific genes continues to increase. However, massive parallel sequencing or next generation sequencing (NGS) is becoming standardized (Ballard et al., 2015), and the cost per patient is rapidly dropping (Palermo et al., 2016). NGS includes whole genome sequencing, whole exome sequencing (WES) and other methods. Because the cost of WES is now less than the cost of sequencing *CFTR*, use of this technology is becoming an attractive alternative to classic targeted gene sequencing or mutation specific genotyping for a genetic counseling workup (LaRusch et al., 2012). In response to this accelerating development of sequencing techniques, several firms have created genetic panels focusing on hereditary pancreatitis. For example, Invitae offers a six-gene panel (*CASR, CFTR, CPA1, CTRC, PRSS1, SPINK1*) for chronic pancreatitis (Invitae, 2023). Other firms offering proprietary panels include ARUP Laboratories (4 genes), LabCorp (3 genes), and Ambry (6 genes) (Ambry, 2023; ARUP, 2023; LabCorp, 2023). Still other firms evaluate as many as 12 genes and more (Schwarzenberg, 2023).

Clinical Utility and Validity

Testing for mutations in the *PRSS1*, *SPINK*, and *CFTR* genes is usually done by either direct sequence analysis or next generation sequencing, both of which have high analytic validity. Several studies have evaluated the clinical validity of genetic testing (Applebaum-Shapiro et al., 2001; Ceppa et al., 2013; Poddar et al., 2015; Sultan et al., 2012). One limitation with some studies was lack of inclusion of patients with clinically defined hereditary pancreatitis. Hence, the true clinical sensitivity and specificity of genetic testing in hereditary pancreatitis cannot be accurately determined and needs to be further researched. Similarly, there is a lack of published literature on the clinical utility of testing. Further research is required to evaluate how genetic testing will impact patient management decision and clinical outcomes.

Kumar et al. (2016) sought to characterize and identify risk factors associated with acute recurrent pancreatitis (ARP) and CP in childhood in a multinational cross-sectional study (INSPPIRE). The authors analyzed 301 children with ARP or CP. They found that "At least 1 gene mutation in pancreatitis-related genes was found in 48% of patients with ARP vs 73% of patients with CP. Children with *PRSS1* or *SPINK1* mutations were more likely to present with CP compared with ARP (*PRSS1*: OR = 4.20 and *SPINK1*: OR = 2.30). Obstructive risk factors presented in 33% in both groups, but toxic/metabolic risk factors were more common in children with ARP (21% overall; 26% ARP, 15% CP). They concluded that "The high disease burden in pediatric CP underscores the importance of identifying predisposing factors for progression of ARP to CP in children" (Kumar et al., 2016).

Grabarczyk et al. (2017) also found that *CTRC* variants are strong CP risk factors in pediatric patients. The authors investigated 136 pediatric patients with CP and compared them to 401 controls. They showed that p.Arg254Trp (4.6%) and p.Lys247_Arg254del (5.3%) heterozygous mutations are frequent and significantly associated with CP risk in pediatric patients (odds ratio [OR] = 19.1; 95% CI 2.8-160; P = 0.001 and OR = 5.5; 95% CI 1.6-19.4; P = 0.001, respectively). The c.180TT genotype of common p.Gly60Gly variant was found to be a strong and independent CP risk factor (OR = 23; 95% CI 7.7-70; P < 0.001) with effect size comparable to p.Arg254Trp mutation (Grabarczyk et al., 2017).

Schwarzenberg et al. (2015) evaluated the genetic spectrum of CP. 76 CP patients were examined, and 51 were found to have a genetic risk factor for CP. Of these 51 mutations, 33 were a *PRSS1* mutation, 14 were a *SPINK1* mutation, 11 were a *CFTR* mutation, and 2 were a *CTRC* mutation. The final 25 patients were found to have an obstructive risk factor (Schwarzenberg et al., 2015).

Zou et al. (2018) evaluated the prevalence of four CP-related genes (*SPINK1, PRSS1, CTRC, CFTR*) in Han Chinese patients. The authors performed next-generation sequencing on 1061 patients and 1196 controls. The 1061 patients were further divided into three categories, idiopathic CP (ICP, 715 patients), alcoholic CP (ACP, 206), and smoking-associated CP (SCP, 140). The impact of rare pathogenic variants on age of onset and clinical outcomes was evaluated. Rare pathogenic variants were found in 535 CP patients compared to 71 controls. Mutation positive patients were found to have earlier age of onset as well additional clinical features such as pancreatic stones and diabetes mellitus compared to mutation negative ICP patients. Overall, pathogenic variants were found in 57.1% of ICP patients, compared to 39.8% of ACP patients and 32.1% of SCP patients. The authors concluded that rare pathogenic variants "significantly" influenced age of onset and clinical outcomes of CP (Zou et al., 2018).

Nabi et al. (2020) evaluated 239 children in a prospective study from January 2015 to May 2018 to examine genetic risk factors in children with idiopathic acute recurrent pancreatitis (IARP). Among the enrollees, 85.35% children had IARP, and found that family history of pancreatitis was found among 4.6% of participants. For specific genes, "mutations/polymorphisms in at least 1 gene were identified in 89.5% (129/144) children including *SPINK1* in 41.9%, *PRSS1* (rs10273639) in 58.2%, *CTRC* in 25.6%, *CTSB* in 54.9%, *CLDN2* in 72.9%, and *CFTR* in 2.3%." This conveys the overlapping genetic nature of IARP with related genes in HP, making genetic testing important for managing potential disease progression.

Suzuki et al. (2020) investigated the currently understood genetic abnormalities in pancreatitis, and found that "patients with these genetic predispositions [*PRSS1* and *SPINK1* genes], both children and adults, have often been initially diagnosed with idiopathic acute pancreatitis, in approximately 20-50% pediatric cases and 28-0% of adult cases… Patients with chronic pancreatitis (CP) due to *SPINK1* gene mutation and HP patients have a potentially high risk of pancreatic exocrine insufficiency, diabetes mellitus, and of particular importance, pancreatic cancer." This conveys the continuously emphasized clinical utility of genetic testing to pursue opportunities for counselling and symptom management with disease progression, despite not having gene therapy options for directly targeting HP causing and associated genes (Suzuki et al., 2020).

Weiss et al. (2020) discussed the potential pitfalls from using next generation sequencing (NGS) to diagnose *PRSS1* mutations in chronic pancreatitis. Due to the "high degree of DNA sequence homology (>91%) between *PRSS1* and other members of the trypsinogen multigene family," there may be erroneous diagnoses of pathologic chronic pancreatitis among patients with benign variants of other *PRSS1*- related genes, like *PRSS2* or *PRSS3P2*. The researchers concluded that sequence homology "can confound the mapping of short NGS reads to a reference genome and lead to technical artefacts." They recommend "careful clinical evaluation, pretest and post-test genetic counselling and confirmation of NGS test results by Sanger sequencing" to confirm a diagnosis of genetically mutated chronic pancreatitis. This presented the precautions that must be accounted for when utilizing genetic testing for hereditary pancreatitis (Weiss et al., 2020).

Zou et al. (2020) performed whole genome sequencing on a population of 464 Chinese CP patients and on a group of 504 control participants. The *Transient receptor potential cation channel, Subfamily V, Member 6 (TRPV6)* gene was identified as a gene significantly associated with chronic pancreatitis through a "burden test of aggregated rare nonsynonymous variants with a combined annotation dependent depletion score > 20 ($p = .020$)." In another phase of the study, Sanger sequencing was used to analyze

the entire coding sequence and exon/intron boundaries of the *TPRV6* gene. Combining the two phases of the study, the authors identified 25 distinct variants of *TPRV6* and noted that loss-of-function variants were over-represented in the chronic pancreatitis group. The authors concluded that *TPRV6* is likely a novel susceptibility gene for chronic pancreatitis.

VI. Guidelines and Recommendations

Consensus Committees of the European Registry of Hereditary Pancreatic Diseases, the Midwest Multi-Center Pancreatic Study Group and the International Association of Pancreatology

A Consensus Committees of the European Registry of Hereditary Pancreatic Diseases, the Midwest Multi-Center Pancreatic Study Group and the International Association of Pancreatology developed guidelines for genetic testing of the *PRSS1* gene and genetic counseling for HP (Ellis et al., 2001). The recommended indications for symptomatic patients included:

- Recurrent (two separate, documented episodes with hyperlipasemia) attacks of acute pancreatitis for which there is no explanation (anatomical anomalies, ampullary or main pancreatic strictures, trauma, viral infection, gallstones, alcohol, drugs, hyperlipidaemia, etc.)
- Unexplained chronic pancreatitis
- A family history of pancreatitis in a first- or second-degree relative
- Unexplained episode of pancreatitis in a child that required hospitalization

Predictive (presymptomatic) genetic testing of unaffected relatives is considered more complex. Predictive testing is recommended only for individuals with a first-degree relative with a defined HP gene mutation, and who are over 16 years of age and capable of making an independent and fully informed decision (Ellis et al., 2001).

American Society of Clinical Oncology (ASCO)

The ASCO states that "genetic testing is sometimes considered for patients who develop recurrent pancreatitis at young ages. Genetic testing is available for mutations in the *PRSS1, SPINK1*, and *CFTR* genes." However, the authors note that "it is unknown if mutations in these genes cause an increased risk of pancreatic cancer. The authors believe that other genes may be associated with HP, and studies are ongoing to learn more about this condition" (ASCO, 2021). It is also noted that "Screening for pancreatic cancer is suggested for people known to have HP beginning at age 40, 20 years after the onset of pancreatitis, or 10 years before the youngest pancreatic cancer diagnosis in the family, whichever is earliest. However, the effectiveness of current screening

techniques for the early diagnosis of pancreatic cancer is not proven." Nevertheless, the most commonly used screening tests include magnetic resonance imaging (MRI) and endoscopic ultrasound (EUS) (ASCO, 2021).

American College of Gastroenterology (ACG)

In 2013, the ACG issued guidelines for the management of acute pancreatitis. They include the following recommendation: "genetic testing may be considered in young patients (< 30 years old) if no cause is evident and a family history of pancreatic disease is present (conditional recommendation, low quality of evidence)." ACG also states that "the role of genetic testing in AP has yet to be determined, but may be useful in patients with more than one family member with pancreatic disease. Individuals with IAP and a family history of pancreatic diseases should be referred for formal genetic counseling" (Tenner et al., 2013).

In 2020, the ACG published clinical guidelines on chronic pancreatitis. There, they state that "In patients with clinical features of CP, a comprehensive review of all risk factors should be performed. This provides information on the underlying mechanisms, identifies both fixed and modifiable risk factors, identifies potential targets for therapies, and provides clinically relevant prognostic information." As part of that initial approach, they recommend genetic testing in patients "with clinical evidence of a pancreatitis-associated disorder or possible CP [chronic pancreatitis] in which the etiology is unclear, especially in younger patients (strong recommendation, low quality of evidence)." The guideline goes on to state that "at minimum, patients with idiopathic CP should be evaluated for *PRSS1*, *SPINK1*, *CFTR*, and *CTRC* gene mutation analysis…" The guideline mentions that assessment of germline mutations is primarily for prognostic and therapeutic purposes, rather than diagnostic (Gardner et al., 2020).

United European Gastroenterology (UEG)

The United European Gastroenterology published evidence-based guidelines for the diagnosis and therapy of chronic pancreatitis which recommend (Lohr et al., 2017):

"All patients with a family history or early onset disease (<20 years) should be offered genetic testing for associated variants."

"Genetic screening for every CP patient cannot be recommended since alcohol abuse is the predominant cause of the disease in up to 60% of adult cases."

"In patients with early onset CP, genetic screening can be offered after informed consent."

"In patients with alcoholic CP, routine genetic testing cannot be recommended."

The working group also noted that "variants in *SPINK1* and *CTRC*, and to a lesser extent, common single-nucleotide polymorphisms (SNPs) in the *PRSS1* and CLDN2-MORC4 loci, are associated with alcoholic CP" (Lohr et al., 2017).

European Pancreatic Club (EPC) and Hungarian Pancreatic Study Group (HPSG)

The European Pancreatic Club, in collaboration with the Hungarian Pancreatic Study Group, organized a consensus guideline meeting on the diagnosis and management of pancreatitis in the pediatric population which state the following:

"Pediatric AP and RAP often develop in the background of genetic susceptibility and genetic testing is warranted in patients with a second episode of idiopathic AP or first episode of idiopathic AP and a family history of AP or CP. Full sequence analysis of *PRSS1*, *SPINK1*, *CTRC*, *CPA1* and *CFTR* gene exons and exon-intron boundaries and testing for the pathogenic CEL hybrid allele are recommended". The authors go further to mention that "Variants in the *PRSS1* and *CPA1* genes may be associated with a family history of pancreatitis or even autosomal dominant hereditary pancreatitis. Children with a single episode of AP are at risk for developing a second episode. However, genetic testing is cumbersome and expensive. There is usually no therapeutic consequence, but it may assist in long term prognosis" (Parniczky et al., 2018).

"The presence of mutations in the above mentioned genes increases the risk of ARP and CP. Hereditary pancreatitis associated with mutations in *PRSS1*, especially p.R122H, that could considerably increase the risk of pancreatic adenocarcinoma. Knowing the genetic risk factors may not alter the therapy, but it helps to understand the disease's etiological background for the disease and may lead to future targeted investigation" (Parniczky et al., 2018).

Regarding the etiological factors in childhood onset CP, the authors assert that "Genetic variations are the most common risk factors for development of pediatric CP. *(GRADE 1/A, full agreement)* However, other risk factors such as obstruction, autoimmune and toxic and metabolic factors also need to be examined. *(GRADE 2/B, full agreement)*". Moreover, as "There is an association between CP and cystic fibrosis (CF), therefore a sweat test should be performed to screen for CF as a possible etiological factor in children. *(GRADE 1/A, strong agreement)*" (Parniczky et al., 2018).

International Study Group of Pediatric Pancreatitis: In search for a cuRE (INSPPIRE) Consortium

This group was formed "to collect detailed information on a cohort of children with ARP and CP with the aim to fill gaps in knowledge and improve clinical care." Their genetic testing-related guidelines are listed below:

- "The search for a genetic cause of ARP or CP should include a sweat chloride test (even if newborn screening for cystic fibrosis (CF) is negative) and *PRSS1* gene mutation testing. Genetic testing for CF should be considered if a sweat test is unable to be performed."
- "Mutation analysis of the genes *SPINK1*, *CFTR* and *CTRC* may identify risk factors for ARP or CP."
- "Patients with ARP or CP and a sweat test <=60 mmol/L should have expanded *CFTR* mutation testing done if there is no other identified cause of their pancreatic disease (such as a *PRSS1* mutation or a clear obstructive etiology)" (Gariepy et al., 2017).

National Comprehensive Cancer Network (NCCN)

The NCCN notes familial pancreatitis and non-hereditary forms of pancreatitis are both linked with an increased risk of pancreatic cancer. Additionally, chronic pancreatitis is another risk factor for pancreatic cancer. The NCCN specifically lists *PRSS1*, *SPINK1*, and *CFTR* as contributing genes to familial pancreatitis. The approximate increase in risk of pancreatic cancer is somewhere between 26-fold and 87-fold in those with the *PRSS1*, *SPINK1*, and *CFTR* gene mutations (NCCN, 2023).

International Association of Pancreatology, American Pancreatic Association, Japan Pancreas Society, and European Pancreatic Club

In 2020, the International Association of Pancreatology, the American Pancreatic Association, the Japan Pancreas Society, and European Pancreatic Club released a set of international consensus guidelines on surveillance for pancreatic cancer in the setting of chronic pancreatitis. Though the working group did not explicitly endorse or oppose genetic testing, it was clear that due to the recommendations separated by genetic variants within chronic pancreatitis, genetic testing would become critical for surveillance. With regards to the conditions by which hereditary pancreatitis would warrant surveillance for cancer, the working group stated:

- "The risk of pancreatic cancer in affected individuals with an autosomal dominant history of hereditary pancreatitis due to inherited *PRSS1* mutations is high enough to justify surveillance. *Quality assessment: high; recommendation: strong*"
- "The risk of pancreatic cancer in affected individuals with an autosomal dominant history of hereditary pancreatitis but without *PRSS1* mutations is high enough to justify surveillance. *Quality assessment: moderate; recommendation: weak*"
- "The risk of pancreatic cancer in patients with chronic pancreatitis associated with *SPINK1* p. N34S is not high enough to justify screening or surveillance. *Quality assessment: moderate; recommendation: strong*"

- "The risk of pancreatic cancer in patients with chronic pancreatitis associated with other germline mutations including those of *CFTR, CTRC, CPA1, and CEL*, is not high enough to justify screening or surveillance. *Quality assessment: moderate; recommendation: conditional*" (Greenhalf et al., 2020).

National Institute for Health and Care Excellence (NICE)

NICE updated their guidelines on pancreatitis in December 2020. With regards to genetic testing for hereditary pancreatitis (acute) and patient information, NICE stated the following:

"Give people with pancreatitis, and their family members or carers (as appropriate), written and verbal information on the following, where relevant, as soon as possible after diagnosis:

- pancreatitis and any proposed investigations and procedures, using diagrams
- hereditary pancreatitis, and pancreatitis in children, including specific information on genetic counselling, genetic testing, risk to other family members, and advice on the impact of their pancreatitis on life insurance and travel
- the long-term effects of pancreatitis, including effects on the person's quality of life
- the harm caused to the pancreas by smoking or alcohol."

For an individual with chronic pancreatitis, NICE recognizes that the cause may not be alcohol-related, but can include "genetic factors; autoimmune disease, in particular IgG4 disease; metabolic causes; [and] structural or anatomical factors" (NICE, 2020).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory

Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81222	CFTR (cystic fibrosis transmembrane conductance regulator) (eg, cystic fibrosis) gene analysis; duplication/deletion variants
81223	CFTR (cystic fibrosis transmembrane conductance regulator) (eg, cystic fibrosis) gene analysis; full gene sequence
81224	CFTR (cystic fibrosis transmembrane conductance regulator) (eg, cystic fibrosis) gene analysis; intron 8 poly-T analysis (eg, male infertility)
81401	Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)
81404	Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81479	Unlisted molecular pathology procedure

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Ambry. (2023). Pancreatitis panel. https://www.ambrygen.com/clinician/genetic-testing/69/exome-and-general-genetics/pancreatitis-panel

Applebaum-Shapiro, S. E., Finch, R., Pfutzer, R. H., Hepp, L. A., Gates, L., Amann, S., Martin, S., Ulrich, C. D., 2nd, & Whitcomb, D. C. (2001). Hereditary pancreatitis in North America: the Pittsburgh-Midwest Multi-Center Pancreatic Study Group Study. *Pancreatology*, *1*(5), 439-443. https://doi.org/10.1159/000055844

ARUP. (2023). *Pancreatitis Panel (CFTR, CTRC, PRSS1, SPINK1), Sequencing*. https://ltd.aruplab.com/Tests/Pub/3004788

ASCO. (2021). *Hereditary Pancreatitis*. https://www.cancer.net/cancer-types/hereditary-pancreatitis

Ballard, D. D., Flueckiger, J. R., Fogel, E. L., McHenry, L., Lehman, G. A., Watkins, J. L., Sherman, S., & Cote, G. A. (2015). Evaluating Adults With Idiopathic Pancreatitis for Genetic Predisposition: Higher Prevalence of Abnormal Results With Use of Complete Gene Sequencing. *Pancreas*, *44*(1), 116-121. https://doi.org/10.1097/mpa.0000000000000225

Brock, C., Nielsen, L. M., Lelic, D., & Drewes, A. M. (2013). Pathophysiology of chronic pancreatitis. *World J Gastroenterol*, *19*(42), 7231-7240. https://doi.org/10.3748/wjg.v19.i42.7231

Ceppa, E. P., Pitt, H. A., Hunter, J. L., Leys, C. M., Zyromski, N. J., Rescorla, F. J., Sandrasegaran, K., Fogel, E. L., McHenry, L. W., Watkins, J. L., Sherman, S., & Lehman, G. A. (2013). Hereditary pancreatitis: endoscopic and surgical management. *J Gastrointest Surg*, *17*(5), 847-856; discussion 856-847. https://doi.org/10.1007/s11605-013-2167-8

Durie, P. R. (1996). Inherited and congenital disorders of the exocrine pancreas. *Gastroenterologist*, *4*(3), 169-187. https://www.ncbi.nlm.nih.gov/pubmed/8891682

Ellis, I., Lerch, M. M., & Whitcomb, D. C. (2001). Genetic testing for hereditary pancreatitis: guidelines for indications, counselling, consent and privacy issues. *Pancreatology*, *1*(5), 405-415. https://www.karger.com/Article/PDF/55840

Etemad, B., & Whitcomb, D. C. (2001). Chronic pancreatitis: diagnosis, classification, and new genetic developments. *Gastroenterology*, *120*(3), 682-707. https://doi.org/10.1053/gast.2001.22586

Gardner, T. B., Adler, D. G., Forsmark, C. E., Sauer, B. G., Taylor, J. R., & Whitcomb, D. C. (2020). ACG Clinical Guideline: Chronic Pancreatitis. *American Journal of Gastroenterology*, *115*(3). https://doi.org/10.14309/ajg.0000000000000535

Gariepy, C. E., Heyman, M. B., Lowe, M. E., Pohl, J. F., Werlin, S. L., Wilschanski, M., Barth, B., Fishman, D. S., Freedman, S. D., Giefer, M. J., Gonska, T., Himes, R., Husain, S. Z., Morinville, V. D., Ooi, C. Y., Schwarzenberg, S. J., Troendle, D. M., Yen, E., & Uc, A. (2017). Causal Evaluation of Acute Recurrent and Chronic Pancreatitis in Children: Consensus From the INSPPIRE Group. *J Pediatr Gastroenterol Nutr*, *64*(1), 95-103. https://doi.org/10.1097/mpg.0000000000001446

Goldstein, A., & Falk, M. (2003, 01/31/2019). *Mitochondrial DNA Deletion Syndromes*. University of Washington, WA. https://www.ncbi.nlm.nih.gov/books/NBK1203/

Grabarczyk, A. M., Oracz, G., Wertheim-Tysarowska, K., Kujko, A. A., Wejnarska, K., Kolodziejczyk, E., Bal, J., Koziel, D., Kowalik, A., Gluszek, S., & Rygiel, A. M. (2017). Chymotrypsinogen C Genetic Variants, Including c.180TT, Are Strongly Associated With Chronic Pancreatitis in Pediatric Patients. *J Pediatr Gastroenterol Nutr*, *65*(6), 652-657. https://doi.org/10.1097/mpg.0000000000001767

Greenhalf, W., Lévy, P., Gress, T., Rebours, V., Brand, R. E., Pandol, S., Chari, S., Jørgensen, M. T., Mayerle, J., Lerch, M. M., Hegyi, P., Kleeff, J., Castillo, C. F., Isaji, S., Shimosegawa, T., Sheel, A., Halloran, C. M., Garg, P., Takaori, K., . . . Neoptolemos, J.

(2020). International consensus guidelines on surveillance for pancreatic cancer in chronic pancreatitis. Recommendations from the working group for the international consensus guidelines for chronic pancreatitis in collaboration with the International Association of Pancreatology, the American Pancreatic Association, the Japan Pancreas Society, and European Pancreatic Club. *Pancreatology*, *20*(5), 910-918. https://doi.org/10.1016/j.pan.2020.05.011

Grendell, J. H. (2003). Genetic factors in pancreatitis. *Curr Gastroenterol Rep*, *5*(2), 105-109. https://doi.org/10.1007/s11894-003-0078-7

Hasan, A., Moscoso, D. I., & Kastrinos, F. (2018). The Role of Genetics in Pancreatitis. *Gastrointest Endosc Clin N Am*, *28*(4), 587-603. https://doi.org/10.1016/j.giec.2018.06.001

Invitae. (2023). Invitae Chronic Pancreatitis Panel. https://www.invitae.com/en/physician/tests/01745/#info-panel-disorders_tested

Kniffin, C., & McKusick, V. (2012, 09/15/2016). *JOHANSON-BLIZZARD SYNDROME; JBS*. Johns Hopkins University. https://omim.org/entry/243800

Kumar, S., Ooi, C. Y., Werlin, S., Abu-El-Haija, M., Barth, B., Bellin, M. D., Durie, P. R., Fishman, D. S., Freedman, S. D., Gariepy, C., Giefer, M. J., Gonska, T., Heyman, M. B., Himes, R., Husain, S. Z., Lin, T. K., Lowe, M. E., Morinville, V., Palermo, J. J., . . . Uc, A. (2016). Risk Factors Associated With Pediatric Acute Recurrent and Chronic Pancreatitis: Lessons From INSPPIRE. *JAMA Pediatr*, *170*(6), 562-569. https://doi.org/10.1001/jamapediatrics.2015.4955

LabCorp. (2023). Pancreatitis: Three-gene Profile (PRSS1, SPINK1, CFTR) (Full Gene Sequencing). https://www.labcorp.com/tests/252794/pancreatitis-three-gene-profile-i-prss1-spink1-cftr-i-full-gene-sequencing

LaRusch, J., Barmada, M. M., Solomon, S., & Whitcomb, D. C. (2012). Whole exome sequencing identifies multiple, complex etiologies in an idiopathic hereditary pancreatitis kindred. *Jop*, *13*(3), 258-262. https://pubmed.ncbi.nlm.nih.gov/22572128/

LaRusch, J., Solomon, S., & Whitcomb, D. C. (2014). Pancreatitis Overview [Text]. *GEneReviews*. https://www.ncbi.nlm.nih.gov/books/NBK190101/

LaRusch, J., & Whitcomb, D. C. (2011). Genetics of pancreatitis. *Curr Opin Gastroenterol*, *27*(5), 467-474. https://doi.org/10.1097/MOG.0b013e328349e2f8

Lerch, M. M., & Gorelick, F. S. (2000). Early trypsinogen activation in acute pancreatitis. *Med Clin North Am*, *84*(3), 549-563, viii. https://doi.org/10.1016/S0025-7125(05)70239-X

Lerch, M. M., Zenker, M., Turi, S., & Mayerle, J. (2006). Developmental and metabolic disorders of the pancreas. *Endocrinol Metab Clin North Am*, *35*(2), 219-241, vii. https://doi.org/10.1016/j.ecl.2006.02.004

Li, Z. S., Wang, W., Liao, Z., Zou, D. W., Jin, Z. D., Chen, J., Wu, R. P., Liu, F., Wang, L. W., Shi, X. G., Yang, Z., & Wang, L. (2010). A long-term follow-up study on endoscopic

management of children and adolescents with chronic pancreatitis. *Am J Gastroenterol*, *105*(8), 1884-1892. https://doi.org/10.1038/ajg.2010.85

Lohr, J. M., Dominguez-Munoz, E., Rosendahl, J., Besselink, M., Mayerle, J., Lerch, M. M., Haas, S., Akisik, F., Kartalis, N., Iglesias-Garcia, J., Keller, J., Boermeester, M., Werner, J., Dumonceau, J. M., Fockens, P., Drewes, A., Ceyhan, G., Lindkvist, B., Drenth, J., . . . Bruno, M. (2017). United European Gastroenterology evidence-based guidelines for the diagnosis and therapy of chronic pancreatitis (HaPanEU). *United European Gastroenterol J*, *5*(2), 153-199. https://doi.org/10.1177/2050640616684695

Masson, E., Le Marechal, C., Delcenserie, R., Chen, J. M., & Ferec, C. (2008). Hereditary pancreatitis caused by a double gain-of-function trypsinogen mutation. *Hum Genet*, *123*(5), 521-529. https://doi.org/10.1007/s00439-008-0508-6

Molven, A., Njolstad, P. R., & Weiss, F. U. (2015). Lipase gene fusion: a new route to chronic pancreatitis. In *Oncotarget* (Vol. 6, pp. 30443-30444). https://doi.org/10.18632/oncotarget.5454

Mullady, D. K., Yadav, D., Amann, S. T., O'Connell, M. R., Barmada, M. M., Elta, G. H., Scheiman, J. M., Wamsteker, E. J., Chey, W. D., Korneffel, M. L., Weinman, B. M., Slivka, A., Sherman, S., Hawes, R. H., Brand, R. E., Burton, F. R., Lewis, M. D., Gardner, T. B., Gelrud, A., . . . Anderson, M. A. (2011). Type of pain, pain-associated complications, quality of life, disability and resource utilisation in chronic pancreatitis: a prospective cohort study. *Gut*, *60*(1), 77-84. https://doi.org/10.1136/gut.2010.213835

Nabi, Z., Talukdar, R., Venkata, R., Aslam, M., Shava, U., & Reddy, D. N. (2020). Genetic Evaluation of Children with Idiopathic Recurrent Acute Pancreatitis. *Dig Dis Sci*, *65*(10), 3000-3005. https://doi.org/10.1007/s10620-019-06026-2

NCCN. (2023, December 13, 2023). *NCCN Clinical Practice Guidelines in Oncology - Pancreatic Adenocarcinoma*

Version 1.2024 - December 13, 2023. https://www.nccn.org/professionals/physician_gls/pdf/pancreatic.pdf

Nelson, A., & Myers, K. (2008, 10/18/2018). *Shwachman-Diamond Syndrome*. University of Washington, Seattle. https://www.ncbi.nlm.nih.gov/books/NBK1756/

NICE. (2020, December 16). *Pancreatitis*. Retrieved April 22 from https://www.nice.org.uk/guidance/ng104/chapter/Recommendations

Nijmeijer, R. M., van Santvoort, H. C., Zhernakova, A., Teller, S., Scheiber, J. A., de Kovel, C. G., Besselink, M. G., Visser, J. T., Lutgendorff, F., Bollen, T. L., Boermeester, M. A., Rijkers, G. T., Weiss, F. U., Mayerle, J., Lerch, M. M., Gooszen, H. G., Akkermans, L. M., & Wijmenga, C. (2013). Association analysis of genetic variants in the myosin IXB gene in acute pancreatitis. *PLoS One*, *8*(12), e85870. https://doi.org/10.1371/journal.pone.0085870

O'Neill, M., Stumpf, A., & McKusick, V. (2013, 09/10/2013). *MATURITY-ONSET DIABETES OF THE YOUNG, TYPE 8, WITH EXOCRINE DYSFUNCTION; MODY8*. Johns Hopkins University. https://omim.org/entry/609812

Ooi, C. Y., Gonska, T., Durie, P. R., & Freedman, S. D. (2010). Genetic testing in pancreatitis. *Gastroenterology*, *138*(7), 2202-2206, 2206.e2201. https://doi.org/10.1053/j.gastro.2010.04.022

Palermo, J. J., Lin, T. K., Hornung, L., Valencia, C. A., Mathur, A., Jackson, K., Fei, L., & Abu-El-Haija, M. (2016). Genophenotypic Analysis of Pediatric Patients With Acute Recurrent and Chronic Pancreatitis. *Pancreas*, *45*(9), 1347-1352. https://doi.org/10.1097/mpa.0000000000000655

Parniczky, A., Abu-El-Haija, M., Husain, S., Lowe, M., Oracz, G., Sahin-Toth, M., Szabo, F. K., Uc, A., Wilschanski, M., Witt, H., Czako, L., Grammatikopoulos, T., Rasmussen, I. C., Sutton, R., & Hegyi, P. (2018). EPC/HPSG evidence-based guidelines for the management of pediatric pancreatitis. *Pancreatology*, *18*(2), 146-160. https://doi.org/10.1016/j.pan.2018.01.001

Poddar, U., Yachha, S. K., Mathias, A., & Choudhuri, G. (2015). Genetic predisposition and its impact on natural history of idiopathic acute and acute recurrent pancreatitis in children. *Dig Liver Dis*, *47*(8), 709-714. https://doi.org/10.1016/j.dld.2015.04.012

Ravi Kanth, V., & Nageshwar Reddy, D. (2014). Genetics of acute and chronic pancreatitis: An update. *World J Gastrointest Pathophysiol*, *5*(4), 427-437. https://doi.org/10.4291/wjgp.v5.i4.427

Schwarzenberg, S. J. (2023, September 13, 2023). *Pancreatitis associated with genetic risk factors*. UpToDate, Inc. https://www.uptodate.com/contents/pancreatitis-associated-with-genetic-risk-factors

Schwarzenberg, S. J., Bellin, M., Husain, S. Z., Ahuja, M., Barth, B., Davis, H., Durie, P. R., Fishman, D. S., Freedman, S. D., Gariepy, C. E., Giefer, M. J., Gonska, T., Heyman, M. B., Himes, R., Kumar, S., Morinville, V. D., Lowe, M. E., Nuehring, N. E., Ooi, C. Y., . . . Uc, A. (2015). Pediatric chronic pancreatitis is associated with genetic risk factors and substantial disease burden. *J Pediatr*, *166*(4), 890-896.e891. https://doi.org/10.1016/j.jpeds.2014.11.019

Sultan, M., Werlin, S., & Venkatasubramani, N. (2012). Genetic prevalence and characteristics in children with recurrent pancreatitis. *J Pediatr Gastroenterol Nutr*, *54*(5), 645-650. https://doi.org/10.1097/mpg.0b013e31823f0269

Sun, C., Liu, M. Y., Liu, X. G., Hu, L. H., Xia, T., Liao, Z., & Li, Z. S. (2015). Serine Protease Inhibitor Kazal Type 1 (SPINK1) c.194+2T > C Mutation May Predict Long-term Outcome of Endoscopic Treatments in Idiopathic Chronic Pancreatitis. *Medicine (Baltimore)*, *94*(47), e2046. https://doi.org/10.1097/md.0000000000002046

Suzuki, M., Minowa, K., Nakano, S., Isayama, H., & Shimizu, T. (2020). Genetic Abnormalities in Pancreatitis: An Update on Diagnosis, Clinical Features, and

Treatment. *Diagnostics (Basel, Switzerland)*, *11*(1), 31. https://doi.org/10.3390/diagnostics11010031

Szmola, R., & Sahin-Toth, M. (2007). Chymotrypsin C (caldecrin) promotes degradation of human cationic trypsin: identity with Rinderknecht's enzyme Y. *Proc Natl Acad Sci U S A*, *104*(27), 11227-11232. https://doi.org/10.1073/pnas.0703714104

Tandan, M., & Nageshwar Reddy, D. (2013). Endotherapy in chronic pancreatitis. *World J Gastroenterol*, *19*(37), 6156-6164. https://doi.org/10.3748/wjg.v19.i37.6156

Tenner, S., Baillie, J., DeWitt, J., & Vege, S. S. (2013). American College of Gastroenterology guideline: management of acute pancreatitis. *Am J Gastroenterol*, *108*(9), 1400-1415; 1416. https://doi.org/10.1038/ajg.2013.218

Walker, N. F., Warren, O. J., Gawn, L., & Jiao, L. R. (2013). The role of genetic testing in management of hereditary chronic pancreatitis. *JRSM Short Rep*, *4*(1), 6. https://doi.org/10.1258/shorts.2012.012071

Weiss, F. U., Laemmerhirt, F., & Lerch, M. M. (2020). Next generation sequencing pitfalls in diagnosing trypsinogen (PRSS1) mutations in chronic pancreatitis. *Gut*. https://doi.org/10.1136/gutjnl-2020-322864

Werlin, S., Konikoff, F. M., Halpern, Z., Barkay, O., Yerushalmi, B., Broide, E., Santo, E., Shamir, R., Shaoul, R., Shteyer, E., Yaakov, Y., Cohen, M., Kerem, E., Ruszniewski, P., Masson, E., Ferec, C., & Wilschanski, M. (2015). Genetic and electrophysiological characteristics of recurrent acute pancreatitis. *J Pediatr Gastroenterol Nutr*, *60*(5), 675-679. https://doi.org/10.1097/mpg.0000000000000623

Wertheim-Tysarowska, K., Oracz, G., & Rygiel, A. M. (2021). Genetic Risk Factors in Early-Onset Nonalcoholic Chronic Pancreatitis: An Update. *Genes*, *12*(5), 785. https://doi.org/10.3390/genes12050785

Whitcomb, D. C. (1999). Early trypsinogen activation in acute pancreatitis. *Gastroenterology*, *116*(3), 770-772. https://www.gastrojournal.org/article/S0016-5085(99)70205-2/fulltext

Whitcomb, D. C. (2004). Mechanisms of disease: Advances in understanding the mechanisms leading to chronic pancreatitis. *Nat Clin Pract Gastroenterol Hepatol*, *1*(1), 46-52. https://doi.org/10.1038/ncpgasthep0025

Whitcomb, D. C. (2019). Pancreatitis: TIGAR-O Version 2 Risk/Etiology Checklist With Topic Reviews, Updates, and Use Primers. *Clin Transl Gastroenterol*, *10*(6), e00027. https://doi.org/10.14309/ctg.0000000000000027

Whitcomb, D. C., Yadav, D., Adam, S., Hawes, R. H., Brand, R. E., Anderson, M. A., Money, M. E., Banks, P. A., Bishop, M. D., Baillie, J., Sherman, S., DiSario, J., Burton, F. R., Gardner, T. B., Amann, S. T., Gelrud, A., Lo, S. K., DeMeo, M. T., Steinberg, W. M., . . . Barmada, M. M. (2008). Multicenter approach to recurrent acute and chronic pancreatitis in the United States: the North American Pancreatitis Study 2 (NAPS2). *Pancreatology*, *8*(4-5), 520-531. https://doi.org/10.1159/000152001

Witt, H., Beer, S., Rosendahl, J., Chen, J. M., Chandak, G. R., Masamune, A., Bence, M., Szmola, R., Oracz, G., Macek, M., Jr., Bhatia, E., Steigenberger, S., Lasher, D., Buhler, F., Delaporte, C., Tebbing, J., Ludwig, M., Pilsak, C., Saum, K., . . . Sahin-Toth, M. (2013). Variants in CPA1 are strongly associated with early onset chronic pancreatitis. *Nat Genet*, *45*(10), 1216-1220. https://doi.org/10.1038/ng.2730

Zou, W. B., Tang, X. Y., Zhou, D. Z., Qian, Y. Y., Hu, L. H., Yu, F. F., Yu, D., Wu, H., Deng, S. J., Lin, J. H., Zhao, A. J., Zhao, Z. H., Wu, H. Y., Zhu, J. H., Qian, W., Wang, L., Xin, L., Wang, M. J., Wang, L. J., . . . Liao, Z. (2018). SPINK1, PRSS1, CTRC, and CFTR Genotypes Influence Disease Onset and Clinical Outcomes in Chronic Pancreatitis. *Clin Transl Gastroenterol*, *9*(11), 204. https://doi.org/10.1038/s41424-018-0069-5

Zou, W. B., Wang, Y. C., Ren, X. L., Wang, L., Deng, S. J., Mao, X. T., Li, Z. S., & Liao, Z. (2020). TRPV6 variants confer susceptibility to chronic pancreatitis in the Chinese population. *Hum Mutat*, *41*(8), 1351-1357. https://doi.org/10.1002/humu.24032

Genetic Testing for Inherited Cardiomyopathies and Channelopathies

Policy Number: AHS – M2025 – Genetic Testing for Inherited Cardiomyopathies and Channelopathies	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Cardiomyopathies are diseases of the heart muscle. These conditions are frequently genetic and do not include muscle abnormalities caused by coronary artery disease, hypertension, valvular disease, and congenital heart disease. Symptoms include arrhythmia, cardiac dysfunction, and heart failure (Cooper Jr, 2022).

Channelopathies, also known as primary electrical disease, are a group of cardiac diseases caused by genetic defects in ion channels of the heart leading to arrhythmias, syncope, and the risk of sudden cardiac death (SCD) (Campuzano et al., 2015).

II. Related Policies

Policy Number	Policy Title
AHS-M2180	Genetic Markers for Assessing Risk of Cardiovascular Disease

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Genetic counseling **IS REQUIRED** for individuals prior to and after undergoing genetic testing for diagnostic, carrier, and/or risk assessment purposes.

2) Sequencing of long QT syndrome (LQTS)-associated genes **MEETS COVERAGE CRITERIA** for the following:
   a) For individuals who have had a syncopal event and who have a Schwartz score greater than 1.
   b) For individuals for whom a cardiologist has a strong clinical suspicion of LQTS based on the individual's clinical history, family history, and electrocardiogram (ECG) findings (e.g., Schwartz score greater than or equal to 3.5).
   c) For individuals with QT prolongation (QTc greater than 480 ms for prepubescent individuals, QTc greater than 500 ms for postpubescent individuals) in the absence of other clinical conditions that might prolong the QT interval (e.g., electrolyte abnormalities, hypertrophy, bundle branch block).
   d) For individuals with a close relative (see Note 1) with confirmed LQTS and for whom the familial mutation is not known.
3) For close relatives (see Note 1) of an individual with a documented LQTS-causing mutation, testing for the known familial likely pathogenic or pathogenic variant **MEETS COVERAGE CRITERIA**.
4) For individuals with a negative sequence analysis where the clinical suspicion of congenital LQTS remains high based on a Schwartz score greater than 1, genetic testing for LQTS with duplication/deletion analysis **MEETS COVERAGE CRITERIA**.
5) Genetic testing for catecholaminergic polymorphic ventricular tachycardia (CPVT) **MEETS COVERAGE CRITERIA** in the following situations:
   a) For individuals who have a close relative (see Note 1) with a known CPVT likely pathogenic or pathogenic variant.
   b) For individuals who have a close relative (see Note 1) diagnosed with CPVT by clinical means but whose genetic status is unavailable.
   c) For individuals with signs and/or symptoms indicating a moderate-to-high pretest probability of CPVT, but for whom a definitive diagnosis cannot be made without genetic testing.
   d) For individuals who display exercise-, catecholamine-, or emotion-induced PVT or ventricular fibrillation in a structurally normal heart.
6) Genetic testing for Brugada syndrome **MEETS COVERAGE CRITERIA** in the following situations:
   a) For individuals with signs and/or symptoms consistent with Brugada Syndrome but for whom a definitive diagnosis cannot be made without genetic testing.

b) For individuals who have a close relative (see Note 1) with a known Brugada Syndrome likely pathogenic or pathogenic variant.

7) Sequencing of short QT syndrome (SQTS)-associated genes **MEETS COVERAGE CRITERIA** for the following:

   a) For individuals for whom a cardiologist has a strong clinical suspicion of SQTS based on the individual's clinical history, family history, and ECG findings (e.g., abnormally short QT intervals [less than or equal to 360 ms in males; less than or equal to 370 ms in females], an increased propensity to develop atrial and ventricular tachyarrhythmia in the absence of structural heart disease).

   b) Testing for the known familial likely pathogenic or pathogenic variant in asymptomatic individuals with a close relative (see Note 1) with a known SQTS likely pathogenic or pathogenic variant.

8) For individuals with dilated cardiomyopathy and significant cardiac conduction disease (i.e., first-, second-, or third-degree heart block) and/or who have one or more family members who experienced sudden cardiac death or developed unexplained heart failure before 60 years of age, genetic testing for dilated cardiomyopathy **MEETS COVERAGE CRITERIA**.

9) In asymptomatic close relatives (see Note 1) of an affected individual, genetic testing for a known familial likely pathogenic or pathogenic variant associated with dilated cardiomyopathy **MEETS COVERAGE CRITERIA**.

10) Genetic testing for arrhythmogenic right ventricular cardiomyopathy (ARVC) **MEETS COVERAGE CRITERIA** in the following situations:

   a) For individuals with signs and/or symptoms consistent with ARVC but for whom a definitive diagnosis cannot be made without genetic testing.

   b) For individuals who have a close relative (see Note 1) with a known ARVC likely pathogenic or pathogenic variant.

11) In asymptomatic close relatives (see Note 1) of an affected individual, genetic testing for a known familial likely pathogenic or pathogenic variant associated with progressive cardiac conduction disease (CCD or Lev-Lenegre disease) **MEETS COVERAGE CRITERIA**.

12) In asymptomatic close relatives (see Note 1) of an affected individual, genetic testing for a known familial likely pathogenic or pathogenic variant associated with restrictive cardiomyopathy (RCM) **MEETS COVERAGE CRITERIA**.

13) Genetic testing for left ventricular noncompaction (LVNC) **MEETS COVERAGE CRITERIA** in the following situations:

   a) For individuals with signs and/or symptoms consistent with LVNC but for whom a definitive diagnosis cannot be made without genetic testing.

   b) For individuals who have a close relative (see Note 1) with a known LVNC likely pathogenic or pathogenic variant.

14) For individuals who meet the diagnostic criteria for hypertrophic cardiomyopathy (HCM), genetic testing for predisposition to HCM **MEETS COVERAGE CRITERIA**.

15) For individuals who have a first-degree relative with established HCM, genetic testing for the known familial likely pathogenic or pathogenic variant **MEETS COVERAGE CRITERIA**.

16) For individuals with a family history of HCM in which a first-degree relative with HCM has tested negative for likely pathogenic or pathogenic variants, genetic testing for a predisposition to HCM **DOES NOT MEET COVERAGE CRITERIA**.

17) For symptomatic individuals with a Schwartz score less than or equal to 1, genetic testing for LQTS **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

18) For individuals with known LQTS, genetic testing for LQTS to determine prognosis and/or direct therapy **DOES NOT MEET COVERAGE CRITERIA**.

19) For all other situations when the above criteria are not met, genetic testing for LQTS or CPVT **DOES NOT MEET COVERAGE CRITERIA**.

20) For all other situations not meeting the criteria outlined above, genetic testing for Brugada Syndrome **DOES NOT MEET COVERAGE CRITERIA**.

21) For all other situations not meeting the criteria outlined above, genetic testing for SQTS **DOES NOT MEET COVERAGE CRITERIA**.

22) Genetic testing for Early Repolarization "J-wave" Syndrome, Sinus Node Dysfunction (SND), and/or other rhythm disorders **DOES NOT MEET COVERAGE CRITERIA**.

23) For all other individuals, genetic testing for a predisposition to HCM **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Close blood relatives include first-degree relatives (e.g., parents, siblings, and children), second-degree relatives (e.g., grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings), and third-degree relatives (great-grandparents, great-aunts, great-uncles, great-grandchildren, and first cousins).

IV. Table of Terminology

Term	Definition
ACC	American College of Cardiology
ACM	Arrhythmic cardiomyopathy
ACCF	American College of Cardiology Foundation
ACMG	American College of Medical Genetics and Genomics
ACTA2	*Actin alpha 2, smooth muscle gene*
ACTC1	*Actin alpha cardiac muscle 1 gene*
AF	Atrial fibrillation
APHRS	Asia Pacific Heart Rhythm Society
APOB	*Apolipoprotein B gene*
ARVC	Arrhythmogenic right ventricular cardiomyopathy
ARVC/D	Arrhythmogenic right ventricular cardiomyopathy/dysplasia
BrS	Brugada syndrome
CASQ2	*Calsequestrin 2 gene*
CCD	Cardiac conduction disease
CLQTS	Congenital long QT syndrome
COL3A1	*Collagen type III alpha 1 chain gene*
CPVT	Catecholaminergic polymorphic ventricular tachycardia
DCM	Dilated cardiomyopathy
DSC2	*Desmocoilin 2 gene*
DSG2	*Desmoglein 2 gene*
DSP	*Desmoplakin gene*
ECG	Electrocardiogram
ECS	European Society of Cardiology
EHRA	European Heart Rhythm Association
ER	Early repolarization
FBN1	*Fibrillin-1 gene*
FLNC	*Filamin C gene*
HCM	Hypertrophic cardiomyopathy

HF	Heart failure
HFSA	Heart Failure Society of America
HRS	Heart Rhythm Society
IVF	Idiopathic ventricular fibrillation
KCNE1	*Potassium voltage-gated channel subfamily E regulatory subunit 1 gene*
KCNE2	*Potassium voltage-gated channel subfamily E regulatory subunit 2 gene*
KCNH2	*Potassium voltage-gated channel subfamily H member 2 gene*
KCNQ1	*Potassium voltage-gated channel subfamily Q member 1 gene*
LDLR	*Low density lipoprotein receptor gene*
LMNA	*Lamin A/C gene*
LQTS	Long QT syndrome
LV	Left ventricle
LVH	Left ventricular hypertrophy
LVNC	Left ventricular noncompaction
MYBPC3	*Myosin binding protein C3 gene*
MYH7	*Myosin heavy chain 7 gene*
MYH11	*Myosin heavy chain 11 gene*
MYL2	*Myosin light chain 2 gene*
MYL3	*Myosin light chain 3 gene*
PCCD	Progressive cardiac conduction disease
PCSK9	*Proprotein convertase subtilisin/kexin type 9 gene*
PKP2	*Plakophilin 2 gene*
PRKAG2	*Protein kinase AMP-activated non-catalytic subunit gamma 2 gene*
RCM	Restrictive cardiomyopathy
RYR2	*Ryanodine receptor 2 gene*
SCA	Sudden cardiac arrest
SCD	Sudden cardiac death
SCN5A	*Sodium voltage-gated channel alpha subunit 5 gene*
SMAD3	*SMAD family member 3 gene*
SND	Sinus node dysfunction
SUDS	Sudden unexplained death syndrome
SQTS	Short QT syndrome
TGFBR1	*Transforming growth factor beta receptor 1 gene*
TGFBR2	*Transforming growth factor beta receptor 2 gene*
TMEM43	*Transmembrane protein 43 gene*
TNNI3	*Troponin I3, cardiac type gene*

TNNT2	*Troponin T2, cardiac type gene*
TPM1	*Tropomyosin 1 gene*
TRDN	*Triadin gene*
TTN	*Titin gene*
WGS	Whole genome sequencing

V. Scientific Background

Cardiomyopathies

In 1995, the World Health Organization (WHO) divided cardiomyopathies into five categories: dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM), arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D), and unclassified cardiomyopathies. Cardiomyopathies have a variety of genotypes and phenotypes that typically require echocardiographic evaluation. Either phase of the heartbeat (systole or diastole) may be affected by cardiomyopathies (Cooper Jr, 2022).

Systolic dysfunction is usually characterized by a decrease in myocardial contractility. This decrease causes a reduction in the ejection fraction of the left ventricle (LV), thereby forcing one of two compensatory mechanisms; either the LV itself increases in size (leading to larger stroke volume) or the contractility of the heart increases in response to increased stretch. However, these compensatory mechanisms will eventually fail, leading to physiological manifestations of heart failure. This dysfunction is often seen in DCM, as well as some HCM patients (Cooper Jr, 2022).

Diastolic dysfunction refers to abnormal LV relaxation and filling, as well as elevated filling pressures. As with systolic dysfunction, the primary issue with diastole is caused by abnormal contractility of the heart muscle. The contractility of the myocardium influences both the LV relaxation phase (the isovolumetric period between the aortic valve's close and mitral valve's opening) and passive compliance phase (variable pressure, starting at mitral valve's opening) of diastole. Due to the impaired contractility of the myocardium, the pressure in each phase is abnormal. Dysfunction in this phase is often seen in HCM, RCM, and DCM (Cooper Jr, 2022).

Hypertrophic cardiomyopathy (HCM)

Hypertrophic cardiomyopathy (HCM) is a commonly inherited cardiovascular disease defined as thickening of the ventricular wall resulting from more than 1500 mutations in 11 or more genes encoding proteins of the cardiac sarcomere (Maron, 2022).

Hypertrophic cardiomyopathy (HCM) is characterized by left ventricular hypertrophy (LVH, thickness of ≥15 mm), observed by echocardiography or magnetic resonance imaging and not otherwise explainable by other cardiovascular issues, such as coronary artery disease, hypertension, valvular disease, and congenital heart disease. Development of LVH usually starts in adolescence and is complete by early adulthood. Symptoms include chest pain, dyspnea and syncope, and severe disease can lead to disabling complications, including heart failure and malignant ventricular arrhythmias. However, many patients with HCM are asymptomatic or have minimal symptoms and are only discovered through means such as family screenings or an abnormal ECG (Maron, 2022). HCM is the most frequent cause of sudden death in young people and can lead to functional disability from heart failure and stroke (Maron, 2003). HCM has a prevalence of approximately 1 in 500 people (Maron et al., 1995). However, estimates of clinically expressed HCM plus gene carriers are as high as 1 in 200 (Semsarian et al., 2015).

More than 90% of HCM is inherited as an autosomal-dominant disease with variable expressivity and age-related penetrance (Frustaci et al., 2018). Currently, relevant genetic abnormalities can be detected in approximately 60 percent of patients with clinically documented HCM (Cirino et al., 2017; Maron et al., 2012). Most of the genetic mutations associated with HCM are found in the genes encoding various proteins that make up the cardiac sarcomere, the basic contractile unit of cardiac myocytes. More than 1500 pathogenic variants have been identified in at least 11 different genes (Cirino, 2014; Maron et al., 2012). Mutations in myosin heavy chain (*MYH7*) and myosin-binding protein C (*MYBPC3*) are the most common and account for roughly 70 percent of HCM. Other genes implicated in HCM are regulatory myosin light chain (*MYL2*) and cardiac troponin T (*TNNT2*). Non-sarcomeric genes encoding plasma membrane or mitochondrial proteins, or Z-disc encoding genes, have also been documented (Frustaci et al., 2018).

Wide phenotypic variability exists, ranging from asymptomatic to severe life-threatening heart failure even within the same mutation. This variability in clinical expression may be related to environmental factors and modifier genes (Alcalai et al., 2008). Moreover, no strong correlation between left ventricular problems and symptoms exists; patients with major obstructions or hypertrophy may be asymptomatic and vice versa. The primary characteristic of LVH is present in multiple conditions, such as systemic hypertension, Fabry disease, aortic stenosis, and more. Such conditions should be excluded before a diagnosis of HCM is made (Maron, 2022).

Diagnostic screening of first-degree relatives is important to identify at-risk patients. Guidelines have been established for clinically unaffected relatives of affected individuals. Clinical screening with physical examination, electrocardiography, and

echocardiography is recommended every 12 to 18 months for individuals between the ages of 12 to 18 years and every three to five years for adults with additional screening recommended for any change in symptoms (Gersh et al., 2011).

Dilated cardiomyopathy (DCM)

Dilated cardiomyopathy (DCM) is characterized by dilation and impaired contraction of one or both ventricles. The dilation often becomes severe and is invariably accompanied by an increase in total cardiac mass. Affected patients have impaired systolic function and clinical presentation is usually with features of heart failure (Cooper Jr, 2022).

Dilated cardiomyopathy is caused by a variety of disorders. The cause is unknown for over 50% of patients with the disease. Familial dilated cardiomyopathy is caused by a genetic mutation in 20-35% with the disease. DCM is transmitted primarily in an autosomal dominant inheritance pattern. Mutations in over 30 genes have been determined to cause familial dilated cardiomyopathy (Hershberger, 2023).

Other common causes for DCM include (AHA, 2024) coronary heart disease, heart attack, high blood pressure, diabetes, thyroid disease, viral hepatitis and HIV, infections, especially viral infections that inflame the heart muscle, alcohol, complications during the last month of pregnancy or within five months of birth, certain toxins such as cobalt, certain drugs (such as cocaine and amphetamines) and two medicines used to treat cancer (doxorubicin and daunorubicin).

Genetic forms of dilated cardiomyopathy are diagnosed by family history and molecular testing. Genes associated with familial dilated cardiomyopathy include *MYH7, MYBPC3, TNNT2, TNNC1, TNNI3, TPM1, MYL2, MYL3, ACTC1, ACTN2, CSRP3, PLN, TTR, PRKG2, LAMP2, GLA, LMNA, BAG3, RBM20, SCN5A, DES, DSC2, DSG2, DSP, JUP, PKP2, RYR2, TMEM43*, and *TTN* (Hershberger et al., 2018).

The frequencies of DCM mutations in any one gene are low (<1% to 6-8%), and a genetic cause is identified in only 30-35% of familial DCM cases. Therefore, routine genetic testing for DCM was only recommended in familial disease (≥2 affected family members) (Yancy et al., 2013). However, as molecular genetic testing laboratories offer DCM genetic testing panels of 12-30 genes utilizing next-generation sequencing methods, testing sensitivity now ranges from 15-25%, has become standard of care (M. J. Ackerman et al., 2011; Hershberger, 2023; Hershberger et al., 2009; Hershberger et al., 2010; Hershberger & Siegfried, 2011).

Guidelines now recommend testing for all patients with cardiomyopathy even if no other disease is evident in the family. Genetic testing is clinically useful in the management of affected individuals, as well as to assess risk in relatives (Hershberger et al., 2018). *LMNA* mutations are associated with high rates of conduction system

disease, ventricular arrhythmias, and sudden cardiac death (SCD), and may consequently lower the threshold for prophylactic ICD implantation. DCM patients with a variant of the *SCN5A* gene exhibit a phenotype associated with significant arrhythmias and frequent premature ventricular complexes. Although such patients responded poorly to conventional HF therapy, treatment with sodium channel blocking drugs produced a dramatic reduction in ectopy and normalization of left ventricular (LV) function (Japp et al., 2016).

Restrictive cardiomyopathy (RCM)

Restrictive cardiomyopathy (RCM) differs from other cardiomyopathies in that there may not be many physical abnormalities (i.e., no dilation or hypertrophy). However, the ventricular filling process is still significantly impaired. RCM may be difficult to see on two-dimensional imaging, and assessment of flow velocity across the mitral valve is more accurate in detecting these filling abnormalities (Cooper Jr, 2022). Some cases of RCM are secondary to a more apparent cardiac condition. However, idiopathic cases of RCM are generally genetic conditions. Pathogenic mutations in sarcomeric or cytoskeletal genes such as *TNNI3, TNNT2,* and *TPN1* have been linked to familial RCM (Ammash, 2024).

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D)

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is characterized by changes in myocardium of the right (and often left as well) ventricular free wall. This myocardium is replaced by fibrous or fibro-fatty tissue, causing dysfunction of the affected ventricles (Cooper Jr, 2022). At the molecular level, the desmosomes are typically impaired due to genetic mutations. This causes the mechanical stress of the heart to detach myocytes, which eventually leads to their death. The initial repair mechanisms of this detachment are what produces the fibrous tissue. Up to 30% of ARVC cases are familial, and mutations in gene products such as plakoglobin and desmoplakin have been associated with ARVC (McKenna, 2024).

Unclassified Cardiomyopathies

Other types of cardiomyopathies that do not fall into one of the other four categories are considered "unclassified." For example, left ventricular noncompaction (LVNC) falls in this category due to its characteristic myocardial wall; the myocardial wall consists of "prominent trabeculae and deep intertrabecular recesses", thereby resulting in two layers of myocardium of different thickness. This condition may be sporadic or familial; up to 50% of LVNC cases are familial. Genes such as alpha-dystrobrevin or other sarcomeric genes may contribute to LVNC (Attenhofer-Jost & Connolly, 2022).

This category also includes cardiomyopathies such as stress-induced cardiomyopathy and cirrhotic cardiomyopathy (Cooper Jr, 2022).

Clinical Utility and Validity (Cardiomyopathies)

A study by Cirino et al. (2017) compared the results from panel genetic testing to whole genome sequencing (WGS). Forty-one patients with HCM who had undergone targeted genetic testing (either multigene panel or familial variant test) were recruited into a clinical trial of WGS. Panel size ranged from 4-62 genes, and all but two subjects were tested for the main eight sarcomeric genes (*MYH7, MYBPC3, TNNT2, TPM1, MYL2, MYL3, TNNI3, ACTC*). The authors stated that WGS detected nearly all variants identified on panel testing and allowed further analysis of posited disease genes. Several variants of uncertain clinical use and other genetic findings were also identified. Panel testing and WGS provided similar results, but WGS provides reanalysis over time; however, WGS also requires genomic expertise to correctly interpret results (Cirino et al., 2017).

A study focusing on the non-sarcomeric genes contributing to HCM was performed by Walsh et al. (2017). A reference sample of 60,706 exomes were analyzed and compared to 6,179 HCM cases. This comparison revealed many gene variants in the main eight sarcomeric genes (*MYH7, MYBPC3, TNNT2, TPM1, MYL2, MYL3, TNNI3, ACTC1*) but very few variants of the non-sarcomeric genes in HCM cases. The authors concluded the variation in most of the non-sarcomeric genes does not affect HCM significantly as 99% of HCM pathogenic variants were found to be in the main eight sarcomeric genes. Four non-sarcomeric genes were found to have an excess of variants, but even these amounted to only 2% of the HCM cases overall; the other 26 non-sarcomeric genes examined were found to have very little or no excess variation over the reference sample of exomes. Furthermore, the authors state that only the well-known variants are symptomatic whereas the other variants are of unknown significance or benign, making clinical sequencing of limited use. The authors recommended that the only genes tested should be the eight sarcomeric genes, the metabolic cardiomyopathy genes, and possibly *ACTN2* and *MYOZ2* (Walsh et al., 2017).

Bhonsale et al. (2015) assessed the impact of genotype on clinical outcomes of ARVC patients. Pathogenic mutations were evaluated in 577 patients. The investigators found that patients with a *desmoplakin*-associated mutation had an over four-fold occurrence of left ventricular (LV) dysfunction and heart failure than *PKP2* carriers. No significant difference was found between clinical outcomes of patients with missense mutations and patients with truncating or splice site mutations. Patients with multiple mutations had more severe symptoms, such as lower survival rate without ventricular fibrillation or tachycardia, more frequent LV dysfunction, heart failure, cardiac transplant, and earlier occurrence of sustained ventricular fibrillation and tachycardia, as compared to those with only one mutation (Bhonsale et al., 2015).

Kostareva et al. (2016) evaluated the "genetic spectrum" of idiopathic RCM. The authors screened for 108 cardiomyopathy and arrhythmia-associated genes in 24 patients with idiopathic RCM. They found pathogenic and "likely-pathogenic" variants in 13 of the 24 patients (54%), and half of these genotype-positive patients carried a combination of pathogenic variants, likely-pathogenic variants, and variants of unknown significance. The most frequent combination included mutations in sarcomeric and cytoskeletal genes (Kostareva et al., 2016).

Kayvanpour et al. (2017) evaluated the genetic-phenotype associations for DCM. The study included 48 studies encompassing 8097 patients, and the authors investigated mutations in *LMNA, PLN, RBM20, MYBPC3*, *MYH7, TNNT2* and *TNNI3*. The authors results were as follows: "The average frequency of mutations in the investigated genes was between 1 and 5 %. The mean age of DCM onset was the beginning of the fifth decade for all genes. Heart transplantation (HTx) rate was highest in *LMNA* mutation carriers (27 %), while *RBM20* mutation carriers were transplanted at a markedly younger age (mean 28.5 years). While 73 % of DCM patients with *LMNA* mutations showed cardiac conduction diseases, low voltage was the reported ECG hallmark in *PLN* mutation carriers. The frequency of ventricular arrhythmia in DCM patients with *LMNA* (50 %) and *PLN* (43 %) mutations was significantly higher. The penetrance of DCM phenotype in subjects with *TTN* truncating variants increased with age and reached 100 % by age of 70" (Kayvanpour et al., 2017).

Genetic testing has been recognized as a valuable part of diagnosis and classification in pediatric cardiomyopathy. Lipshultz et al. (2019) identified a single-center study of 63 children with cardiomyopathy that reported that 42% of the illness could be attributed to a genetic cause, as defined by the presence of an affected first-degree family member having tested positive for an HCM or DCM gene panel mutation. However, it was noted that though the morphofunctional phenotype in children may be similar to those of adults, the causes and proportion resulting from them differ. These differences are believed to be neuromuscular, metabolic, mitochondrial, and syndromic causes also playing important roles in children. Lipshultz et al. (2019) identified another study to corroborate this finding: in 916 children with an identified case of cardiomyopathy, an underlying metabolic or syndromic cause was present in more than a third, and a metabolic or syndromic cause was present in 40% to 50% of 61 children with HCM and 8% of 1731 children with DCM in the Pediatric Cardiomyopathy Registry (Lipshultz Steven et al., 2019).

Parker and Landstrom (2021) reviewed current literature on what role genetic testing play in diagnosing and managing patients and families with a history of pediatric CM. With relation to HCM, the researchers identified multiple studies demonstrating that patients with "sarcomere variants tend to be more severely affected than patients with

non-sarcomeric variants" and "within the sarcomere genes, some argue that specific sarcomere variants are inherently more pathogenic than others." On top of that, there appear to be some other genetic and environmental factors that influence the HCM phenotype in each patient, thus indicating that the genotype-phenotype correlation could greatly affect the clinical course and prognosis. Especially for metabolic forms of HCM and patients with a clear genetic etiology, treatment may be adopted based on these genetic findings; for instance, "in *GAA*- positive Pompe disease, alpha-glucosidase enzyme replacement therapy is associated with HCM reversal in affected children" (Parker & Landstrom, 2021).

Similar concepts were mentioned for DCM, as some genotypes yielded worse phenotypes. For example, "*TNNC1* variants are associated with early onset DCM, and increased rates of heart transplant and SCD compared to other genes." Gene therapy could also be explored as an option, such as for Duchenne muscular dystrophy, to "target and rescue functional expression of pathogenic variants." For arrhythmic cardiomyopathy (ACM), Parker and Landstrom (2021) specifically mention how confirming an ACM diagnosis via genetic testing "versus an arrhythmic syndrome secondary to a cardiac channelopathy" could aid clinical management. Variants in the *PKP2* and *TMEM43* genes have been found to be associated with exercise-induced disease progression and risk of sudden cardiac arrest (SCA), so this could impact chosen involvement in sports (Parker & Landstrom, 2021).

Grondin et al. (2022) studied the clinical utility of genetic testing in unexplained cardiac arrest. The study included 228 unexplained cardiac arrest survivors. Whole exome sequencing was performed on each participant to identify pathogenic or likely-pathogenic variants. In total, 23 of the 228 participants (10%) had a pathogenic or likely-pathogenic variant. Genetic testing increased the proportion of "explained" cases from 9%, with only phenotyping, to 18%, with phenotyping and WES. "The majority of disease-causing variants was located in cardiomyopathy-associated genes, highlighting the arrhythmogenic potential of such variants in the absence of an overt cardiomyopathy diagnosis" (Grondin et al., 2022).

Cardiac Ion Channelopathies

The electromechanical pumping action of the heart maintains circulation and ensures the delivery of blood and nutrients to all organs to support their normal function. Synchronized contraction of the myocardium is necessary to generate sufficient pressure to drive blood flow (Voorhees & Han, 2015). Mechanical contraction of cardiac myocytes is coordinated by the generation and propagation of an action potential (Fernandez-Falgueras et al., 2017) through the synergistic activation and inactivation of several voltage-dependent ion channels. Membrane depolarization

during the action potential leads to the opening of the voltage-gated calcium channels resulting in an inward current, followed by the efflux of potassium ions, generation of an outward current, and cell repolarization (Garcia-Elias & Benito, 2018). Action potential duration is determined by the magnitude and timing of inward and outward currents (Kirk & Kass, 2015). Differential expression, selectivity, and gating properties of cardiac ion channels in distinct regions of the heart promote unidirectional propagation of electrical activity (Fernandez-Falgueras et al., 2017).

Mutations in genes encoding these specific channels or associated proteins may impair ionic conduction resulting in changes in action potential, synchronization, and/or propagation of electrical impulse and predispose to potentially malignant arrhythmias (Nerbonne & Kass, 2005; Roden et al., 2002). Dyssynchronous contraction of the ventricle, arising from electrical activation delays, also significantly worsens morbidity and mortality in heart failure (HF) patients (Kirk & Kass, 2015). Ion channelopathies have been identified as a significant cause of sudden cardiac death (SCD) in patients with structurally normal hearts (Campuzano et al., 2015; Magi et al., 2017), and some cases of otherwise unexplained stillbirth (Munroe et al., 2018).

Patients can show early symptoms of palpitations or hemodynamic compromise, including dizziness, seizure, or syncope, particularly following exertion; however, in many cases SCD is the only sign of cardiac trouble (Martin et al., 2013). Electrical disturbances in the heart rhythm that can be detected on electrocardiogram (ECG) of some patients with channelopathies result in diagnosis of:

- Long QT Syndrome (LQTS) is characterized by prolonged ventricular repolarization and electrocardiographic prolongation of the QT interval (QTc ≥ 480 ms in repeated 12-lead ECG, although a QTc ≥ 460 ms is sufficient in the presence of unexplained syncope). The variable clinical manifestations of LQTS range from asymptomatic patients diagnosed through family screening, to SCD, syncope, convulsions, malignant ventricular arrhythmias, VF, and *torsade de pointes* (Fernandez-Falgueras et al., 2017). The prevalence of LQTS in infants is approximately 1 in 2000 (Schwartz et al., 2009).
    - Diagnosis of LQTS is made using the Schwartz Score, which indicates high probability of LQTS with a score ≥3.5. The table of diagnostic criteria is shown below (Schwartz & Crotti, 2011):

Electrocardiographic findings #	
A QTc^	
≥480 ms	3
460–479 ms	2
450–459 ms (in males)	1
B QTc^ 4th minute of recovery from exercise stress test ≥480 ms	1
C Torsade de pointes*	2
D T wave alternans	1
E Notched T wave in 3 leads	1
F Low heart rate for age@	0.5
Clinical history	
A Syncope*	
With stress	2
Without stress	1
B Congenital deafness	0.5
Family history	
A Family members with definite LQTS$	1
B Unexplained sudden cardiac death below age 30 among immediate family members$	0.5

In the absence of medications or disorders known to affect these electrocardiographic features.
^ QTc calculated by Bazett's formula where QTc = QT/√RR.
* Mutually exclusive.
Resting heart rate below the 2nd percentile for age.
The same family member cannot be counted in A and B.
SCORE: ≤1 point: low probability of LQTS.
1.5 to 3 points: intermediate probability of LQTS.
≥3.5 points high probability.

- Brugada Syndrome (BrS) is clinically characterized by right ventricular conduction delay and ST-segment elevation in the anterior right precordial leads. Syncope is one of the main clinical manifestations; individuals with BrS develop a monomorphic ventricular tachycardia that may precipitate during sleep, rest, or fever (Magi et al., 2017). Recent reports suggest that BrS could be responsible for 4%–12% of all sudden death (SD) and up to 20% of SD in patients with structurally normal hearts (Fernandez-Falgueras et al., 2017).
- Short QT Syndrome (SQTS) is characterized by abnormally short QT intervals and an increased propensity to develop atrial and ventricular tachyarrhythmia in the absence of structural heart disease. Cardiac arrest seems to be the most frequent symptom (up to 40%). Palpitations are a common symptom (30%), followed by syncope (25%) and atrial fibrillation (AF), which are the first symptoms of the disease in up to 20% of patients. The episodes may occur in a wide range of situations, such as in reaction to loud noise, at rest, during exercise, and during daily activity (Fernandez-Falgueras et al., 2017)

- Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is characterized by a normal ECG and ventricular arrhythmia in genetically predisposed individuals during intense physical exercise or acute emotional stress. Typical clinical manifestations of CPVT include dizziness and syncope. Ventricular arrhythmia, however, may degenerate into rapid polymorphic ventricular tachycardia and ventricular fibrillation, leading to SCD (Magi et al., 2017).
- Progressive Cardiac Conduction Disease (PCCD) is characterized by problems with the cardiac impulse of the heart. These conduction abnormalities may be accompanied by structural problems such as fibrous or fatty calcification, and an ECG may display unusual patterns such as a prolonged P-wave or prolonged QRS interval. These arrhythmias may lead to sudden cardiac death. Mutations in ion channel proteins such as *TRPM4* or *SCN5A* have been associated with CCDs (M. J. Ackerman et al., 2011; Wilde et al., 2005).

Not all cases are accompanied by changes in ECG, which makes them more difficult to diagnose. Genetic testing can contribute substantially both to the diagnosis of affected patients and with the identification of asymptomatic individuals at risk (Bastiaenen & Behr, 2011; Priori et al., 2013).

Currently, mutations associated with SCD have been identified in sodium, potassium and calcium channels and associated proteins (Fernandez-Falgueras et al., 2017). A general overview of the main genetic variants that have been linked to the major cardiac channelopathies is displayed in the table below [adapted from (Garcia-Elias & Benito, 2018; Magi et al., 2017; Munroe et al., 2018; Tester & Ackerman, 2011)].

Gene	Protein	Prevalence	Other Associations
Brugada Syndrome (BrS)			
	Ion Channel Subunits		
*SCN5A**	NaV1.5 (α-subunit of the voltage-dependent Na^+channel)	≃25% (BrS1)	DCM, ARVC, Heart block, LQTS, SSS, SIDS
*SCN1B**	β1-subunit of the voltage-dependent Na^+ channel	<1%	CCD, Epilepsy
*SCN2B**	β2-subunit of the voltage-dependent Na^+ channel	<1%	AF
*SCN3B**	β3-subunit of the voltage-dependent Na^+ channel	<1%	AF, VF, SIDS
*SCN10A**	NaV1.8 (α-subunit of the neuronal voltage-dependent Na^+ channel)	≃10%	LQTS, AF, painful small-fiber peripheral neuropathy

Gene	Protein	Prevalence	Other Associations
*CACNA1C**	CaV1.2 (α1C-subunit of the voltage-dependent L-type Ca^{2+} channel)	<1%	Timothy syndrome, LQTS
*CACNB2b**	β2-subunit of the voltage-dependent L-type Ca^{2+}channel	<1%	SQTS
*KCND3**	KV4.3 (α-subunit of the voltage-dependent K^+ channel)	<1%	SIDS, Spinocerebellar ataxia
*KCNE3**	minK-related peptide 2 (β-subunit of the voltage-dependent K^+ channel)	<1%	
KCNAB2	β2-subunit of the voltage-dependent K^+ channel	<1%	
KCND2	KV4.2 (voltage-dependent K^+ channel)	<1%	Epilepsy
*KCNE5**	minK-related peptide 4 (β-subunit of the voltage-dependent K^+ channel)	<1%	AF, VF
*KCNJ8**	Kir6.1 (inward-rectifier K^+ channel, subunit of the ATP-sensitive K^+ channel)	<1%	VF, SIDS, Cantu syndrome
*ABCC9**	SUR2 (sulfonylurea receptor, subunit of the ATP-sensitive K^+ channel)	<1%	DCM, ERS, Cantu syndrome and related disorders
*KCNH2**	KV11.1/hERG (α-subunit of the voltage-dependent K^+ channel)	<1%	LQTS, SQTS
*CACNA2D1**	α2/δ subunit of the volatge-dependent L-type Ca^{2+}channel	<1%	Epilepsy
*HCN4**	hyperpolarization-activated, cyclic nucleotide-gated ion channel 4	<1%	SSS, AF, AV block, Bradycardia, Tachycardia, NCC
*TRPM4**	Transient receptor potential melastatin 4	<1%	Herat Block, LQTS
	Auxiliary Proteins		
FGF12	fibroblast growth factor 12	<1%	Epilepsy
*GPD1L**	glycerol-3-phosphate dehydrogenase 1-like	<1%	
SLMAP	sarcolemma associated protein (striatin-interacting phosphatase and kinase complex)	<1%	
*PKP2**	plakophillin-2	<1%	ARVC

Gene	Protein	Prevalence	Other Associations
SEMA3A	semaphorin-3A	<1%	
*RANGRF**	MOG1 (multicopy suppressor of *Gsp1*)	<1%	histiocytoid cardiomyopathy
HEY2	CHF1 (cardiovascular helix-loop-helix factor 1)	<1%	
Long QT Syndrome (LQTS)			
	Ion Channel Subunits		
*KCNQ1**	KV7.1 (α-subunit of the voltage-dependent K^+ channel)	≃40% (LQT1)	JLNS, SQTS
*KCNH2**	KV11.1/hERG (α-subunit of the voltage-dependent K^+ channel)	≃30% (LQT2)	SQTS
*SCN5A**	NaV1.5 (α-subunit of the voltage-dependent Na^+ channel)	≃10% (LQT3)	BrS, DCM, ARVC, Heart block, SSS, SIDS
*KCNE1**	minK (β1-subunit of the voltage-dependent K^+ channel)	<1%	JLNS
*KCNE2**	MiRP1 (β2-subunit of the voltage-dependent K^+ channel)	<1%	
*KCNJ2**	Kir2.1 (inward rectifying K^+ channel)	<1% (LQT7)	Andersen-Tawil syndrome, SQTS, AF
*KCNJ5**	Kir3.4 (G protein-activated inward rectifying K^+ channel 4)	<1%	LQTS, Hyperaldosteronism
*SCN1B**	β1-subunit of the voltage-dependent Na^+ channel	<1%	BrS, CCD, Epilepsy
*SCN4B**	β4-subunit of the voltage-dependent Na^+ channel	<1%	AF
*CACNA1C**	CaV1.2 (α1C-subunit of the voltage-dependent L-type Ca^{2+} channel)	<1% (LQT8)	BrS, Timothy syndrome
	Auxiliary Proteins		
*AKAP9**	A-kinase anchor protein-9	<1%	
*ANK2**	ankyrin B	<1%	Arrhythmia
*CALM1**	calmodulin (CaM)	<1%	CVPT
*CALM2**	calmodulin (CaM)	<1%	CVPT
*CALM3**	calmodulin (CaM)	<1%	CVPT
*SNTA1**	α1-syntrophin	<1%	
*TRDN**	triadin	<1%	CVPT

Gene	Protein	Prevalence	Other Associations
*CAV3**	caveolin-3	<1%	HCM, LGMD, Rippling muscle disease, Tateyama-type distal myopathy, SIDS
*TRPM4**	Transient receptor potential melastatin 4	<1%	Herat Block, BrS
*RYR2**	ryanodine receptor 2 (RyR2)	<1%	ARVC, CPVT
Short QT Syndrome (SQTS)			
	Ion Channel Subunits		
*KCNH2**	KV11.1/hERG (α-subunit of the voltage-dependent K^+ channel)	≃15% (SQT1)	LQTS
*KCNQ1**	KV7.1 (α-subunit of the voltage-dependent K^+ channel)	<1%	JLNS, LQTS
*KCNJ2**	Kir2.1 (inward rectifying K^+ channel)	<1%	Andersen-Tawil syndrome, AF
*CACNA1C**	CaV1.2 (α1C-subunit of the voltage-dependent L-type Ca^{2+} channel)	<1%	BrS, Timothy syndrome
*CACNB2b**	β2-subunit of the voltage-dependent L-type Ca^{2+}channel	<1%	BrS
*CACNA2D1**	α2/δ-subunit of the voltage-dependent L-type Ca^{2+} channel	<1%	BrS, Epilepsy
Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)			
*RYR2**	ryanodine receptor 2 (RyR2)	≃50–60% (CPVT1)	ARVC
KCNJ2	Kir2.1	10%	
*CASQ2**	calsequestrin 2	≃5%	
*TRDN**	triadin	<1%	LQTS
*CALM1**	calmodulin (CaM)	<1%	LQTS
*CALM2**	calmodulin (CaM)	<1%	LQTS
*CALM3**	calmodulin (CaM)	<1%	LQTS
TECLR	trans-2,3-enoyl-CoA reductase- like	<1%	

* - *commercially available test*

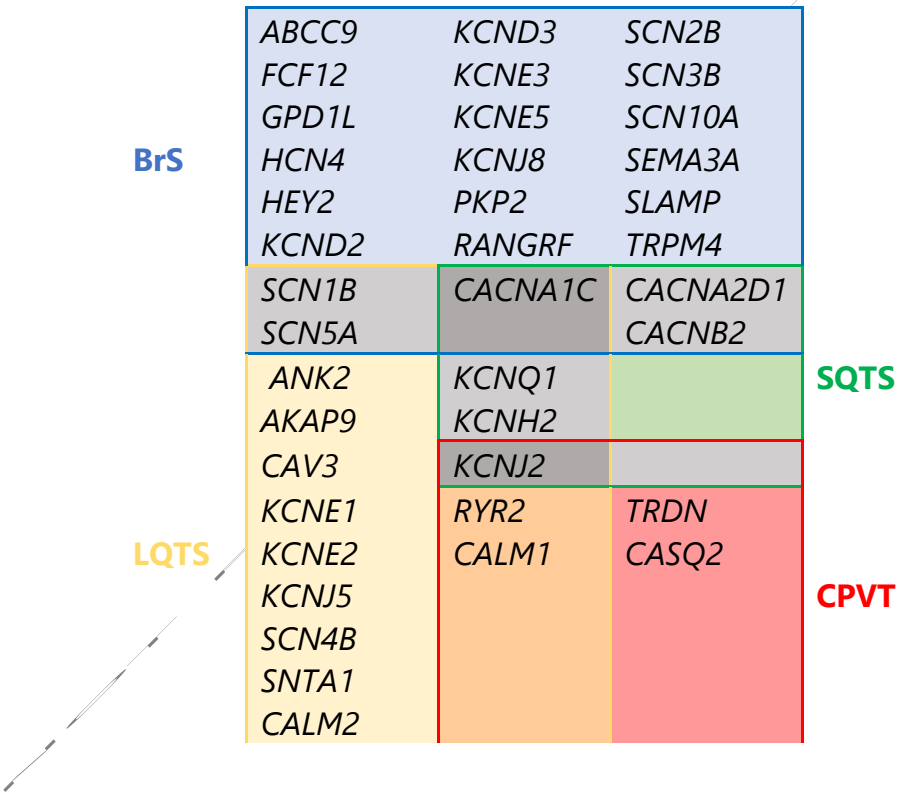
Abbreviations: AF – Atrial fibrillation; ARVC- Arrhythmogenic right ventricular cardiomyopathy; AV – Atrioventricular; BrS – Brugada syndrome; CCD – Cardiac conduction defect; CHD – Congenital heart defects; CPVT –Catecholaminergic polymorphic ventricular tachycardia; DCM – Dilated cardiomyopathy; EMD – Emery Dreifuss muscular dystrophy; ERS –Early repolarization syndrome; HCM – Hypertrophic cardiomyopathy; HCC -

Gene	Protein	Prevalence	Other Associations

histiocytoid cardiomyopathy; JLNS – Jervell and Lange-Nielsen syndrome; LGMD – Limb girdle muscular dystrophy; LQTS – Long QT syndrome; SIDS – Sudden infant death syndrome; SQTS – Short QT syndrome; SSS – Sick sinus syndrome; SUDS – Sudden unexpected death syndrome; VF – Ventricular fibrillation

The clinical presentations of these conditions overlap as shown below (adapted from Campuzano, 2015), and genetic testing may clarify diagnoses, etiologies, and treatments in symptomatic individuals (Spoonamore & Johnson, 2016). However, predicting clinical presentation based on genetic mutation is also challenging (Bezzina et al., 2015) due to the incomplete penetrance of most of these genes (Giudicessi & Ackerman, 2013).

Gene Mutations and their Associated Conditions



Proprietary Testing (Channelopathies)

Proprietary gene panels exist for the assessment of cardiac ion channelopathies. For example, GeneDX offers several customizable panels for various channelopathies and cardiomyopathies. Conditions such as ARVC, Brugada Syndrome, and CPVT are available as separate panels, and GeneDX offers combined panels such as a "Custom Arrhythmia Panel", a "Custom Cardiomyopathy Panel", and a "Combined Cardiac

Panel" (GeneDX, 2024). Other commercially available panels include offerings from Invitae (Invitae, 2024), Fulgent, (Fulgent, 2024) and BluePrint Genetics (BluePrint, 2024).

Analytical Validity (Channelopathies)

Ware et al. (2013) compared two NGS approaches for diagnostic sequencing inherited arrhythmia syndromes. PCR-based target enrichment and long-read sequencing (PCR-LR) was compared to in-solution hybridization-based enrichment and short-read sequencing (Hyb-SR). The PCR-LR assay comprehensively assessed five long QT genes (*KCNQ1, KCNH2, SCN5A, KCNE1* and *KCNE2*) and "hot spots" in *RYR2*. The Hyb-SR assay targeted 49 genes, including those in the PCR-LR assay. The sensitivity for detection of control variants was identical. In both assays, the major limitation was upstream target capture, particularly in regions of extreme GC content. These initial experiences with NGS cardiovascular diagnostics achieved up to 89% sensitivity at a fraction of current costs (Ware et al., 2013).

Proost et al. (2017) validated a targeted gene panel for next-generation sequencing of 51 genes associated with primary electrical disease with 20 Human Polymorphism Study Center samples and 19 positive control samples with a total of 1479 variants. "An analytical sensitivity and specificity of 100% and 99.9% were obtained." After validation, the assay was applied to "114 PED patients which identified 107 variants in 36 different genes, 18 of which were classified as pathogenic or likely pathogenic, 54 variants were of unknown significance, and 35 were classified as likely benign." They concluded "that the PED Multiplex Amplification of Specific Targets for Resequencing Plus assay is a proficient and highly reliable test to routinely screen patients experiencing primary arrhythmias" (Proost et al., 2017).

Clinical Utility and Validity (Channelopathies)

Garcia et al. (2016) proposed a framework for establishing clinical validity for assessing polymorphisms of inherited cardiac conditions, as well as evaluating the strength of association between genotype and phenotype, from a logical argument. Clinical validity of a gene is established when a gene is known to cause disease; since a specific variant must be responsible for causing the disease, a gene variant must be known to cause that disease. Conversely, if no variants can be established to cause disease, the clinical validity association has not been established. Variants of unknown significance (VUS) would, therefore, not have established clinical validity. Garcia et al. (2016) proposed three categories of strength of association: strong, suggested, and emerging.

- "Strong" refers to "cases where there exists at least one clinically observed variant supported by sufficient evidence to classify that variant as pathogenic. "Strong" indicates that the relationship has been proven."

- "Suggested" is used in cases where some preliminary evidence exists suggesting a causal relationship, but the relationship has not yet been formally proven."
- "Emerging" is used to describe a growing suspicion that a specific condition is caused by a gene that has already been proven to cause disease.

The authors go on to state that if one "strong" relationship exists, then clinical validity has been established. If not, the gene should be considered of "uncertain significance" (Garcia et al., 2016).

The authors also provide recommendations for gene panels to test for certain conditions. They "propose that a comprehensive panel test designed for the molecular diagnosis of a particular condition should include the following classes of genes:

- Genes that have been conclusively proven to cause the condition in question.
- Genes suspected but not yet proven to cause the condition in question.
- Genes that have been conclusively proven to cause a condition within the clinical differential. This category should include genes that cause a condition that can progress into the condition in question, genes that cause a condition that can be confused with the condition in question, and genes that cause a syndrome that include the condition in question as a primary feature.

They suggest that the clinical validity of a panel is established when that panel includes a set of genes that account for a substantial proportion of the genetic causes of the disease in question. Conversely, a panel is NOT valid if it omits certain genes that account for a substantial proportion of the known genetic risk. A clinically valid panel may also include genes for which some preliminary evidence of clinical validity exists ("preliminary evidence genes") (Garcia et al., 2016).

Hofman et al. (2013) analyzed the yield of DNA testing over 15 years. They analyzed results from 7021 individuals who were counseled, 6944 from 2298 different families (aged 41±19 years; 49% male). In 702 families (31%), a possible disease-causing mutation was detected. The yield of DNA testing of probands with primary electric diseases was 47% in LQTS, 26% in BrS, and 37% in CPVT. Cascade screening revealed 1539 mutation-positive subjects, and in 372 families counseled after sudden unexplained death an inherited arrhythmia syndrome was diagnosed in 29% (n=108) (Hofman et al., 2013).

Le Scouarnec et al. (2015) et al. examined 167 index cases presenting with a Brugada pattern on the electrocardiogram as well as 167 individuals aged over 65-years old and showing no history of cardiac arrhythmia. They found that "a significant enrichment in rare coding variation (with a minor allele frequency below 0.1%) was observed only for *SCN5A*, with rare coding variants carried by 20.4% of cases with BrS versus 2.4% of

control individuals. No significant enrichment was observed for any other arrhythmia-susceptibility gene, including *SCN10A* and *CACNA1C*. These results indicate that, except for *SCN5A*, rare coding variation in previously reported arrhythmia-susceptibility genes do not contribute significantly to the occurrence of BrS in a population with European ancestry. Extreme caution should thus be taken when interpreting genetic variation in molecular diagnostic setting, since rare coding variants were observed in a similar extent among cases versus controls, for most previously reported BrS-susceptibility genes" (Le Scouarnec et al., 2015).

Tester et al. (2012) examined 173 cases of sudden unexplained death that were referred for cardiac channel molecular autopsy. The mutational analysis included the long QT syndrome genes (*KCNQ1*, *KCNH2, SCN5A, KCNE1*, and *KCNE2*) and a catecholaminergic polymorphic ventricular tachycardia (CPVT) type 1–associated gene (*RYR2*). Overall, 45 putative pathogenic mutations absent in 400 to 700 controls were identified in 45 autopsy-negative SUD cases (26.0%) (Tester et al., 2012).

Seidelmann et al. (2017) evaluated the use of whole exome sequencing for clinical diagnosis, risk stratification, and management of inherited CVD. They found that genetic diagnosis was reached with a success rate of 26.5% (53/200 patients). This compares to 18% (36/200) that would have been diagnosed using commercially available genetic panels; although, this finding was not statistically significant. The authors concluded, "Whole exome sequencing was particularly useful for clinical diagnosis in patients with aborted sudden cardiac death, in whom the primary insult for the presence of both depressed cardiac function and prolonged QT had remained unknown. The analysis of the remaining cases using genome annotation and disease segregation led to the discovery of novel candidate genes in another 14% of the cases" (Seidelmann et al., 2017).

Munroe et al. (2018) examined tissue from 242 stillbirths (≥22 weeks), including those where no definite cause of death could be confirmed after a full autopsy. Seventy cases were examined, which were then sequenced for a custom panel of 35 genes. One putative pathogenic variant was found, and several novel variants of uncertain significance resulting in cardiac channelopathies was identified in some cases of otherwise unexplained stillbirth. The authors concluded "these variants may have a role in fetal demise" (Munroe et al., 2018).

Wang et al. (2017) examined the "genetic spectrum" of LVNC. The authors sequenced 73 cardiomyopathy-related genes in 102 patients, and 43 pathogenic variants were identified in 16 genes in 39 patients. Sarcomeric variants accounted for 63% of these variants whereas variants associated with channelopathies accounted for 12%. *MYH7* and *TAZ* pathogenic variants were the most common, and patients with pathogenic

variants showed more severe symptoms such as earlier age of onset (Wang et al., 2017).

van Lint et al. (2019) evaluated the detection rates for variants of unknown (class 3), likely (class 4), and certain (class 5) pathogenicity in cardiogenetic gene panels. The study included 936 patients that were evaluated with the arrhythmia panels (four versions), and 1970 patients were evaluated with the cardiomyopathy panels (six versions). The arrhythmia panels detected class 3 variants in 34.8% of patients, class 4 variants in 4.2% of patients, and class 5 variants in 4.6% of patients. The cardiomyopathy panels detected class 3 variants in 40.8% of patients, class 4 variants in 7.9% of patients, and class 5 variants in 12% of patients. Overall, the arrhythmia panels detected variants of interest in 44% of patients, and the cardiomyopathy panels detected variants of interest in 61% of patients. The authors concluded that "larger gene panels can increase the detection rate of likely pathogenic and pathogenic variants, but mainly increase the frequency of variants of unknown pathogenicity" (van Lint et al., 2019).

VI. Guidelines and Recommendations

American Heart Association, American College of Cardiology, and the Heart Rhythm Society

In 2017 (Al-Khatib Sana et al., 2018) the American Heart Association (AHA), the American College of Cardiology (ACC), and the Heart Rhythm Society (HRS) published the *Guideline for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death* that recommends the following:

Genetic Considerations in Arrhythmia Syndromes

- In patients and family members in whom genetic testing for risk stratification for SCA or SCD is recommended, genetic counseling is beneficial. (I)
- The diagnosis of most inherited arrhythmia syndromes is based on clinical features and family history. The availability of genetic testing for inherited arrhythmia syndromes can: 1) provide opportunity to confirm a suspected clinical diagnosis and sometimes provide prognostic information for the proband and 2) offer cascade screening of potentially affected family members when a disease-causing mutation is identified in the proband. The yield of genetic testing varies by disease.
- Genotyping is frequently most useful when a pathogenic mutation is identified in the proband, such that screening can be applied to relatives who are in a preclinical phase, allowing institution of lifestyle changes, therapy, or ongoing monitoring for those who are gene mutation-positive.

- In young patients (<40 years of age) without structural heart disease who have unexplained cardiac arrest, unexplained near drowning, or recurrent exertional syncope, genetic testing may be important to identify an inherited arrhythmia syndrome as a likely cause.

Arrhythmogenic Right Ventricular Cardiomyopathy

- "Selected first-degree relatives refers to relatives who are willing to undergo further testing and who could benefit from further screening and testing (and not the terminally ill patients or those who do not want to be screened and tested)."
- "Arrhythmogenic right ventricular cardiomyopathy is detected clinically in approximately 35% to 40% of first-degree relatives, most commonly in siblings or symptomatic first-degree relatives"
- "The proband with arrhythmogenic right ventricular cardiomyopathy is usually diagnosed by the presence of clinical symptoms along with the presence of arrhythmogenic right ventricular cardiomyopathy Task Force criteria"
- "A pathogenic genetic mutation was added to the major Task Force criteria in 2010. The yield of genetic testing in probands with suspected arrhythmogenic right ventricular cardiomyopathy is generally 30% to 54% and is up to 58% among patients with a strong family history of SCD in multiple members. A negative genetic test for arrhythmogenic right ventricular cardiomyopathy does not exclude the disease, and a positive genetic test currently does not guide therapy."

Hypertrophic Cardiomyopathy

- "In first-degree relatives of patients with HCM due to a known causative mutation, genetic counseling and mutation-specific genetic testing are recommended."
- "In patients with clinically suspected or diagnosed HCM, genetic counseling and genetic testing are reasonable."
- "When genetic testing reveals a mutation in the index patient, ascertainment of genetic status in first- and second-degree relatives can be predictive of risk for developing HCM. Relatives with overt HCM will have the same pathogenic HCM mutation as the index patient."

Cardiac Channelopathies

- In first-degree relatives of patients who have a causative mutation for long QT syndrome, catecholaminergic polymorphic ventricular tachycardia, short QT syndrome, or Brugada syndrome, genetic counseling and mutation-specific genetic testing are recommended (I)

- Clinical screening of first-degree relatives of patients with inherited arrhythmia syndromes is crucial to identifying affected family members. Due to the increased risk of adverse cardiac events in genotype-positive patients with long QT syndrome, catecholaminergic polymorphic ventricular tachycardia, and Brugada syndrome, targeted screening for the identified family-specific mutation can identify individuals who are at risk for these adverse outcomes

Congenital Long QT Syndrome

- In patients with clinically diagnosed long QT syndrome, genetic counseling and genetic testing are recommended (I)
- Genetic testing for disease-causing mutations in long QT syndrome offers important diagnostic, prognostic, and therapeutic information in addition to the clinical evaluation, and a positive test can facilitate establishing risk for family members. The yield of genetic testing in long QT syndrome phenotype-positive patients is 50% to 86%, with the higher range present in patients with marked QT prolongation or positive family history of SCD. A negative genetic test does not exclude the diagnosis of long QT syndrome, which relies on the clinical evaluation.

Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)

- In patients with catecholaminergic polymorphic ventricular tachycardia and with clinical VT or exertional syncope, genetic counseling and genetic testing are reasonable (IIa)
- Genetic testing may be useful to confirm the diagnosis of catecholaminergic polymorphic ventricular tachycardia, which is suggested by the development of bidirectional VT with exertion or stress. Recognition of catecholaminergic polymorphic ventricular tachycardia as the cause for exertional symptoms should prompt aggressive therapy to prevent the significant risk of SCD. Therapy for catecholaminergic polymorphic ventricular tachycardia is not guided by genotype status, but screening of first-degree relatives may be facilitated with genetic testing.

Brugada Syndrome

- In patients with suspected or established Brugada syndrome, genetic counseling and genetic testing may be useful to facilitate cascade screening of relatives (IIb)
- The yield of genetic testing in phenotype-positive patients is approximately 20% to 30% in Brugada syndrome. *SCN5A* variants account for most of this subset of genotype-positive Brugada syndrome. However, 2% to 10% of otherwise healthy individuals host a rare variant of *SCN5A*. A negative genetic test does not exclude

the diagnosis of Brugada syndrome, which is usually based on electrocardiographic and clinical characteristics. Risk stratification is based on symptoms and clinical findings; genotype status is not correlated with the risk of adverse events. Identification of a pathogenetic mutation may help facilitate recognition of carrier status in family members, allowing for lifestyle modification and potential treatment.

Short QT syndrome

- In patients with short QT syndrome, genetic testing may be considered to facilitate screening of first-degree relatives (IIb)
- Pathogenic mutations in potassium channels have been identified in approximately 10% to 20% of patients with short QT syndrome, including in *KCNH2* (SQT1), *KCNQ1* (SQT2), and *KCNJ2* (SQT3).

Early Repolarization "J-wave" Syndrome

- In patients with early repolarization pattern on ECG, genetic testing is not recommended (III-no benefit)

Postmortem Evaluation of SCD

- In first-degree relatives of SCD victims who were 40 years of age or younger, cardiac evaluation is recommended, with genetic counseling and genetic testing performed as indicated by clinical findings (I)
- For family risk profiling, it is important to use the disease-specific genetic test panel that corresponds to the autopsy findings. Risk profiling of family members of an SCD victim suspected of having an inherited cardiomyopathy at autopsy is important. Although phenotyping of surviving family members is crucial, genotyping of the SCD proband provides a mechanism for efficient follow-up evaluation of those relatives with the disease-causing mutation found in the proband (Al-Khatib Sana et al., 2018).

American Heart Association/American College of Cardiology/Heart Failure Society of America (AHA/ACC/HFSA) Guideline for the Management of Heart Failure

These 2022 guidelines provide guidelines on genetic evaluation and testing for the management of heart failure. The relevant recommendations are captured below:

- "In first-degree relatives of selected patients with genetic or inherited cardiomyopathies, genetic screening and counseling are recommended to

detect cardiac disease and prompt consideration of treatments to decrease HF progression and sudden death."

- "In select patients with nonischemic cardiomyopathy, referral for genetic counseling and testing is reasonable to identify conditions that could guide treatment for patients and family members."

These joint recommendations also urge that "In patients in whom a genetic or inherited cardiomyopathy is suspected, a family history should be performed, including at least 3 generations and ideally diagrammed as a family tree pedigree" (Heidenreich et al., 2022).

American College of Cardiology Foundation (ACCF)/American College of Cardiology (ACC) and American Heart Association (AHA)

In 2020, the AHA released a joint guideline with the ACC for the diagnosis and treatment of patients with hypertrophic cardiomyopathy (Ommen et al., 2020). This guideline replaced the 2011 guideline released jointly by the ACCF and the AHA. The updated 2020 guideline has also been endorsed by the Heart Rhythm Society (HRS). With relation to genetic testing, they released the following with a level B-NR quality of evidence, signifying "moderate-quality evidence from 1 or more well-designed, well-executed nonrandomized studies, observational studies, or registry studies; meta-analyses of such studies":

Class 1 (Benefit >>> Risk)

- "In patients with HCM, evaluation of familial inheritance, including a 3-generation family history, is recommended as part of the initial assessment
- In patients with HCM, genetic testing is beneficial to elucidate the genetic basis to facilitate the identification of family members at risk for developing HCM (cascade testing)
- In patients with an atypical clinical presentation of HCM or when another genetic condition is suspected to be the cause, a work-up including genetic testing for HCM and other genetic causes of unexplained cardiac hypertrophy ("HCM phenocopies") is recommended
- In patients with HCM who choose to undergo genetic testing, pre- and post-test genetic counseling by an expert in the genetics of cardiovascular disease is recommended so that risks, benefits, results, and their clinical significance can be reviewed and discussed with the patient in a shared decision-making process
- When performing genetic testing in an HCM proband, the initial tier of genes tested should include genes with strong evidence to be disease-causing in HCM*

- In first-degree relatives of patients with HCM, both clinical screening (ECG and 2D echocardiogram) and cascade genetic testing (when a pathogenic/likely pathogenic variant has been identified in the proband) should be offered
- In families where a sudden unexplained death has occurred with a postmortem diagnosis of HCM, postmortem genetic testing is beneficial to facilitate cascade genetic testing and clinical screening in first-degree relatives
- In patients with HCM who have undergone genetic testing, serial reevaluation of the variant(s) identified is recommended to assess for variant reclassification, which may impact diagnosis and cascade genetic testing in family members
- In affected families with HCM, preconception and pre-natal reproductive and genetic counseling should be offered"
- NOTE: "Strong evidence HCM genes include, at the time of this publication, *MYH7, MYBPC3, TNNI3, TNNT2, TPM1, MYL2, MYL3,* and *ACTC1*"

Class 2b (Benefit ≥ Risk)

- "In patients with HCM, the usefulness of genetic testing in the assessment of risk of SCD [sudden cardiac death] is uncertain
- In patients with HCM who harbor a variant of uncertain significance, the usefulness of clinical genetic testing of phenotype-negative relatives for the purpose of variant reclassification is uncertain"

No benefit

- "For patients with HCM who have undergone genetic testing and were found to have no pathogenic variants (i.e., harbor only benign/likely benign variants), cascade genetic testing of the family is not useful
- Ongoing clinical screening is not indicated in genotype-negative relatives in families with genotype-positive HCM, unless the disease-causing variant is downgraded to variant of uncertain significance, likely benign, or benign variant during follow-up" (Ommen et al., 2020).

The ACCF and AHA also published a joint guideline, regarding the "Management of Heart Failure", which states that "Increasingly, it is recognized that many (20% to 35%) patients with an idiopathic DCM have a familial cardiomyopathy (defined as 2 closely related family members who meet the criteria for idiopathic DCM)." "Advances in technology permitting high-throughput sequencing and genotyping at reduced costs have brought genetic screening to the clinical arena" and refers to the 2009 and 2011 published guidelines. The guidelines further note that genetic testing may be considered in conjunction with counseling in familial DCM (Yancy et al., 2013).

American Heart Association (AHA)

The AHA published a guideline regarding cardiomyopathy in children. In it, they state that "genetic testing should first be performed in the individual known to have a specific cardiomyopathy phenotype and should be informed by the child's overall presentation, with a detailed examination looking for dysmorphic features, muscle weakness, scoliosis, or specific laboratory findings." They also state that indications for genetic testing include "determining the cause of HCM, predicting the clinical course and severity, screening first-degree relatives, and determining recurrence risk."

This guideline addresses HCM, DCM, RCM, LVNC, Arrhythmogenic Ventricular Cardiomyopathy (both right and left ventricles), as well as cardiomyopathy caused by channelopathies such as Long-QT syndrome (Lipshultz et al., 2019). However, though it addresses the many manifestations of cardiomyopathy, few suggestions for clinical practice are made due to "the lack of evidence and consensus in the classification and diagnosis of children with cardiomyopathy," such that the document takes the form of a scientific statement instead of clinical guidelines.

In a 2023 scientific statement regarding cardiomyopathy in children, the AHA goes on to state that when "cardiomyopathy is identified in a child without a known preexisting risk, panel genetic testing or whole exome sequencing is recommended. A pathogenic or likely pathogenic variant in the proband should prompt cascade genetic testing of first-degree relatives who are at risk for cardiomyopathy" (Bogle et al., 2023).

The AHA also published a guideline discussing early repolarization. In it, they remark that the biological basis for early repolarization pattern "remains incompletely understood." Therefore, the AHA proposes "large-scale, unbiased (e.g., genome-wide association studies), family-guided genetic discovery approaches, as well as efforts aimed at understanding both the mechanisms underlying ER and the associated arrhythmogenesis" (Patton et al., 2016).

In a 2020 statement from the AHA titled "Genetic Testing for Inherited Cardiovascular Diseases: A Scientific Statement from the American Heart Association", the AHA pointed towards a 2018 conjoint guideline by the HFSA with the ACMG for guidance for genetic testing of cardiomyopathies. The pertinent recommendations were extracted and are presented below:

"A family history of at least 3 generations should be obtained for all patients with a primary cardiomyopathy. Second, clinical screening for cardiomyopathy is recommended for at-risk first-degree relatives. Third, patients with genetic, familial, or other unexplained forms of cardiomyopathy should be referred to expert centers. Genetic counseling is recommended for all patients with cardiomyopathy and their family members.

In a family, testing should be directed to the most clearly affected family member. If that individual is found to have a gene variant that is judged to be pathogenic or likely pathogenic, then cascade genetic testing for that variant should be offered to at-risk family members. For infants with cardiomyopathy, in addition to any routine newborn screening tests that might have been performed, the specialized evaluation is likely to include genetic testing and should also include an evaluation for syndromic or metabolic conditions for which a specific intervention or therapy might be warranted" (Musunuru et al., 2020).

Finally, the guideline notes that the management of certain conditions may benefit or otherwise be influenced by the results of genetic testing. Long-QT syndrome, HCM, DCM, and RCM are listed as having variants that may be specifically targeted with certain treatments (Musunuru et al., 2020).

Heart Failure Society of American and American College of Medical Genetics and Genomics (ACMG)

In 2018, the Heart Failure Society of America and the American College of Medical Genetics and Genomics published a clinical practice resource for the genetic evaluation of cardiomyopathy. There,

"Recommendation 1. Genetic testing is recommended for patients with cardiomyopathy

(a) Genetic testing is recommended for the most clearly affected family member.
(b) Cascade genetic testing of at-risk family members is recommended for pathogenic and likely pathogenic variants.
(c) In addition to routine newborn screening tests, specialized evaluation of infants with cardiomyopathy is recommended, and genetic testing should be considered."

On the point of whom to test, the societies have the following to say:

"To yield the most conclusive, informative results, diagnostic genetic testing is optimally initiated on a confirmed affected individual. Furthermore, as there are sometimes multiple genetic variants contributing to disease in a single family, the testing should ideally be initiated on the person who is most likely to harbor the disease-causing variant or variants. This is frequently the individual in the family with the most severe disease and/or the earliest disease onset. This is a well established principle in clinical genetics, as selecting the individual with the most evident disease increases the likelihood of finding a genetic cause. If the ideal person for initiation of genetic testing in a family is unavailable or unwilling to proceed, then comprehensive

genetic testing should be considered for another unequivocally affected family member."

As for when to test, ACMG "recommend[s] genetic testing at the time a new cardiomyopathy diagnosis is made, but it can be conducted at any time following diagnosis. Education and counseling regarding genetic testing options are a key component of the process. For those who have had genetic testing in the past, retesting may be appropriate if the previous testing produced negative or inconclusive results and the test's detection rate has improved. This latter point is particularly relevant for DCM as the gene panels have rapidly expanded (e.g., *TTN* and others) and are anticipated to continue" (Hershberger et al., 2018). A chart of selected genes implicated in various cardiomyopathies is included below.

Table 1 Selected genes in association with cardiomyopathy

	Core genes[a]	Estimates of genetic testing diagnostic yield	ACMG secondary findings gene list	Metabolic causes of cardiomyopathty	Examples of genetic syndromes
HCM	*MYH7, MYBPC3, TNNT2, TNNC1, TNNI3, TPM1, MYL2, MYL3, ACTC1, ACTN2, CSRP3, PLN, TTR, PRKAG2, LAMP2, GLA*	30–60%	*MYBPC3, MYH7, TNNT2, TNNI3, TPM1, MYL3, ACTC1, PRKAG2, GLA, MYL2, LMNA*	*GAA* (Pompe); Mitochondrial disease genes	RASopathies (e.g., Noonan syndrome, others); Friedreich ataxia
DCM	*TTN*[b], *LMNA, MYH7, TNNT2, BAG3, RBM20, TNNC1, TNNI3, TPM1, SCN5A, PLN*. For testing, all HCM, ARVC genes are recommended to be included	10–40%		Mitochondrial disease genes	Muscular dystrophies; Alström syndrome
ARVC	*DES, DSC2, DSG2, DSP, JUP, LMNA, PKP2, PLN, RYR2, SCN5A, TMEM43, TTN*[b]; consider full DCM panel	10–50%	*PKP2, DSP, DSC2, TMEM43, DSG2, RYR2 SCN5A*		Naxos syndrome; Carvajal syndrome
RCM	Consider HCM or DCM gene panel	10–60%			
LVNC	Use the gene panel for the cardiomyopathy identified in association with the LVNC phenotype	Unknown		Mitochondrial disease genes including *TAZ* in Barth syndrome	1p36 deletion syndrome; RASopathies

[a]Core gene lists represent genes with the highest diagnostic yield and/or strongest evidence of the gene in association with the listed phenotype; the genes listed are not exhaustive and should be considered illustrative for the type of cardiomyopathy. Considerable overlap of genes between cardiomyopathy phenotypes is well established. Genes known to cause metabolic disease or genetic syndromes are often included in testing panels, but vary depending on the clinical laboratory. Gene lists therefore need to be reviewed carefully before ordering testing. Metabolic and genetic syndrome columns provide examples only and are not intended to be comprehensive

[b]Only *TTN* truncating variants are thought relevant for cardiomyopathy

HCM hypertrophic cardiomyopathy, *DCM* dilated cardiomyopathy, *ARVC* arrhythmogenic right ventricular cardiomyopathy, *RCM* restrictive cardiomyopathy, *LVNC* left ventricular noncompaction, *ACMG* American College of Medical Genetics and Genomics

(Table 1 above appropriated from *(Hershberger et al., 2018)*)

The specific cardiomyopathy comments are as follows:

HCM

- "Beyond sarcomeric genes, core genes to screen in patients with HCM include *GLA, PRKAG2*, and *LAMP2*."
- "Consultation with a geneticist is indicated."

DCM

- "Core genes to be tested in individuals with DCM include genes encoding sarcomeric and cytoskeletal proteins."
- "Genetic testing is important in mothers of individuals with Duchenne or Becker to determine carrier status because carrier females may develop DCM in the third to fifth decade of life."

LVNC

- "Genetic testing is not recommended when the LVNC phenotype is identified serendipitously in asymptomatic individuals with otherwise normal cardiovascular structure and function."

As such, these genes may form an important backdrop upon which the following is performed:

"Recommendation 2. Focused cardiovascular phenotyping is recommended when pathogenic or likely pathogenic variants in cardiomyopathy genes, designated for reporting of secondary findings by the ACMG, are identified in an individual.

(a) If a cardiovascular phenotype is identified as would be predicted by currently available knowledge of the gene/ variant pair, all usual approaches described in this document for a genetic evaluation, including family-based approaches, are recommended.
(b) If no cardiovascular disease phenotype is identified in the individual, recommendations for surveillance screening at intervals should be considered.
(c) If no cardiovascular phenotype is identified in the individual, cascade evaluation of at-risk relatives may be considered, tempered by the strength of evidence supporting the pathogenicity of the variant, the usual age of onset of the gene/variant pair, and pedigree information (e.g., the ages of at-risk family members, other previously known cardiovascular clinical data in the pedigree, and related information)" (Hershberger et al., 2018).

American College of Medical Genetics and Genomics (ACMG)

In 2023 version 3.2, ACMG referenced several myopathies and channelopathies in their "minimum" list of genes for which mutations should be reported when whole genome sequencing is performed for other primary purposes (incidental findings). Those genes are listed below:

- *FBN1, TGFBR1, TGFBR2, SMAD3, ACTA2, MYH11* for aortopathies
- *PKP2, DSP, DSC2, TMEM43*, and *DSG2* for ARVC
- *RYR2, CASQ2,* and *TRDN* for Catecholaminergic polymorphic ventricular tachycardia (CPVT)
- *TNNT2, LMNA, FLNC, TTN, BAG3, DES, RBM20, and TNNC1* for DCM
- *COL3A1* for Ehlers-Danlos syndrome, vascular type
- *LDLR, APOB,* and *PCSK9* for Familial hypercholesterolemia
- *MYH7, MYBP3, TNNI3, TPM1, MYL3, ACTC1, PRKAG2,* and *MYL2* for HCM
- *KCNQ1* and *KCNH2* for Long QT syndrome types 1 and 2

- *SCN5A* for Long QT syndrome 3; Brugada syndrome
- *CALM1, CALM2, CALM3* for Long QT syndrome types 14-16 (Miller et al., 2023).

In an erratum published in 2021, the authors appended a series of new gene-phenotype pairings, each associated with the cardiovascular phenotype "Risk of sudden death with preventive interventions available": *CASQ2*/catecholaminergic polymorphic ventricular tachycardia (CPVT), *TRDN*/catecholaminergic polymorphic ventricular tachycardia (CPVT) & long QT syndrome, *FLNC*/cardiomyopathy, and *TTN*/cardiomyopathy (Miller et al., 2021).

The Heart Rhythm Society (HRS)/The European Heart Rhythm Association (EHRA) Expert Consensus Statement

Hypertrophic Cardiomyopathy (HCM)

Class I recommendations (is recommended):

- "Comprehensive or targeted (*MYBPC3, MYH7, TNNI3, TNNT2, TPM1*) HCM genetic testing is recommended for any patient in whom a cardiologist has established a clinical diagnosis of HCM based on examination of the patient's clinical history, family history, and electrocardiographic/echocardiographic phenotype."
- "Mutation-specific genetic testing is recommended for family members and appropriate relatives following the identification of the HCM-causative mutation in an index case."

Dilated Cardiomyopathy (DCM)

Class I recommendations:

- "Comprehensive or targeted (*LMNA* and *SCN5A*) DCM genetic testing is recommended for patients with DCM and significant cardiac conduction disease (i.e., first-, second-, or third- degree heart block) and/or with a family history of premature unexpected sudden death."
- "Mutation-specific testing is recommended for family members and appropriate relatives following the identification of a DCM-causative mutation in the index case."

Class IIa (can be useful) recommendations:

- Genetic testing can be useful for patients with familial DCM to confirm the diagnosis, to recognize those who are highest risk of arrhythmia and syndromic

features, to facilitate cascade screening within the family, and to help with family planning.

Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC)

Class I recommendations:

- "Mutation-specific genetic testing is recommended for family members and appropriate relatives following the identification of the ACM/ARVC- causative mutation in an index case."

Class II recommendations:

- "Comprehensive or targeted (*DSC2*, *DSG2*, *DSP*, *JUP*, *PKP2*, and *TMEM43*) ACM/ARVC genetic testing can be useful for patients satisfying task force diagnostic criteria for ACM/ARVC." (II-A, can be useful)
- "Genetic testing may be considered for patients with possible ACM/ARVC (1 major or 2 minor criteria) according to the 2010 task force criteria (European Heart Journal)." (II-B, may be considered)

Left Ventricular Noncompaction (LVNC)

Class I recommendations:

- "Mutation-specific genetic testing is recommended for family members and appropriate relatives following the identification of a LVNC-causative mutation in the index case."

Class IIa recommendations:

- LVNC genetic testing can be useful for patients in whom a cardiologist has established a clinical diagnosis of LVNC based on examination of the patient's clinical history, family history, and electrocardiographic/echocardiographic phenotype. (II-A)

Restrictive Cardiomyopathy (RCM)

Class I recommendations:

- "Mutation-specific genetic testing is recommended for family members and appropriate relatives following the identification of a RCM-causative mutation in the index case."

Class IIb (may be considered) recommendations:

- "RCM genetic testing may be considered for patients in whom a cardiologist has established a clinical index of suspicion for RCM based on examination of the patient's clinical history, family history, and electrocardiographic/echocardiographic phenotype."

Long-QT Syndrome

Class I recommendations:

- "Comprehensive or LQT1-3 (*KCNQ1, KCNH2*, and *SCN5A*) targeted LQTS genetic testing is recommended for any patient in whom a cardiologist has established a strong clinical index of suspicion for LQTS based on examination of the patient's clinical history, family history, and expressed electrocardiographic (resting 12-lead ECGs and/or provocative stress testing with exercise or catecholamine infusion) phenotype.
- Comprehensive or LQT1-3 (*KCNQ1, KCNH2*, and *SCN5A*) targeted LQTS genetic testing is recommended for any asymptomatic patient with QT prolongation in the absence of other clinical conditions that might prolong the QT interval (such as electrolyte abnormalities, hypertrophy, bundle branch block, etc., i.e., otherwise idiopathic) on serial 12-lead ECGs defined as QTc >480 ms (prepuberty) or >500 ms (adults).
- Mutation-specific genetic testing is recommended for family members and other appropriate relatives subsequently following the identification of the LQTS-causative mutation in an index case."

Class IIb recommendations:

- "Comprehensive or LQT1-3 (*KCNQ1, KCNH2,* and *SCN5A*) targeted LQTS genetic testing may be considered for any asymptomatic patient with otherwise idiopathic QTc values >460 ms (prepuberty) or >480 ms (adults) on serial 12-lead ECGs."

Catecholaminergic polymorphic ventricular tachycardia (CPVT)

Class I recommendations:

- Comprehensive or CPVT1 and CVPT2 (*RYR2* and *CASQ2*) targeted CPVT genetic testing is recommended for any patient in whom a cardiologist has established a clinical index of suspicion for CPVT based on examination of the patient's clinical history, family history, and expressed electrocardiographic phenotype during provocative stress testing with cycle, treadmill, or catecholamine infusion.

- Mutation-specific genetic testing is recommended for family members and appropriate relatives following the identification of the CPVT-causative mutation in an index case.

Brugada Syndrome

Class I recommendations:

- Mutation-specific genetic testing is recommended for family members and appropriate relatives following the identification of the BrS-causative mutation in an index case.

Class IIa recommendations:

- Comprehensive or BrS1 (*SCN5A*) targeted BrS genetic testing can be useful for any patient in whom a cardiologist has established a clinical index of suspicion for BrS based on examination of the patient's clinical history, family history, and expressed electrocardiographic (resting 12-lead ECGs and/or provocative drug challenge testing) phenotype

Genetic testing is not indicated in the setting of an isolated type 2 or type 3 Brugada ECG pattern.

Short QT syndrome

Class I recommendations:

- Mutation-specific genetic testing is recommended for family members and appropriate relatives following the identification of the SQTS-causative mutation in an index case.

Class IIb recommendations:

- Comprehensive or SQT1-3 (*KCNH2, KCNQ1*, and *KCNJ2*) targeted SQTS genetic testing may be considered for any patient in whom a cardiologist has established a strong clinical index of suspicion for SQTS based on examination of the patient's clinical history, family history, and electrocardiographic phenotype.

Sinus Node Dysfunction (SND)

- "In idiopathic sinus node dysfunction (SND), mutations in the cardiac pacemaker channel gene *HCN4* and in sodium channel genes can be identified in an unknown portion. However, because non-genetic causes appear more frequently

in idiopathic SND, genetic testing for idiopathic SND should be considered on an individual basis".

Progressive Cardiac Conduction Disease (CCD)

- "Genetic testing may be considered as part of the diagnostic evaluation for patients with either isolated CCD or CCD with concomitant congenital heart disease, especially when there is documentation of a positive family history of CCD."
- "Mutation-specific genetic testing is recommended for family members and appropriate relatives following the identification of the CCD-causative mutation in an index case."
- "Taken together, tiered genetic testing for patients with CCD and congenital heart disease or cardiomyopathies is useful because other cardiac and non-cardiac disease features may be present or may develop; individual genes should be considered after discussion of clinical features with an experienced cardiogenetic center".

Post-Mortem Genetic Testing in Sudden Unexpected Death Cases

- "In the setting of autopsy-negative SUDS, comprehensive or targeted (*RYR2, KCNQ1, KCNH2*, and *SCN5A*) ion channel genetic testing **may be considered** in an attempt to establish probable cause and manner of death and to facilitate the identification of potentially at-risk relatives and **is recommended** if circumstantial evidence points toward a clinical diagnosis of LQTS or CPVT specifically (such as emotional stress, acoustic trigger, drowning as the trigger of death)."
- "Mutation-specific genetic testing is recommended for family members and other appropriate relatives following the identification of a SUDS-causative mutation in the decedent" (Ackerman et al., 2011; M. J. Ackerman et al., 2011).

Heart Rhythm Society (HRS), European Heart Rhythm Association (EHRA), and Asia Pacific Heart Rhythm Society (APHRS)

In an international consensus statement released by the HRS, EHRA, and APHRS, the three medical societies provide expert recommendations for diagnosing "inherited primary arrhythmia syndromes." The pertinent guidelines are listed below:

- "Expert Consensus Recommendations on LQTS Diagnosis
  1. LQTS is diagnosed:
     a. In the presence of an LQTS risk score ≥3.5 in the absence of a secondary cause for QT prolongation *and/or*

b. In the presence of an unequivocally pathogenic mutation in one of the LQTS genes *or*
c. In the presence of a QT interval corrected for heart rate using Bazett's formula (QTc) ≥500 ms in repeated 12-lead electrocardiogram (ECG) and in the absence of a secondary cause for QT prolongation.

2. LQTS can be diagnosed in the presence of a QTc between 480-499 ms in repeated 12-lead ECGs in a patient with unexplained syncope in the absence of a secondary cause for QT prolongation and in the absence of a pathogenic mutation."

- "Expert Consensus Recommendations on Brugada Syndrome Diagnosis
  1. BrS *is diagnosed* in patients with ST-segment elevation with type 1 morphology ≥2 mm in ≥1 lead among the right precordial leads V1, V2, positioned in the 2nd, 3rd or 4th intercostal space occurring either spontaneously or after provocative drug test with intravenous administration of Class I antiarrhythmic drugs.
  2. BRS ***is diagnosed*** in patients with type 2 or type 3 ST-segment elevation in ≥1 lead among the right precordial leads V1, V2 positioned in the 2nd, 3rd or 4th intercostal space when a provocative test with intravenous administration of Class I antiarrhythmic drugs induces a **type I** ECG morphology."
- "Expert Consensus Recommendations on [Catecholaminergic Polymorphic Ventricular Tachycardia] Diagnosis
  1. CPVT ***is diagnosed*** in the presence of a structurally normal heart, normal ECG, and unexplained exercise or catecholamine-induced bidirectional VT or polymorphic ventricular premature beats or VT in an individual < 40 years of age.
  2. CPVT ***is diagnosed*** in patients (index case or family member) who have a pathogenic mutation.
  3. CPVT ***is diagnosed*** in family members of a CPVT index case with a normal heart who manifest exercise-induced premature ventricular contractions (PVCs) or bidirectional/ polymorphic VT.
  4. CPVT ***can be diagnosed*** in the presence of a structurally normal heart and coronary arteries, normal ECG, and unexplained exercise or catecholamine-induced bidirectional VT or polymorphic ventricular premature beats or VT in an individual >40 years of age."
- "Expert Consensus Recommendations on Short QT Syndrome Diagnosis
  1. SQTS ***is diagnosed*** in the presence of a QTc ≤330 ms.
  2. SQTS ***can be diagnosed*** in the presence of a QTc <360 ms and one or more of the following: a pathogenic mutation, family history of SQTS, family history of sudden death at age ≤40, survival of a VT/VF episode in the absence of heart disease."

- "Expert Consensus Recommendations on Early Repolarization Diagnosis
  1. ER ***syndrome is diagnosed*** in the presence of J-point elevation ≥1mm in ≥2 contiguous inferior and/or lateral leads of a standard 12-lead ECG in a patient resuscitated from otherwise unexplained VF/polymorphic VT.
  2. ER syndrome ***can be diagnosed*** in an SCD victim with a negative autopsy and medical chart review with a previous ECG demonstrating J-point elevation ≥1mm in ≥2 contiguous inferior and/or lateral leads of a standard 12-lead ECG
  3. ER syndrome ***can be diagnosed*** in the presence of J-point elevation ≥1mm in ≥2 contiguous inferior and/or lateral leads of a standard 12-lead ECG."
- "Expert Consensus Recommendations on Progressive Cardiac Conduction Disease Diagnosis
  1. Progressive cardiac conduction disease (PCCD) is diagnosed in the presence of unexplained progressive conduction abnormalities in young (<50 years) individuals with structurally normal hearts in the absence of skeletal myopathies, especially if there is a family history of PCCD."
- "Unexplained Cardiac Arrest:
  1. Expert Consensus Recommendations on Idiopathic Ventricular Fibrillation (IVF) Diagnosis
     a. IVF ***is defined*** as a resuscitated cardiac arrest victim, preferably with documentation of VF, in whom known cardiac, respiratory, metabolic and toxicological etiologies have been excluded through clinical evaluation.
  2. Expert Consensus Recommendations on Idiopathic Ventricular Fibrillation Evaluation
     a. **Class IIa** - Genetic testing in IVF ***can be useful*** when there is a suspicion of a specific genetic disease following clinical evaluation of the IVF patient and/or family members.
     b. **Class III** - Genetic screening of a large panel of genes in IVF patients in whom there is no suspicion of an inherited arrhythmogenic disease after clinical evaluation ***should not be performed***.
- "Unexplained Sudden Cardiac Death: Expert Consensus Recommendations on Sudden Unexplained Death Syndrome Evaluation
  1. Class I – Collection of blood and/or suitable tissue for molecular autopsy/postmortem genetic testing **is recommended** in all SUDS victims.
  2. Class IIa – An arrhythmia syndrome focused molecular autopsy/postmortem genetic testing **can be useful** for all SUDS victims" (Priori et al., 2013).

Heart Rhythm Society (HRS)

Arrhythmogenic Cardiomyopathies

The HRS published an "Expert Consensus Statement" on "Evaluation, Risk Stratification, and Management of Arrhythmogenic Cardiomyopathy." In it, they include recommendations on genetic testing for this set of cardiac disorders.

"For individuals and decedents with either a clinical or necropsy diagnosis of ACM, genetic testing of the established ACM-susceptibility genes is recommended."

"For genetic testing of the established ACM-susceptibility genes, comprehensive analysis of all established genes with full coverage is recommended."

"When a likely pathogenic or pathogenic genetic variant has been identified in the proband, cascade genetic testing can be offered to first-degree at-risk relatives… Cascade genetic testing is therefore only offered to family members where a likely pathogenic or pathogenic variant in a known disease-associated gene is identified in the proband…" (Towbin et al., 2019).

Canadian Cardiovascular Society/Canadian Heart Rhythm Society

Long QT Syndrome

Genetic testing is recommended in the cardiac arrest survivor with LQTS for the primary purpose of screening first-degree relatives, in the patient with syncope and QTc prolongation that is attributed to LQTS, and in the asymptomatic patient with consistent QTc prolongation that is clinically suspected to represent LQTS.

Brugada Syndrome

Genetic testing in the cardiac arrest survivor, patient with syncope, or asymptomatic patient with a persistent or provocable type 1 Brugada ECG pattern is recommended for the primary purpose of screening of family members. However, patients with types 2 or 3 Brugada ECG patterns are not recommended for genetic testing.

CPVT

Genetic testing is recommended for the primary purpose of screening family members (Gollob et al., 2011).

-

European Society of Cardiology (ECS)

Genetic counselling is recommended for all HCM patients when the HCM is not explained solely by a non-genetic cause.

Genetic testing is recommended for patients fulfilling the diagnostic criteria for HCM, both as a confirmatory test and to enable genetic testing for relatives. Both cascade genetic screening and a clinical evaluation are recommended for first-degree relatives that carry the same mutation as the HCM patient ("proband"). Even if a mutation is absent, relatives should consider reassessment should symptoms appear, or other clinical data emerges. A genetic analysis, such as pedigree analysis and high-throughput sequencing, should include the most implicated sarcomere protein genes. If a rarer condition is suspected, the analysis should include the gene responsible for that condition.

Pre-natal genetic testing is not recommended due to phenotypic variability (Elliott et al., 2014).

In 2023, the ESC released clinical practice guidelines delineating the management of cardiomyopathies. The pertinent recommendations from this update are listed below:

- "Recommendations for diagnostic work-up in cardiomyopathies:
  - It is recommended that all patients with suspected or established cardiomyopathy undergo systematic evaluation using a multiparametric approach that includes clinical evaluation, pedigree analysis, ECG, Holter monitoring, laboratory tests, and multimodality imaging (Class I recommendation, evidence level C)
  - It is recommended that all patients with suspected cardiomyopathy undergo evaluation of family history and that a three- to four-generation family tree is created to aid in diagnosis, provide clues to underlying aetiology, determine inheritance pattern, and identify at-risk relatives (Class I recommendation, evidence level C)"
- "Recommendations for genetic counselling and testing in cardiomyopathies
  - Genetic counselling, provided by an appropriately trained healthcare professional and including genetic education to inform decision-making and psychosocial support, is recommended for families with an inherited or suspected inherited cardiomyopathy, regardless of whether genetic testing is being considered (Class I recommendation, evidence level B)
  - It is recommended that genetic testing for cardiomyopathy is performed with access to a multidisciplinary team, including those with expertise in genetic testing methodology, sequence variant interpretation, and clinical application of genetic testing, typically in a specialized cardiomyopathy service or in a

network model with access to equivalent expertise (Class I recommendation, evidence level B)

- Pre- and post-test genetic counselling is recommended in all individuals undergoing genetic testing for cardiomyopathy (Class I recommendation, evidence level B)
- If pre-natal diagnostic testing is to be pursued by the family, it is recommended that this is performed early in pregnancy, to allow decisions regarding continuation or co-ordination of pregnancy to be made (Class I recommendation, evidence level C)
- For index patients:
  - Genetic testing is recommended in patients fulfilling diagnostic criteria for cardiomyopathy in cases where it enables diagnosis, prognostication, therapeutic stratification, or reproductive management of the patient, or where it enables cascade genetic evaluation of their relatives who would otherwise be enrolled into long-term surveillance (Class I recommendation, evidence level B)
  - Genetic testing is recommended for a deceased individual identified to have cardiomyopathy at post-mortem if a genetic diagnosis would facilitate management of surviving relatives (Class I recommendation, evidence level C)
  - Genetic testing may be considered in patients fulfilling diagnostic criteria for cardiomyopathy when it will have a net benefit to the patient, considering the psychological impact and preference, even if it does not enable diagnosis, prognostication, or therapeutic stratification, or cascade genetic screening of their relatives (Class IIb recommendation, evidence level C)
  - Genetic testing in patients with a borderline phenotype not fulfilling diagnostic criteria for a cardiomyopathy may be considered only after detailed assessment by specialist teams (Class IIb recommendation, evidence level C)
- For family members:
  - It is recommended that cascade genetic testing, with pre- and post-test counselling, is offered to adult at-risk relatives if a confident genetic diagnosis (i.e. a P/LP variant) has been established in an individual with cardiomyopathy in the family (starting with first-degree relatives if available, and cascading out sequentially) (Class I recommendation, evidence level B)
  - Cascade genetic testing with pre- and post-test counselling should be considered in paediatric at-risk relatives if a confident genetic diagnosis (i.e. a P/LP variant) has been established in an individual with

cardiomyopathy in the family (starting with first-degree relatives, if available, and cascading out sequentially), considering the underlying cardiomyopathy, expected age of onset, presentation in the family, and clinical/legal consequences (Class IIa recommendation, evidence level B)

- Testing for the presence of a familial variant of unknown significance, typically in parents and/or affected relatives, to determine if the variant segregates with the cardiomyopathy phenotype should be considered if this might allow the variant to be interpreted with confidence (Class IIa recommendation, evidence level C)
- Diagnostic genetic testing is not recommended in a phenotype-negative relative of a patient with cardiomyopathy in the absence of a confident genetic diagnosis (i.e. a P/LP variant) in the family (Class III recommendation, evidence level C)" (Arbelo et al., 2023).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81401	Molecular pathology procedure, Level 2 (e.g., 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)
81403	Molecular pathology procedure, Level 4 (e.g., analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more

	independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)
81404	Molecular pathology procedure, Level 5 (e.g., analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)
81405	Molecular pathology procedure, Level 6 (e.g., analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81406	Molecular pathology procedure, Level 7 (e.g., analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons, cytogenomic array analysis for neoplasia)
81407	Molecular pathology procedure, Level 8 (e.g., analysis of 26-50 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of >50 exons, sequence analysis of multiple genes on one platform)
81408	Molecular pathology procedure, Level 9 (e.g., analysis of >50 exons in a single gene by DNA sequence analysis)
81413	Cardiac ion channelopathies (e.g., Brugada syndrome, long QT syndrome, short QT syndrome, catecholaminergic polymorphic ventricular tachycardia); genomic sequence analysis panel, must include sequencing of at least 10 genes, including ANK2, CASQ2, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNQ1, RYR2, and SCN5A
81414	Cardiac ion channelopathies (e.g., Brugada syndrome, long QT syndrome, short QT syndrome, catecholaminergic polymorphic ventricular tachycardia); duplication/deletion gene analysis panel, must include analysis of at least 2 genes, including KCNH2 and KCNQ1
81439	Hereditary cardiomyopathy (e.g., hypertrophic cardiomyopathy, dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy), genomic sequence analysis panel, must include sequencing of at least 5 cardiomyopathy-related genes (e.g., DSG2, MYBPC3, MYH7, PKP2, TTN)
81479	Unlisted molecular pathology procedure
0237U	Cardiac ion channelopathies (e.g., Brugada syndrome, long QT syndrome, short QT syndrome, catecholaminergic polymorphic ventricular tachycardia), genomic sequence analysis panel including ANK2, CASQ2, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNQ1, RYR2, and SCN5A, including small sequence changes in exonic and intronic regions, deletions, duplications, mobile element insertions, and variants in non-uniquely mappable regions Proprietary test: Genomic Unity® Cardiac Ion Channelopathies Analysis Lab/Manufacturer: Variantyx Inc

S3861	Genetic testing, sodium channel, voltage-gated, type V, alpha subunit (SCN5A) and variants for suspected brugada syndrome
S3865	Comprehensive gene sequence analysis for hypertrophic cardiomyopathy
S3866	Genetic analysis for a specific gene mutation for hypertrophic cardiomyopathy (HCM) in an individual with a known HCM mutation in the family

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Ackerman, Hamilton, R., Hershberger, R. E., Le Marec, H., McKenna, W. J., Schulze-Bahr, E., Semsarian, C., Towbin, J. A., Watkins, H., Wilde, A., Priori, S. G., Wolpert, C., Zipes, D. P., Willems, S., Berul, C., Brugada, R., Calkins, H., Judge, D. P., Camm, A. J., . . . Gollob, M. (2011). HRS/EHRA Expert Consensus Statement on the State of Genetic Testing for the Channelopathies and Cardiomyopathies: This document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). *EP Europace*, *13*(8), 1077-1109. https://doi.org/10.1093/europace/eur245

Ackerman, M. J., Priori, S. G., Willems, S., Berul, C., Brugada, R., Calkins, H., Camm, A. J., Ellinor, P. T., Gollob, M., Hamilton, R., Hershberger, R. E., Judge, D. P., Le Marec, H., McKenna, W. J., Schulze-Bahr, E., Semsarian, C., Towbin, J. A., Watkins, H., Wilde, A., . . . Zipes, D. P. (2011). HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). *Heart Rhythm*, *8*(8), 1308-1339. https://doi.org/10.1016/j.hrthm.2011.05.020

AHA. (2024, May 28). *Dilated Cardiomyopathy (DCM)*. https://www.heart.org/en/health-topics/cardiomyopathy/what-is-cardiomyopathy-in-adults/dilated-cardiomyopathy-dcm

Al-Khatib Sana, M., Stevenson William, G., Ackerman Michael, J., Bryant William, J., Callans David, J., Curtis Anne, B., Deal Barbara, J., Dickfeld, T., Field Michael, E., Fonarow Gregg, C., Gillis Anne, M., Granger Christopher, B., Hammill Stephen, C., Hlatky Mark, A., Joglar José, A., Kay, G. N., Matlock Daniel, D., Myerburg Robert, J., & Page Richard, L. (2018). 2017 AHA/ACC/HRS Guideline for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death. *Circulation*, *138*(13), e272-e391. https://doi.org/10.1161/CIR.0000000000000549

Alcalai, R., Seidman, J. G., & Seidman, C. E. (2008). Genetic basis of hypertrophic cardiomyopathy: from bench to the clinics. *J Cardiovasc Electrophysiol*, *19*(1), 104-110. https://doi.org/10.1111/j.1540-8167.2007.00965.x

Ammash, N. M. (2024, January 10). *Restrictive cardiomyopathies*. https://www.uptodate.com/contents/restrictive-cardiomyopathies

Arbelo, E., Protonotarios, A., Gimeno, J. R., Arbustini, E., Barriales-Villa, R., Basso, C., Bezzina, C. R., Biagini, E., Blom, N. A., de Boer, R. A., De Winter, T., Elliott, P. M., Flather, M., Garcia-Pavia, P., Haugaa, K. H., Ingles, J., Jurcut, R. O., Klaassen, S., Limongelli, G., . . . Kaski, J. P. (2023). 2023 ESC Guidelines for the management of cardiomyopathies. *Eur Heart J*, *44*(37), 3503-3626. https://doi.org/10.1093/eurheartj/ehad194

Attenhofer-Jost, C., & Connolly, H. (2022, May 5). *Isolated left ventricular noncompaction in adults: Clinical manifestations and diagnosis*. https://www.uptodate.com/contents/isolated-left-ventricular-noncompaction-in-adults-clinical-manifestations-and-diagnosis

Bastiaenen, R., & Behr, E. R. (2011). Sudden death and ion channel disease: pathophysiology and implications for management. *Heart*, *97*(17), 1365-1372. https://doi.org/10.1136/hrt.2011.223883

Bezzina, C. R., Lahrouchi, N., & Priori, S. G. (2015). Genetics of Sudden Cardiac Death. https://doi.org/10.1161/CIRCRESAHA.116.304030

Bhonsale, A., Groeneweg, J. A., James, C. A., Dooijes, D., Tichnell, C., Jongbloed, J. D., Murray, B., te Riele, A. S., van den Berg, M. P., Bikker, H., Atsma, D. E., de Groot, N. M., Houweling, A. C., van der Heijden, J. F., Russell, S. D., Doevendans, P. A., van Veen, T. A., Tandri, H., Wilde, A. A., . . . Hauer, R. N. (2015). Impact of genotype on clinical course in arrhythmogenic right ventricular dysplasia/cardiomyopathy-associated mutation carriers. *Eur Heart J*, *36*(14), 847-855. https://doi.org/10.1093/eurheartj/ehu509

BluePrint. (2024). *Comprehensive Cardiology Panel*. Retrieved February 19 from https://blueprintgenetics.com/tests/panels/cardiology/comprehensive-cardiology-panel/

Bogle, C., Colan, S. D., Miyamoto, S. D., Choudhry, S., Baez-Hernandez, N., Brickler, M. M., Feingold, B., Lal, A. K., Lee, T. M., Canter, C. E., Lipshultz, S. E., on behalf of the American Heart Association Young Hearts Pediatric Heart, F., Transplantation Committee of the Council on Lifelong Congenital Heart, D., & Heart Health in the, Y. (2023). Treatment Strategies for Cardiomyopathy in Children: A Scientific Statement From the American Heart Association. *Circulation*, *148*(2), 174-195. https://doi.org/10.1161/CIR.0000000000001151

Campuzano, O., Sarquella-Brugada, G., Brugada, R., & Brugada, J. (2015). Genetics of channelopathies associated with sudden cardiac death. *Glob Cardiol Sci Pract*, *2015*(3), 39. https://doi.org/10.5339/gcsp.2015.39

Cirino, A. L., Ho, Carolyn. (2014). *Hypertrophic Cardiomyopathy Overview* [Text]. https://www.ncbi.nlm.nih.gov/books/NBK1768/

Cirino, A. L., Lakdawala, N. K., McDonough, B., Conner, L., Adler, D., Weinfeld, M., O'Gara, P., Rehm, H. L., Machini, K., Lebo, M., Blout, C., Green, R. C., MacRae, C. A., Seidman, C. E., & Ho, C. Y. (2017). A Comparison of Whole Genome Sequencing to Multigene Panel Testing in Hypertrophic Cardiomyopathy Patients. *Circ Cardiovasc Genet*, *10*(5). https://doi.org/10.1161/circgenetics.117.001768

Cooper Jr, L. T. (2022, August 16). *Definition and classification of the cardiomyopathies*. https://www.uptodate.com/contents/definition-and-classification-of-the-cardiomyopathies

Elliott, P. M., Anastasakis, A., Borger, M. A., Borggrefe, M., Cecchi, F., Charron, P., Hagege, A. A., Lafont, A., Limongelli, G., Mahrholdt, H., McKenna, W. J., Mogensen, J., Nihoyannopoulos, P., Nistri, S., Pieper, P. G., Pieske, B., Rapezzi, C., Rutten, F. H., Tillmanns, C., & Watkins, H. (2014). 2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy: the Task Force for the Diagnosis and Management of Hypertrophic Cardiomyopathy of the European Society of Cardiology (ESC). *Eur Heart J*, *35*(39), 2733-2779. https://doi.org/10.1093/eurheartj/ehu284

Fernandez-Falgueras, A., Sarquella-Brugada, G., Brugada, J., Brugada, R., & Campuzano, O. (2017). Cardiac Channelopathies and Sudden Death: Recent Clinical and Genetic Advances. *Biology (Basel)*, *6*(1). https://doi.org/10.3390/biology6010007

Frustaci, A., De Luca, A., Guida, V., Biagini, T., Mazza, T., Gaudio, C., Letizia, C., Russo, M. A., Galea, N., & Chimenti, C. (2018). Novel alpha-Actin Gene Mutation p.(Ala21Val) Causing Familial Hypertrophic Cardiomyopathy, Myocardial Noncompaction, and Transmural Crypts. Clinical-Pathologic Correlation. *J Am Heart Assoc*, *7*(4). https://doi.org/10.1161/jaha.117.008068

Fulgent. (2024). *Hypertrophic Cardiomyopathy NGS Panel*. https://www.fulgentgenetics.com/hypertrophic-cardiomyopathy

Garcia-Elias, A., & Benito, B. (2018). Ion Channel Disorders and Sudden Cardiac Death [Review]. *International Journal of Molecular Sciences*, *19*(3), 692. https://doi.org/10.3390/ijms19030692

Garcia, J., Tahiliani, J., Johnson, N. M., Aguilar, S., Beltran, D., Daly, A., Decker, E., Haverfield, E., Herrera, B., Murillo, L., Nykamp, K., & Topper, S. (2016). Clinical Genetic Testing for the Cardiomyopathies and Arrhythmias: A Systematic Framework for Establishing Clinical Validity and Addressing Genotypic and Phenotypic Heterogeneity. *Front Cardiovasc Med*, *3*, 20. https://doi.org/10.3389/fcvm.2016.00020

GeneDX. (2024). *GeneDx Testing Directory*. Retrieved 1/14/21 from https://www.genedx.com/

Gersh, B. J., Maron, B. J., Bonow, R. O., Dearani, J. A., Fifer, M. A., Link, M. S., Naidu, S. S., Nishimura, R. A., Ommen, S. R., Rakowski, H., Seidman, C. E., Towbin, J. A., Udelson, J. E., & Yancy, C. W. (2011). 2011 ACCF/AHA guideline for the diagnosis and treatment of hypertrophic cardiomyopathy: a report of the American College of Cardiology

Foundation/American Heart Association Task Force on Practice Guidelines. *J Thorac Cardiovasc Surg*, *142*(6), e153-203. https://doi.org/10.1016/j.jtcvs.2011.10.020

Giudicessi, J. R., & Ackerman, M. J. (2013). Determinants of incomplete penetrance and variable expressivity in heritable cardiac arrhythmia syndromes. *Transl Res*, *161*(1), 1-14. https://doi.org/10.1016/j.trsl.2012.08.005

Gollob, M. H., Blier, L., Brugada, R., Champagne, J., Chauhan, V., Connors, S., Gardner, M., Green, M. S., Gow, R., Hamilton, R., Harris, L., Healey, J. S., Hodgkinson, K., Honeywell, C., Kantoch, M., Kirsh, J., Krahn, A., Mullen, M., Parkash, R., . . . Woo, A. (2011). Recommendations for the use of genetic testing in the clinical evaluation of inherited cardiac arrhythmias associated with sudden cardiac death: Canadian Cardiovascular Society/Canadian Heart Rhythm Society joint position paper. *Can J Cardiol*, *27*(2), 232-245. https://doi.org/10.1016/j.cjca.2010.12.078

Grondin, S., Davies, B., Cadrin-Tourigny, J., Steinberg, C., Cheung, C. C., Jorda, P., Healey, J. S., Green, M. S., Sanatani, S., Alqarawi, W., Angaran, P., Arbour, L., Antiperovitch, P., Khan, H., Leather, R., Guerra, P. G., Rivard, L., Simpson, C. S., Gardner, M., . . . Tadros, R. (2022). Importance of genetic testing in unexplained cardiac arrest. *Eur Heart J*, *43*(32), 3071-3081. https://doi.org/10.1093/eurheartj/ehac145

Heidenreich, P. A., Bozkurt, B., Aguilar, D., Allen, L. A., Byun, J. J., Colvin, M. M., Deswal, A., Drazner, M. H., Dunlay, S. M., Evers, L. R., Fang, J. C., Fedson, S. E., Fonarow, G. C., Hayek, S. S., Hernandez, A. F., Khazanie, P., Kittleson, M. M., Lee, C. S., Link, M. S., . . . Yancy, C. W. (2022). 2022 AHA/ACC/HFSA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. *Circulation*, *145*(18), e895-e1032. https://doi.org/10.1161/cir.0000000000001063

Hershberger, R. E. (2023, April 17). *Familial dilated cardiomyopathy: Prevalence, diagnosis and treatment*. https://www.uptodate.com/contents/familial-dilated-cardiomyopathy-prevalence-diagnosis-and-treatment

Hershberger, R. E., Givertz, M. M., Ho, C. Y., Judge, D. P., Kantor, P. F., McBride, K. L., Morales, A., Taylor, M. R. G., Vatta, M., & Ware, S. M. (2018). Genetic evaluation of cardiomyopathy: a clinical practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. https://doi.org/10.1038/s41436-018-0039-z

Hershberger, R. E., Lindenfeld, J., Mestroni, L., Seidman, C. E., Taylor, M. R., & Towbin, J. A. (2009). Genetic evaluation of cardiomyopathy--a Heart Failure Society of America practice guideline. *J Card Fail*, *15*(2), 83-97. https://doi.org/10.1016/j.cardfail.2009.01.006

Hershberger, R. E., Morales, A., & Siegfried, J. D. (2010). Clinical and genetic issues in dilated cardiomyopathy: a review for genetics professionals. *Genet Med*, *12*(11), 655-667. https://doi.org/10.1097/GIM.0b013e3181f2481f

Hershberger, R. E., & Siegfried, J. D. (2011). Update 2011: clinical and genetic issues in familial dilated cardiomyopathy. *J Am Coll Cardiol*, *57*(16), 1641-1649. https://doi.org/10.1016/j.jacc.2011.01.015

Hofman, N., Tan, H. L., Alders, M., Kolder, I., de Haij, S., Mannens, M. M., Lombardi, M. P., Dit Deprez, R. H., van Langen, I., & Wilde, A. A. (2013). Yield of molecular and clinical testing for arrhythmia syndromes: report of 15 years' experience. *Circulation*, *128*(14), 1513-1521. https://doi.org/10.1161/circulationaha.112.000091

Invitae. (2024). *Invitae Cardiomyopathy Comprehensive Panel*. https://www.invitae.com/en/physician/tests/02251/

Japp, A. G., Gulati, A., Cook, S. A., Cowie, M. R., & Prasad, S. K. (2016). The Diagnosis and Evaluation of Dilated Cardiomyopathy. *J Am Coll Cardiol*, *67*(25), 2996-3010. https://doi.org/10.1016/j.jacc.2016.03.590

Kayvanpour, E., Sedaghat-Hamedani, F., Amr, A., Lai, A., Haas, J., Holzer, D. B., Frese, K. S., Keller, A., Jensen, K., Katus, H. A., & Meder, B. (2017). Genotype-phenotype associations in dilated cardiomyopathy: meta-analysis on more than 8000 individuals. *Clin Res Cardiol*, *106*(2), 127-139. https://doi.org/10.1007/s00392-016-1033-6

Kirk, J. A., & Kass, D. A. (2015). Cellular and Molecular Aspects of Dyssynchrony and Resynchronization. *Card Electrophysiol Clin*, *7*(4), 585-597. https://doi.org/10.1016/j.ccep.2015.08.011

Kostareva, A., Kiselev, A., Gudkova, A., Frishman, G., Ruepp, A., Frishman, D., Smolina, N., Tarnovskaya, S., Nilsson, D., Zlotina, A., Khodyuchenko, T., Vershinina, T., Pervunina, T., Klyushina, A., Kozlenok, A., Sjoberg, G., Golovljova, I., Sejersen, T., & Shlyakhto, E. (2016). Genetic Spectrum of Idiopathic Restrictive Cardiomyopathy Uncovered by Next-Generation Sequencing. *PLoS One*, *11*(9), e0163362. https://doi.org/10.1371/journal.pone.0163362

Le Scouarnec, S., Karakachoff, M., Gourraud, J. B., Lindenbaum, P., Bonnaud, S., Portero, V., Duboscq-Bidot, L., Daumy, X., Simonet, F., Teusan, R., Baron, E., Violleau, J., Persyn, E., Bellanger, L., Barc, J., Chatel, S., Martins, R., Mabo, P., Sacher, F., . . . Redon, R. (2015). Testing the burden of rare variation in arrhythmia-susceptibility genes provides new insights into molecular diagnosis for Brugada syndrome. *Hum Mol Genet*, *24*(10), 2757-2763. https://doi.org/10.1093/hmg/ddv036

Lipshultz, S., E., Law Yuk, M., Asante-Korang, A., Austin Eric, D., Dipchand Anne, I., Everitt Melanie, D., Hsu Daphne, T., Lin Kimberly, Y., Price Jack, F., Wilkinson James, D., Colan Steven, D., & null, n. (2019). Cardiomyopathy in Children: Classification and Diagnosis: A Scientific Statement From the American Heart Association. *Circulation*, *140*(1), e9-e68. https://doi.org/10.1161/CIR.0000000000000682

Lipshultz Steven, E., Law Yuk, M., Asante-Korang, A., Austin Eric, D., Dipchand Anne, I., Everitt Melanie, D., Hsu Daphne, T., Lin Kimberly, Y., Price Jack, F., Wilkinson James, D., Colan Steven, D., & null, n. (2019). Cardiomyopathy in Children: Classification and

Diagnosis: A Scientific Statement From the American Heart Association. *Circulation*, *140*(1), e9-e68. https://doi.org/10.1161/CIR.0000000000000682

Magi, S., Lariccia, V., Maiolino, M., Amoroso, S., & Gratteri, S. (2017). Sudden cardiac death: focus on the genetics of channelopathies and cardiomyopathies. *J Biomed Sci*, *24*. https://doi.org/10.1186/s12929-017-0364-6

Maron, B. J. (2003). Sudden death in young athletes. *N Engl J Med*, *349*(11), 1064-1075. https://doi.org/10.1056/NEJMra022783

Maron, B. J., Gardin, J. M., Flack, J. M., Gidding, S. S., Kurosaki, T. T., & Bild, D. E. (1995). Prevalence of hypertrophic cardiomyopathy in a general population of young adults. Echocardiographic analysis of 4111 subjects in the CARDIA Study. Coronary Artery Risk Development in (Young) Adults. *Circulation*, *92*(4), 785-789. https://doi.org/10.1161/01.CIR.92.4.785

Maron, B. J., Maron, M. S., & Semsarian, C. (2012). Genetics of hypertrophic cardiomyopathy after 20 years: clinical perspectives. *J Am Coll Cardiol*, *60*(8), 705-715. https://doi.org/10.1016/j.jacc.2012.02.068

Maron, M. S. (2022, October 4). *Hypertrophic cardiomyopathy: Clinical manifestations, diagnosis, and evaluation*. https://www.uptodate.com/contents/hypertrophic-cardiomyopathy-clinical-manifestations-diagnosis-and-evaluation

Martin, C. A., Huang, C. L., & Matthews, G. D. (2013). The role of ion channelopathies in sudden cardiac death: implications for clinical practice. *Ann Med*, *45*(4), 364-374. https://doi.org/10.3109/07853890.2013.783994

McKenna, W. J. (2024, October 8). *Arrhythmogenic right ventricular cardiomyopathy: Pathogenesis and genetics*. https://www.uptodate.com/contents/arrhythmogenic-right-ventricular-cardiomyopathy-pathogenesis-and-genetics

Miller, D. T., Lee, K., Abul-Husn, N. S., Amendola, L. M., Brothers, K., Chung, W. K., Gollob, M. H., Gordon, A. S., Harrison, S. M., Hershberger, R. E., Klein, T. E., Richards, C. S., Stewart, D. R., & Martin, C. L. (2023). ACMG SF v3.2 list for reporting of secondary findings in clinical exome and genome sequencing: A policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genetics in Medicine*, *25*(8). https://doi.org/10.1016/j.gim.2023.100866

Miller, D. T., Lee, K., Chung, W. K., Gordon, A. S., Herman, G. E., Klein, T. E., Stewart, D. R., Amendola, L. M., Adelman, K., Bale, S. J., Gollob, M. H., Harrison, S. M., Hershberger, R. E., McKelvey, K., Richards, C. S., Vlangos, C. N., Watson, M. S., & Martin, C. L. (2021). Correction to: ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *23*(8), 1582-1584. https://doi.org/10.1038/s41436-021-01278-8

Munroe, P. B., Addison, S., Abrams, D. J., Sebire, N. J., Cartwright, J., Donaldson, I., Cohen, M. M., Mein, C., Tinker, A., Harmer, S. C., Aziz, Q., Terry, A., Struebig, M., Warren, H. R., Vadgama, B., Fowler, D. J., Peebles, D., Taylor, A. M., Lally, P. J., & Thayyil, S. (2018).

Postmortem Genetic Testing for Cardiac Ion Channelopathies in Stillbirths. https://doi.org/10.1161/CIRCGEN.117.001817

Musunuru, K., Hershberger, R. E., Day, S. M., Klinedinst, N. J., Landstrom, A. P., Parikh, V. N., Prakash, S., Semsarian, C., & Sturm, A. C. (2020). Genetic Testing for Inherited Cardiovascular Diseases: A Scientific Statement From the American Heart Association. *Circulation: Genomic and Precision Medicine*, *13*(4), e000067. https://doi.org/doi:10.1161/HCG.0000000000000067

Nerbonne, J. M., & Kass, R. S. (2005). Molecular physiology of cardiac repolarization. *Physiol Rev*, *85*(4), 1205-1253. https://doi.org/10.1152/physrev.00002.2005

Ommen, S. R., Mital, S., Burke, M. A., Day, S. M., Deswal, A., Elliott, P., Evanovich, L. L., Hung, J., Joglar, J. A., Kantor, P., Kimmelstiel, C., Kittleson, M., Link, M. S., Maron, M. S., Martinez, M. W., Miyake, C. Y., Schaff, H. V., Semsarian, C., & Sorajja, P. (2020). 2020 AHA/ACC Guideline for the Diagnosis and Treatment of Patients With Hypertrophic Cardiomyopathy: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. *Circulation*, *142*(25), e558-e631. https://doi.org/10.1161/cir.0000000000000937

Parker, L. E., & Landstrom, A. P. (2021). The clinical utility of pediatric cardiomyopathy genetic testing: From diagnosis to a precision medicine-based approach to care. *Prog Pediatr Cardiol*, *62*. https://doi.org/10.1016/j.ppedcard.2021.101413

Patton, K. K., Ellinor, P. T., Ezekowitz, M., Kowey, P., Lubitz, S. A., Perez, M., Piccini, J., Turakhia, M., Wang, P., & Viskin, S. (2016). Electrocardiographic Early Repolarization: A Scientific Statement From the American Heart Association. *Circulation*, *133*(15), 1520-1529. https://doi.org/10.1161/cir.0000000000000388

Priori, S. G., Wilde, A. A., Horie, M., Cho, Y., Behr, E. R., Berul, C., Blom, N., Brugada, J., Chiang, C. E., Huikuri, H., Kannankeril, P., Krahn, A., Leenhardt, A., Moss, A., Schwartz, P. J., Shimizu, W., Tomaselli, G., & Tracy, C. (2013). HRS/EHRA/APHRS expert consensus statement on the diagnosis and management of patients with inherited primary arrhythmia syndromes: document endorsed by HRS, EHRA, and APHRS in May 2013 and by ACCF, AHA, PACES, and AEPC in June 2013. *Heart Rhythm*, *10*(12), 1932-1963. https://doi.org/10.1016/j.hrthm.2013.05.014

Proost, D., Saenen, J., Vandeweyer, G., Rotthier, A., Alaerts, M., Van Craenenbroeck, E. M., Van Crombruggen, J., Mortier, G., Wuyts, W., Vrints, C., Del Favero, J., Loeys, B., & Van Laer, L. (2017). Targeted Next-Generation Sequencing of 51 Genes Involved in Primary Electrical Disease. *J Mol Diagn*, *19*(3), 445-459. https://doi.org/10.1016/j.jmoldx.2017.01.010

Roden, D. M., Balser, J. R., George, A. L., Jr., & Anderson, M. E. (2002). Cardiac ion channels. *Annu Rev Physiol*, *64*, 431-475. https://doi.org/10.1146/annurev.physiol.64.083101.145105

Schwartz, P. J., & Crotti, L. (2011). QTc behavior during exercise and genetic testing for the long-QT syndrome. *Circulation*, *124*(20), 2181-2184. https://doi.org/10.1161/circulationaha.111.062182

Schwartz, P. J., Stramba-Badiale, M., Crotti, L., Pedrazzini, M., Besana, A., Bosi, G., Gabbarini, F., Goulene, K., Insolia, R., Mannarino, S., Mosca, F., Nespoli, L., Rimini, A., Rosati, E., Salice, P., & Spazzolini, C. (2009). Prevalence of the congenital long-QT syndrome. *Circulation*, *120*(18), 1761-1767. https://doi.org/10.1161/circulationaha.109.863209

Seidelmann, S. B., Smith, E., Subrahmanyan, L., Dykas, D., Abou Ziki, M. D., Azari, B., Hannah-Shmouni, F., Jiang, Y., Akar, J. G., Marieb, M., Jacoby, D., Bale, A. E., Lifton, R. P., & Mani, A. (2017). Application of Whole Exome Sequencing in the Clinical Diagnosis and Management of Inherited Cardiovascular Diseases in Adults. *Circ Cardiovasc Genet*, *10*(1). https://doi.org/10.1161/circgenetics.116.001573

Semsarian, C., Ingles, J., Maron, M. S., & Maron, B. J. (2015). New perspectives on the prevalence of hypertrophic cardiomyopathy. *J Am Coll Cardiol*, *65*(12), 1249-1254. https://doi.org/10.1016/j.jacc.2015.01.019

Spoonamore, K. G., & Johnson, N. M. (2016). Who Pays? Coverage Challenges for Cardiovascular Genetic Testing in U.S. Patients. *Front Cardiovasc Med*, *3*. https://doi.org/10.3389/fcvm.2016.00014

Tester, D. J., & Ackerman, M. J. (2011). Genetic Testing for Potentially Lethal, Highly Treatable Inherited Cardiomyopathies/Channelopathies in Clinical Practice. *Circulation*, *123*(9), 1021-1037. https://doi.org/10.1161/circulationaha.109.914838

Tester, D. J., Medeiros-Domingo, A., Will, M. L., Haglund, C. M., & Ackerman, M. J. (2012). Cardiac Channel Molecular Autopsy: Insights From 173 Consecutive Cases of Autopsy-Negative Sudden Unexplained Death Referred for Postmortem Genetic Testing. In *Mayo Clin Proc* (Vol. 87, pp. 524-539). https://doi.org/10.1016/j.mayocp.2012.02.017

Towbin, J. A., McKenna, W. J., Abrams, D. J., Ackerman, M. J., Calkins, H., Darrieux, F. C. C., Daubert, J. P., de Chillou, C., DePasquale, E. C., Desai, M. Y., Estes, N. A. M., 3rd, Hua, W., Indik, J. H., Ingles, J., James, C. A., John, R. M., Judge, D. P., Keegan, R., Krahn, A. D., . . . Zareba, W. (2019). 2019 HRS expert consensus statement on evaluation, risk stratification, and management of arrhythmogenic cardiomyopathy. *Heart Rhythm*, *16*(11), e301-e372. https://doi.org/10.1016/j.hrthm.2019.05.007

van Lint, F. H. M., Mook, O. R. F., Alders, M., Bikker, H., Lekanne Dit Deprez, R. H., & Christiaans, I. (2019). Large next-generation sequencing gene panels in genetic heart disease: yield of pathogenic variants and variants of unknown significance. *Neth Heart J*, *27*(6), 304-309. https://doi.org/10.1007/s12471-019-1250-5

Voorhees, A. P., & Han, H. C. (2015). Biomechanics of Cardiac Function. *Compr Physiol*, *5*(4), 1623-1644. https://doi.org/10.1002/cphy.c140070

Walsh, R., Buchan, R., Wilk, A., John, S., Felkin, L. E., Thomson, K. L., Chiaw, T. H., Loong, C. C. W., Pua, C. J., Raphael, C., Prasad, S., Barton, P. J., Funke, B., Watkins, H., Ware, J. S., & Cook, S. A. (2017). Defining the genetic architecture of hypertrophic cardiomyopathy: re-evaluating the role of non-sarcomeric genes. *Eur Heart J*, *38*(46), 3461-3468. https://doi.org/10.1093/eurheartj/ehw603

Wang, C., Hata, Y., Hirono, K., Takasaki, A., Ozawa, S. W., Nakaoka, H., Saito, K., Miyao, N., Okabe, M., Ibuki, K., Nishida, N., Origasa, H., Yu, X., Bowles, N. E., & Ichida, F. (2017). A Wide and Specific Spectrum of Genetic Variants and Genotype-Phenotype Correlations Revealed by Next-Generation Sequencing in Patients with Left Ventricular Noncompaction. *J Am Heart Assoc*, *6*(9). https://doi.org/10.1161/jaha.117.006210

Ware, J. S., John, S., Roberts, A. M., Buchan, R., Gong, S., Peters, N. S., Robinson, D. O., Lucassen, A., Behr, E. R., & Cook, S. A. (2013). Next generation diagnostics in inherited arrhythmia syndromes : a comparison of two approaches. *J Cardiovasc Transl Res*, *6*(1), 94-103. https://doi.org/10.1007/s12265-012-9401-8

Wilde, A. A. M., Veldkamp, M. W., & Smits, J. P. P. (2005). Mechanisms of inherited cardiac conduction disease. *EP Europace*, *7*(2), 122-137. https://doi.org/10.1016/j.eupc.2004.11.004

Yancy, C. W., Jessup, M., Bozkurt, B., Butler, J., Casey, D. E., Jr., Drazner, M. H., Fonarow, G. C., Geraci, S. A., Horwich, T., Januzzi, J. L., Johnson, M. R., Kasper, E. K., Levy, W. C., Masoudi, F. A., McBride, P. E., McMurray, J. J., Mitchell, J. E., Peterson, P. N., Riegel, B., . . . Wilkoff, B. L. (2013). 2013 ACCF/AHA guideline for the management of heart failure: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*, *62*(16), e147-239. https://doi.org/10.1016/j.jacc.2013.05.019

Genetic Testing for Lactase Insufficiency

Policy Number: AHS – M2080 – Genetic Testing for Lactase Insufficiency	Prior Policy Name and Number, as applicable:
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Lactose intolerance (LI) is a common clinical syndrome defined by abdominal pain, flatulence, bloating, borborygmus and osmotic diarrhea; LI is caused by the breakdown of nondigested lactose by the gut microflora (Ponte et al., 2016a).

Lactose malabsorption (LM) is the non-digestion of lactose caused by low expression of the enzyme lactase and is a physiologic feature occurring in most mammals after infancy (Di Rienzo et al., 2013; Ponte et al., 2016b).

II. Related Policies

Policy Number	Policy Title
N/A	Not Applicable

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

1) For the prediction of lactase insufficiency, targeted mutation analysis of *LCT* -13910 C>T **DOES NOT MEET COVERAGE CRITERIA**.

2) For individuals with lactose intolerance and/or lactase insufficiency, genetic testing of *LCT* and/or *MCM6* **DOES NOT MEET COVERAGE CRITERIA**.

IV. Table of Terminology

Term	Definition
AAP	American Academy of Pediatrics
ACG	American College of Gastroenterology
BSACI	British Society for Allergy & Clinical Immunology
C/T-13910	Eurasian lactase persistence variant
CLIA	Clinical Laboratory Improvement Amendments
CMS	Centers for Medicare and Medicaid Services
FODMAP	Fermentable oligo-, di- and monosaccharides and polyols
FGID	Functional gastrointestinal disorders
HBT	Hydrogen breath test
IBD	Inflammatory bowel disease
LBT	Lactose breath test
LCT	Lactase/lactase-phlorizin hydrolase
LD	Lactase deficiency
LDTs	Laboratory developed tests
LI	Lactose intolerance
LIP	Lactose-intolerant phenotype
LM	Lactose malabsorption
LNP	Lactase non-persistence
LTT	Lactose tolerance test
MCM6	*Minichromosome maintenance complex component 6*
NIH	National Institutes of Health
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
RT-PCR	Real-time polymerase chain reaction
SNPs	Single nucleotide polymorphisms

V. Scientific Background

Lactose is a disaccharide, which is a class of sugars comprised of two monosaccharides or simple sugars. Lactose consists of galactose bound to glucose and is the main source of carbohydrates from mammalian milk. Intestinal absorption of lactose

requires initial hydrolysis by the enzyme lactase. Low lactase activity results in undigested lactose and colonic bacterial fermentation of that lactose. This leads to the characteristic symptoms of lactose intolerance, such as bloating and flatulence (Luyt et al., 2014).

Lactase expression decreases as consequence of the normal maturational down-regulation after weaning, ultimately to undetectable levels in most populations (Swallow, 2003). Lactase expression persists, however, in descendants of populations that traditionally practice cattle domestication who maintain the ability to digest milk and other dairy products into adulthood (Deng et al., 2015). Adult expression of the gene encoding lactase (LCT), located on chromosome two at 2q21 appears to be regulated by *cis*-acting elements (Wang et al., 1995) and is inherited as an autosomal recessive trait (Enattah et al., 2002). The LCT gene is regulated by the nearby *MCM6* gene (minichromosome maintenance complex component 6), which encodes a helicase complex. A few different *MCM6* variants have been identified in affecting LCT gene expression (NIH, 2024). Single nucleotide polymorphisms (SNPs) associated with the lactase persistence vary by region. In European populations it is associated with C/T-13910 and G/A-22018 mutations of the LCT gene (Enattah et al., 2002; Hogenauer et al., 2005; Poulter et al., 2003; Ridefelt & Hakansson, 2005). Additional mutations have been identified in Saudi Arabia populations with G-13915 (Imtiaz et al., 2007), and in African tribes with the G-14010, G-13915, and G-13907 polymorphisms (Ingram et al., 2007; Tishkoff et al., 2007). No SNP associated with lactase persistence has been identified in the lactase gene regulatory sequence in Chinese populations (Zheng et al., 2016). In adult patients with homozygous lactase persistence, enzyme levels are 10-times higher than for patients with homozygous non-persistence, and heterozygous individuals (Deng et al., 2015; Enattah et al., 2007).

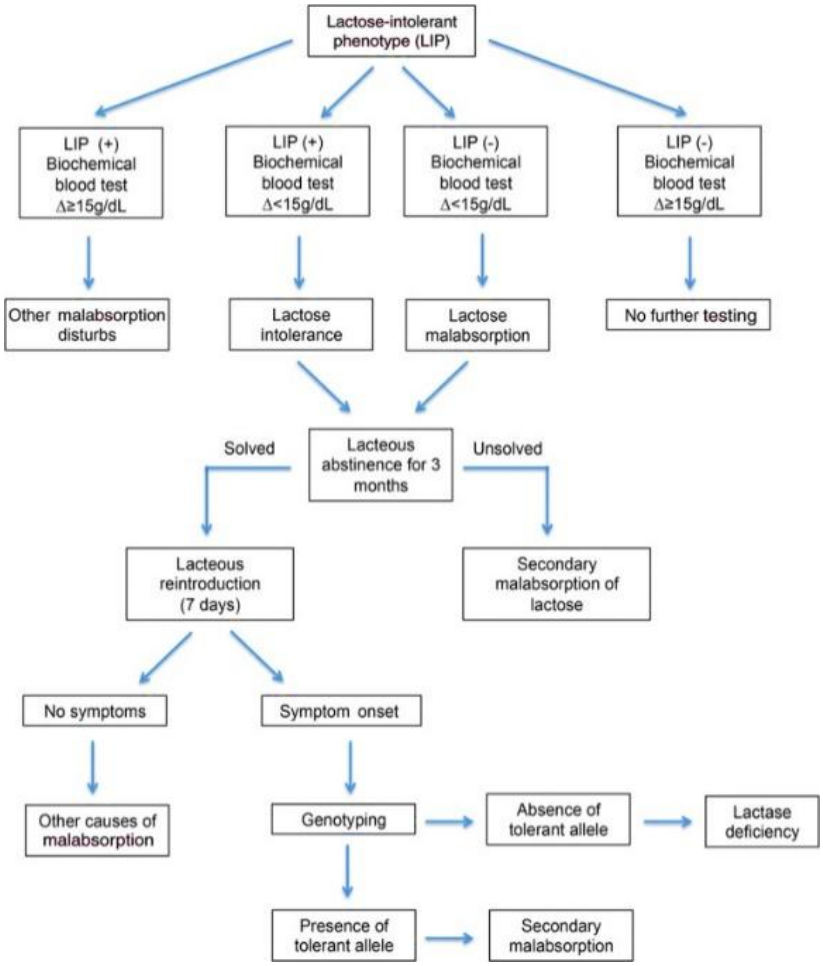
Lactase deficiency Baffour-Awuah et al. (2015) is defined as markedly reduced brush-border lactase activity relative to the activity observed in infants (Deng et al., 2015). Continued dairy consumption despite low expression of lactase results in unabsorbed lactose being present in the intestinal tract (lactose malabsorption [LM]), which can lead to symptoms of lactose intolerance (LI) in susceptible individuals (Hammer & Hogenauer, 2024).

Lactose intolerance is defined by patient reports of abdominal pain, bloating, borborygmi, and diarrhea induced by dairy consumption. Unabsorbed lactose increases the osmotic load thus increasing the intestinal water content, resulting in osmotic diarrhea. Additionally, lactose and other poorly-absorbed oligosaccharides, disaccharides, monosaccharides, and polyols ubiquitous in the diet are readily fermented by the colonic microbiome, leading to production of short-chain fatty acids and gas (mainly hydrogen [H_2], carbon dioxide [CO_2], and methane [CH_4]) (Magge &

Lembo, 2012; Shepherd et al., 2013). LI may be associated with nonspecific symptoms, abdominal pain, bloating, flatulence, diarrhea, or vomiting (Hammer & Hogenauer, 2024); however, it is unclear whether these symptoms are directly due to lactose ingestion. Although LM is nearly always attributable to LD, it is not possible to make a definitive diagnosis on clinical presentation alone because double-blind trials have shown that the reliability of self-reported LI is very poor (Deng et al., 2015; Suarez et al., 1995; Zheng et al., 2016).

Determining if reported symptoms of LI are resultant from LD can be approached through several different methods. The gold standard is the measurement of lactase, sucrase, and maltase activity through intestinal biopsies. However, this method is not commonly used due to its invasive nature (Di Rienzo et al., 2013). Other tests, such as the lactose breath test or biochemical blood tests are more frequently used (Furnari et al., 2013; Mattar et al., 2013; Ponte et al., 2016b). In addition to biochemical blood tests, genetic markers may be useful for LI diagnosis; however, a positive genetic test indicates whether lactase activity decline may represent a clinical problem for the patient, but the test does not give information on actual patient symptoms, making it inappropriate as an initial screening as not all patients with LM will develop symptoms of LI (Ponte et al., 2016a).

On the contrary, this information is more readily accessible by combining the lactose breath test with intolerance symptom evaluation (Di Stefano et al., 2009). Usual LI management involves excluding milk and milk products from the diet, while ensuring adequate calcium intake (Misselwitz, 2014; Ponte et al., 2016b; Usai-Satta et al., 2012). The use of genetic tests has been proposed as an adjunct to LI diagnosis to differentiate primary LD from secondary causes (Bodlaj et al., 2006) as depicted below in the figure taken from Ponte et al. (2016b).



Clinical Utility and Validity

Marton et al. (2012) compared the common polymorphism C/T 13910 with the lactase breath test and lactose tolerance test to assess each test's ability to predict genotype/phenotype relationships. The agreement of the breath test and genotype was 0.88 sensitivity and 0.85 specificity whereas the agreement between genotype and tolerance test was 0.94 sensitivity and 0.90 specificity (Marton et al., 2012).

Baffour-Awuah et al. (2015) studied the association of genotypes at the -13910 and -22018 SNPs with clinical characteristics, RNA quantification, and enzymatic phenotypes among a range of European ethnicities within the U.S. population. The authors concluded that "13910T/T genotype will frequently, but not perfectly, predict lactase persistence in this mixed European-ancestry population; a -13910T/C genotype will not predict the phenotype" (Baffour-Awuah et al., 2015).

Misselwitz et al. (2013) stated that genetic testing for the −13910*T genotype in certain African, Arabic, or Asian subpopulations has limited value because lactase persistence may be linked to different polymorphisms. The authors also stated that genetic tests

will be negative in patients with secondary causes of lactase deficiency and that no information about clinical symptoms lactose intolerance will be obtained during testing (Misselwitz et al., 2013).

Brasen et al. (2017) genotyped 3395 routine samples using real-time polymerase chain reaction (PCR) for the -13910C > T-variant to determine the prevalence of the variants in a Danish cohort examined for lactose intolerance as well as to improve the real-time PCR analysis for detection of the different variants. The researchers found that "Using real-time PCR resulted in 100% successful genotyping of the -13910C > T variant. By using a quality value of 99% and sequencing the undetermined samples we improved the ability of the assay to identify variants other than -13910C > T. This resulted in a reduction of the diagnostic error rate by a factor of 2.4 while increasing the expenses only 3%" (Brasen et al., 2017).

Coluccia et al. (2019) compared results from a lactose breath test and a lactase-gene polymorphism test in a total of 158 symptomatic adults to identify lactose intolerance. Whole blood samples were used for genetic testing purposes. Lactose breath testing resulted in positive results in 75.9% of participants, while genetic testing identified lactase-gene polymorphisms in 82.3% of participants. In conclusion, the authors state, "We suggest considering the use of the genetic test after LBT [lactose breath test] administration, when secondary hypolactasia is suspected, for completion of diagnostic procedures" (Coluccia et al., 2019).

Muendlein et al. (2019) developed a real-time PCR protocol for the detection of the LCT-13910C>T variant from whole blood samples. Lactose tolerance in adults is known to be strongly associated with the genetic variant LCT-13910C>T, which lies near the lactose encoding gene. Results from real-time PCR were compared to those from Sanger sequencing. A total of 105 whole blood samples were analyzed. When compared to Sanger sequencing, the real-time PCR protocol was determined to be a reliable method for the detection of the LCT-13910C>T (Muendlein et al., 2019).

In a cross-sectional study by Couce et al. (2020), 493 children suspected of functional gastrointestinal disorders (FGID) were studied using the exhaled H2 test, gastrointestinal symptoms, and SNP C/T-13910 genetic testing to observe the correlation between the genotype and phenotype of the C/T-13910 lactase non-persistence (LNP) gene. "The C/T-13910 genotype distribution was as follows: CC, 46.0%; CT, 39.5%; TT 14.4%. The frequency of the LP allele was 34.1%." A significant increase in LNP genotype, H2 test, and gastrointestinal symptoms were observed with increasing age. According to phenotype, CC carriers were mainly lactose non-absorbers (75.4%), TT carriers were lactose-absorbers (91.6%), and CT heterozygotes were predominantly lactose absorbers (90.7%) and lactose tolerant (74.3%). According

to these results and its prevalence in older age, the authors state that the C/T-13910 polymorphism is significantly correlated with the phenotype and the authors suggest that the practical value of genetic testing is greater in older children (Couce et al., 2020).

Nardone et al. (2021) studied the prevalence of lactose intolerance assessed by hydrogen breath test (H-BT) and investigated the prevalence of three single genetic polymorphisms of the lactase gene. A total of 54 IBD patients were recruited, and H-BT was positive in 64.8% of IBD patients and 62.3% control patients. The genetic analysis revealed that 46 IBD patients (85.2%) had the wild-type genotype (LCT-13910 CC) while the other polymorphisms (CT-22018, AG-13910, and CT-22018/AG-13910) were less common in IBD patients. In the control group, the wild genotype was found in 87% of the patients. Therefore, the prevalence of polymorphisms did not differ between the IBD group and the control group. The correlation between positive H-BT and genetic analysis showed that the "wild-type genotype was associated with higher rate of lactose intolerance in the total population" (Nardone et al., 2021).

Usai-Satta et al. (2021) reviewed the usefulness of hydrogen breath testing in the nutritional management of carbohydrate digestive disorders, including lactose malabsorption and intolerance, using the online databases PubMed, Medline, and Cochrane. According to the authors, the literature search yielded that though lactose breath testing is an indirect test for lactose malabsorption, "Lactose BT showed good sensitivity and optimal specificity for lactose malabsorption", leading them to conclude that "Before starting a low FODMAP [fermentable oligo-, di- and monosaccharides and polyols] diet, lactose BT should be suggested in a population with low prevalence of hypolactasia," helped in part by its non-invasiveness and inexpensiveness (Usai-Satta et al., 2021).

Cavichio et al. (2024) studied the agreement between C/T-13910 polymorphism genotyping results and lactose tolerance test (LTT) results in Brazil. LTT is the most commonly used LI diagnostic test in Brazil. The C/T-13910 polymorphism has mostly been studied in Caucasian populations; therefore, the authors of this study aimed to investigate the accuracy of C/T-13910 polymorphism genotyping in Brazil's "highly mixed" population. The authors included 404 patients who had undergone both C/T-13910 polymorphism genotyping and an LTT. Overall, "there was agreement between the genotyping and LTT results in 325 (80.4%) patients and discordance in 79 (19.6%) patients." The authors classified these results as "moderate agreement" and recommend additional studies to further investigate (Cavichio et al., 2024).

VI. Guidelines and Recommendations

American Academy of Pediatrics (AAP)

The AAP published guidelines (Heyman, 2006) on the evaluation of Lactose Intolerance in Infants, Children and Adolescents which recommend:

"Children with suspected lactose intolerance can be assessed clinically by dietary lactose elimination or by tests including noninvasive hydrogen breath testing or invasive intestinal biopsy determination of lactase (and other disaccharidase) concentrations. Treatment consists of use of lactase-treated dairy products or oral lactase supplementation, limitation of lactose-containing foods, or dairy elimination… If dairy products are eliminated, other dietary sources of calcium or calcium supplements need to be provided."

The AAP also reported that "Recent studies suggest that in the future, genetic testing may be useful for identifying individuals at increased risk of lactase deficiency and consequent diminished bone mineral density, potentially allowing early intervention with dietary manipulation or nutrient supplementation" (Heyman, 2006).

This statement was reaffirmed in 2012.

American College of Gastroenterology (ACG)
The ACG has a webpage focused on lactose intolerance in children. Regarding diagnoses, the ACG states that "Lactose intolerance is diagnosed by a simple test called a hydrogen breath test" (ACG, 2024). Genetic testing was not mentioned.

The 1st Rome Consensus Conference

The 1st Rome Consensus Conference issued their opinion on H_2-breath testing for sugar malabsorption. They found that "The determination of lactase activity in jejunal biopsy is currently considered the gold standard for lactose malabsorption. However, its results can be influenced by the irregular dissemination of lactase activity throughout the small intestine mucosa. On the basis of literature review, the lactose breath test is a reliable, non-invasive technique, which is provided with good sensitivity and optimal specificity."

Though it is acknowledged that there is no unequivocal reference test available for lactose malabsorption testing, the Conference graded the quality of evidence for the above as Class I ("Conditions with evidence or general accord that a particular procedure or treatment is useful or effective") of Strength A ("Data derived from multiple large and intermediate RCT") (Gasbarrini et al., 2009).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations

(LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81400	Molecular pathology procedure, Level 1 (eg, identification of single germline variant [eg, SNP] by techniques such as restriction enzyme digestion or melt curve analysis)
81479	Unlisted molecular pathology procedure

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

ACG. (2024). Lactose Intolerance in Children. https://gi.org/topics/lactose-intolerance-in-children/

Baffour-Awuah, N. Y., Fleet, S., Montgomery, R. K., Baker, S. S., Butler, J. L., Campbell, C., Tischfield, S., Mitchell, P. D., Allende-Richter, S., Moon, J. E., Fishman, L., Bousvaros, A., Fox, V., Kuokkanen, M., Grand, R. J., & Hirschhorn, J. N. (2015). Functional significance of single nucleotide polymorphisms in the lactase gene in diverse US patients and evidence for a novel lactase persistence allele at -13909 in those of European ancestry. *J Pediatr Gastroenterol Nutr*, *60*(2), 182-191. https://doi.org/10.1097/mpg.0000000000000595

Bodlaj, G., Stocher, M., Hufnagl, P., Hubmann, R., Biesenbach, G., Stekel, H., & Berg, J. (2006). Genotyping of the lactase-phlorizin hydrolase -13910 polymorphism by

LightCycler PCR and implications for the diagnosis of lactose intolerance. *Clin Chem*, *52*(1), 148-151. https://doi.org/10.1373/clinchem.2005.057240

Brasen, C. L., Frischknecht, L., Ornskov, D., Andreasen, L., & Madsen, J. S. (2017). Combination of real-time PCR and sequencing to detect multiple clinically relevant genetic variations in the lactase gene. *Scand J Clin Lab Invest*, *77*(1), 60-65. https://doi.org/10.1080/00365513.2016.1261408

Cavichio, M. W. E., Quaio, C., Baratela, W., Oliveira, P. M. C., & Tahan, S. (2024). EVALUATION OF AGREEMENT BETWEEN C/T-13910 POLYMORPHISM GENOTYPING RESULTS AND LACTOSE TOLERANCE TEST RESULTS: A RETROSPECTIVE POPULATION-BASED STUDY IN BRAZIL. *Arq Gastroenterol*, *61*, e23104. https://doi.org/10.1590/s0004-2803.24612023-104

Coluccia, E., Iardino, P., Pappalardo, D., Brigida, A. L., Formicola, V., De Felice, B., Guerra, C., Pucciarelli, A., Amato, M. R., Riegler, G., & De Magistris, L. (2019). Congruency of Genetic Predisposition to Lactase Persistence and Lactose Breath Test. *Nutrients*, *11*(6). https://doi.org/10.3390/nu11061383

Couce, M. L., Sánchez-Pintos, P., González-Vioque, E., & Leis, R. (2020). Clinical Utility of LCT Genotyping in Children with Suspected Functional Gastrointestinal Disorder. *Nutrients*, *12*(10), 3017. https://www.mdpi.com/2072-6643/12/10/3017

Deng, Y., Misselwitz, B., Dai, N., & Fox, M. (2015). Lactose Intolerance in Adults: Biological Mechanism and Dietary Management. *Nutrients*, *7*(9), 8020-8035. https://doi.org/10.3390/nu7095380

Di Rienzo, T., D'Angelo, G., D'Aversa, F., Campanale, M. C., Cesario, V., Montalto, M., Gasbarrini, A., & Ojetti, V. (2013). Lactose intolerance: from diagnosis to correct management. *Eur Rev Med Pharmacol Sci*, *17 Suppl 2*, 18-25. https://www.europeanreview.org/wp/wp-content/uploads/018-025.pdf

Di Stefano, M., Terulla, V., Tana, P., Mazzocchi, S., Romero, E., & Corazza, G. R. (2009). Genetic test for lactase non-persistence and hydrogen breath test: is genotype better than phenotype to diagnose lactose malabsorption? *Dig Liver Dis*, *41*(7), 474-479. https://doi.org/10.1016/j.dld.2008.09.020

Enattah, N. S., Kuokkanen, M., Forsblom, C., Natah, S., Oksanen, A., Jarvela, I., Peltonen, L., & Savilahti, E. (2007). Correlation of intestinal disaccharidase activities with the C/T-13910 variant and age. *World J Gastroenterol*, *13*(25), 3508-3512. https://pubmed.ncbi.nlm.nih.gov/17659699/

Enattah, N. S., Sahi, T., Savilahti, E., Terwilliger, J. D., Peltonen, L., & Jarvela, I. (2002). Identification of a variant associated with adult-type hypolactasia. *Nat Genet*, *30*(2), 233-237. https://doi.org/10.1038/ng826

Furnari, M., Bonfanti, D., Parodi, A., Franze, J., Savarino, E., Bruzzone, L., Moscatelli, A., Di Mario, F., Dulbecco, P., & Savarino, V. (2013). A comparison between lactose breath test and quick test on duodenal biopsies for diagnosing lactase deficiency in patients

with self-reported lactose intolerance. *J Clin Gastroenterol*, *47*(2), 148-152. https://doi.org/10.1097/MCG.0b013e31824e9132

Gasbarrini, A., Corazza, G. R., Gasbarrini, G., Montalto, M., Di Stefano, M., Basilisco, G., Parodi, A., Usai-Satta, P., Vernia, P., Anania, C., Astegiano, M., Barbara, G., Benini, L., Bonazzi, P., Capurso, G., Certo, M., Colecchia, A., Cuoco, L., Di Sario, A., . . . Tursi, A. (2009). Methodology and indications of H2-breath testing in gastrointestinal diseases: the Rome Consensus Conference. *Aliment Pharmacol Ther*, *29 Suppl 1*, 1-49. https://doi.org/10.1111/j.1365-2036.2009.03951.x

Hammer, H. F., & Hogenauer, C. (2024, January 9). *Lactose intolerance and malabsorption: Clinical manifestations, diagnosis, and management*. https://www.uptodate.com/contents/lactose-intolerance-clinical-manifestations-diagnosis-and-management

Heyman, M. B. (2006). Lactose intolerance in infants, children, and adolescents. *Pediatrics*, *118*(3), 1279-1286. https://doi.org/10.1542/peds.2006-1721

Hogenauer, C., Hammer, H. F., Mellitzer, K., Renner, W., Krejs, G. J., & Toplak, H. (2005). Evaluation of a new DNA test compared with the lactose hydrogen breath test for the diagnosis of lactase non-persistence. *Eur J Gastroenterol Hepatol*, *17*(3), 371-376. https://europepmc.org/article/MED/15716664

Imtiaz, F., Savilahti, E., Sarnesto, A., Trabzuni, D., Al-Kahtani, K., Kagevi, I., Rashed, M. S., Meyer, B. F., & Jarvela, I. (2007). The T/G 13915 variant upstream of the lactase gene (LCT) is the founder allele of lactase persistence in an urban Saudi population. *J Med Genet*, *44*(10), e89. https://doi.org/10.1136/jmg.2007.051631

Ingram, C. J., Elamin, M. F., Mulcare, C. A., Weale, M. E., Tarekegn, A., Raga, T. O., Bekele, E., Elamin, F. M., Thomas, M. G., Bradman, N., & Swallow, D. M. (2007). A novel polymorphism associated with lactose tolerance in Africa: multiple causes for lactase persistence? *Hum Genet*, *120*(6), 779-788. https://doi.org/10.1007/s00439-006-0291-1

Luyt, D., Ball, H., Makwana, N., Green, M. R., Bravin, K., Nasser, S. M., & Clark, A. T. (2014). BSACI guideline for the diagnosis and management of cow's milk allergy. *Clinical & Experimental Allergy*, *44*(5), 642-672. https://doi.org/10.1111/cea.12302

Magge, S., & Lembo, A. (2012). Low-FODMAP Diet for Treatment of Irritable Bowel Syndrome. *Gastroenterol Hepatol (N Y)*, *8*(11), 739-745. https://pubmed.ncbi.nlm.nih.gov/24672410/

Marton, A., Xue, X., & Szilagyi, A. (2012). Meta-analysis: the diagnostic accuracy of lactose breath hydrogen or lactose tolerance tests for predicting the North European lactase polymorphism C/T-13910. *Aliment Pharmacol Ther*, *35*(4), 429-440. https://doi.org/10.1111/j.1365-2036.2011.04962.x

Mattar, R., Basile-Filho, A., Kemp, R., & Santos, J. S. (2013). Comparison of Quick Lactose Intolerance Test in duodenal biopsies of dyspeptic patients with single nucleotide polymorphism LCT-13910C>T associated with primary hypolactasia/lactase-

persistence. *Acta Cir Bras*, *28 Suppl 1*, 77-82. https://pubmed.ncbi.nlm.nih.gov/23381829/
Misselwitz, B. (2014). Lactose intolerance: new insights due to blinded testing? *Digestion*, *90*(1), 72-73. https://doi.org/10.1159/000365144
Misselwitz, B., Pohl, D., Fruhauf, H., Fried, M., Vavricka, S. R., & Fox, M. (2013). Lactose malabsorption and intolerance: pathogenesis, diagnosis and treatment. *United European Gastroenterol J*, *1*(3), 151-159. https://doi.org/10.1177/2050640613484463
Muendlein, A., Leiherer, A., Zach, C., Brandtner, E. M., Fraunberger, P., Drexel, H., & Geiger, K. (2019). Real-time PCR based detection of the lactase non-persistence associated genetic variant LCT-13910C>T directly from whole blood. *Mol Biol Rep*, *46*(2), 2379-2385. https://doi.org/10.1007/s11033-019-04696-9
Nardone, O. M., Manfellotto, F., D'Onofrio, C., Rocco, A., Annona, G., Sasso, F., De Luca, P., Imperatore, N., Testa, A., de Sire, R., Biffali, E., & Castiglione, F. (2021). Lactose Intolerance Assessed by Analysis of Genetic Polymorphism, Breath Test and Symptoms in Patients with Inflammatory Bowel Disease. *Nutrients*, *13*(4), 1290. https://www.mdpi.com/2072-6643/13/4/1290
NIH. (2024, 03/19/2019). *MCM6 gene*. NIH. https://ghr.nlm.nih.gov/gene/MCM6
Ponte, P. R., de Medeiros, P. H., Havt, A., Caetano, J. A., Cid, D. A., Prata Mde, M., Soares, A. M., Guerrant, R. L., Mychaleckyj, J., & Lima, A. (2016a). Clinical evaluation, biochemistry and genetic polymorphism analysis for the diagnosis of lactose intolerance in a population from northeastern Brazil. *Clinics (Sao Paulo)*, *71*(2), 82-89. https://doi.org/10.6061/clinics/2016(02)06
Ponte, P. R., de Medeiros, P. H., Havt, A., Caetano, J. A., Cid, D. A., Prata Mde, M., Soares, A. M., Guerrant, R. L., Mychaleckyj, J., & Lima, A. A. (2016b). Clinical evaluation, biochemistry and genetic polymorphism analysis for the diagnosis of lactose intolerance in a population from northeastern Brazil. *Clinics (Sao Paulo)*, *71*(2), 82-89. https://doi.org/10.6061/clinics/2016(02)06
Poulter, M., Hollox, E., Harvey, C. B., Mulcare, C., Peuhkuri, K., Kajander, K., Sarner, M., Korpela, R., & Swallow, D. M. (2003). The causal element for the lactase persistence/non-persistence polymorphism is located in a 1 Mb region of linkage disequilibrium in Europeans. *Ann Hum Genet*, *67*(Pt 4), 298-311. https://pubmed.ncbi.nlm.nih.gov/12914565/
Ridefelt, P., & Hakansson, L. D. (2005). Lactose intolerance: lactose tolerance test versus genotyping. *Scand J Gastroenterol*, *40*(7), 822-826. https://doi.org/10.1080/00365520510015764
Shepherd, S. J., Lomer, M. C., & Gibson, P. R. (2013). Short-chain carbohydrates and functional gastrointestinal disorders. *Am J Gastroenterol*, *108*(5), 707-717. https://doi.org/10.1038/ajg.2013.96
Suarez, F. L., Savaiano, D. A., & Levitt, M. D. (1995). A comparison of symptoms after the consumption of milk or lactose-hydrolyzed milk by people with self-reported severe

lactose intolerance. *N Engl J Med*, *333*(1), 1-4. https://doi.org/10.1056/nejm199507063330101

Swallow, D. M. (2003). Genetics of lactase persistence and lactose intolerance. *Annu Rev Genet*, *37*, 197-219. https://doi.org/10.1146/annurev.genet.37.110801.143820

Tishkoff, S. A., Reed, F. A., Ranciaro, A., Voight, B. F., Babbitt, C. C., Silverman, J. S., Powell, K., Mortensen, H. M., Hirbo, J. B., Osman, M., Ibrahim, M., Omar, S. A., Lema, G., Nyambo, T. B., Ghori, J., Bumpstead, S., Pritchard, J. K., Wray, G. A., & Deloukas, P. (2007). Convergent adaptation of human lactase persistence in Africa and Europe. *Nat Genet*, *39*(1), 31-40. https://doi.org/10.1038/ng1946

Usai-Satta, P., Oppia, F., Lai, M., & Cabras, F. (2021). Hydrogen Breath Tests: Are They Really Useful in the Nutritional Management of Digestive Disease? *Nutrients*, *13*(3). https://doi.org/10.3390/nu13030974

Usai-Satta, P., Scarpa, M., Oppia, F., & Cabras, F. (2012). Lactose malabsorption and intolerance: What should be the best clinical management? *World J Gastrointest Pharmacol Ther*, *3*(3), 29-33. https://doi.org/10.4292/wjgpt.v3.i3.29

Wang, Y., Harvey, C. B., Pratt, W. S., Sams, V. R., Sarner, M., Rossi, M., Auricchio, S., & Swallow, D. M. (1995). The lactase persistence/non-persistence polymorphism is controlled by a cis-acting element. *Hum Mol Genet*, *4*(4), 657-662. https://pubmed.ncbi.nlm.nih.gov/7543318/

Zheng, X., China, Z. U. D. o. G. H., Chu, H., China, Z. U. D. o. G. H., Cong, Y., China, Z. U. D. o. G. H., Deng, Y., China, Z. U. D. o. G. H., Long, Y., China, Z. U. D. o. G. H., Zhu, Y., China, Z. U. D. o. G. H., Pohl, D., Switzerland, U. H. Z. D. o. G. H. Z., Switzerland, U. o. Z. Z. C. f. I. H. P. Z. Z., Fried, M., Switzerland, U. H. Z. D. o. G. H. Z., Switzerland, U. o. Z. Z. C. f. I. H. P. Z. Z., Dai, N., . . . Switzerland, U. o. Z. Z. C. f. I. H. P. Z. Z. (2016). Self-reported lactose intolerance in clinic patients with functional gastrointestinal symptoms: prevalence, risk factors, and impact on food choices. *Neurogastroenterology & Motility*, *27*(8), 1138-1146. https://doi.org/10.1111/nmo.12602

Genetic Testing for Li-Fraumeni Syndrome

Policy Number: AHS – M2081 – Genetic Testing for Li-Fraumeni Syndrome	Prior Policy Name and Number, as applicable:
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Li-Fraumeni syndrome (LFS) is an autosomal dominant cancer predisposition syndrome characterized by a wide range of malignancies that appear at an unusually early age and is generally associated with defects in the tumor protein p53 gene (*TP53*) (Evans, 2024; Mai et al., 2016; Malkin, 2011).

II. Related Policies

Policy Number	Policy Title
AHS-M2003	Genetic Testing for Breast, Ovarian, Pancreatic, and Prostate Cancers

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals who have received genetic counseling and who have a close blood relative (see Note 1) with a *TP53* mutation (see Note 2), the following germline testing **MEETS COVERAGE CRITERIA**:
   a) Testing restricted to the known familial mutation.
   b) Comprehensive *TP53* gene sequencing when the specific *TP53* mutation is unknown.

2) For individuals who have received genetic counseling, germline testing for *TP53* mutations (single gene or multi-gene panel testing) **MEETS COVERAGE CRITERIA** under **any** of the following conditions:

   a) For an individual who meets either the classic or the Chompret clinical diagnostic criteria for LFS:

      i) Classic LFS is defined by the presence of **all** of the following criteria:

         (a) An individual with a sarcoma before 45 years of age.

         (b) Having a first-degree relative (see Note 1) with any cancer before 45 years of age.

         (c) Having an additional first- or second-degree relative (see Note 1) with any cancer before 45 years of age, or a sarcoma at any age.

      ii) Chompret clinical diagnostic criteria is defined by the presence of any **one** of the following:

         (a) Individual with a tumor belonging to the LFS tumor spectrum (e.g., soft tissue sarcoma, osteosarcoma, central nervous system (CNS) tumor, breast cancer, adrenocortical carcinoma) before age 46 years **AND** at least one first- or second-degree relative (see Note 1) with a LFS tumor (except breast cancer if proband has breast cancer) before 56 years of age or with multiple tumors at any age.

         (b) Individual with multiple tumors (except multiple breast tumors), two of which belong to the LFS tumor spectrum described above, with the first tumor having occurred before 46 years of age.

         (c) Individual with adrenocortical carcinoma (ACC), or choroid plexus tumor or rhabdomyosarcoma of embryonal anaplastic subtype, at any age, irrespective of family history.

         (d) Individual was diagnosed with breast cancer before 31 years of age.

   b) For an individual with a personal or family history of pediatric hypodiploid acute lymphoblastic leukemia.

   c) For individuals who have been diagnosed with any cancer before 30 years of age and for whom a pathogenic or likely pathogenic *TP53* variant has been identified on tumor-only genomic testing.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

3) For all other situations not addressed above, single gene testing for a germline *TP53* mutation **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Close blood relatives include 1st-degree relatives (e.g., parents, siblings, and children), 2nd-degree relatives (e.g., grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings), and 3rd-degree relatives (great-grandparents, great-aunts, great-uncles, great-grandchildren, and first cousins).

Note 2: At the present time, there are no specific, evidence-based, standardized guidelines for recommendations of which "at risk" relatives should be tested. In relatives of an index case, the risk of having a pathologic mutation, and developing disease, is influenced by numerous factors that should be considered in evaluating risk:

1. Proximity of relation to index case (first-, second-, or third-degree)
2. Mode of inheritance of mutation (autosomal dominant versus autosomal recessive)
3. Degree of penetrance of mutation (high, intermediate or low)
4. Results of detailed pedigree analysis
5. De novo mutation rate

If a proband has a *TP53* mutation, the risk to the proband's offspring of inheriting the mutation is 50 percent. If a proband has a *TP53* mutation, the risk to other relatives may depend on the genetic status of the proband's parents (that is, it is not a de novo mutation in the proband). Most *TP53* mutations are inherited from 1 of a proband's parents. After a mutation has been identified in a proband, the proband's parent with any pertinent cancer history of family history should be tested first to establish the lineage of the mutation; otherwise, both parents should be tested. A family history could appear to be negative because of incomplete penetrance of the mutation, limited family members available for testing, early death of a parent, etc.

IV. Table of Terminology

Term	Definition
ACC	Adrenocortical carcinoma
ACMG	American College of Medical Genetics and Genomics
ASBRS	American Society of Breast Surgeons
BAX	*BCL2 associated x* gene

BC	Breast cancer
BCL10	*BCL10 immune signaling adaptor* gene
BRCA 1	*Breast cancer 1* gene
BRCA 2	*Breast cancer 2* gene
CDKN2A	*Cyclin dependent kinase inhibitor 2A* gene
CHEK2	*Checkpoint kinase 2 gene*
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CNS	Central nervous system
DNA	Deoxyribonucleic acid
ERN	European Reference Network
FDA	Food and Drug Administration
GIST	Gastrointestinal stromal cell tumors
LDT	Laboratory-developed test
LFL	Li-Fraumeni-like syndrome
LFS	Li-Fraumeni syndrome
LFSA	Li-Fraumeni Syndrome Association
NCCN	National Comprehensive Cancer Network
NORD	National Organization of Rare Diseases
PALB2	*Partner and localizer of BRCA2* gene
PGV	Pathogenic germline variant
PTEN	*Phosphatase and tensin homolog* gene
PV	Pathogenic variant
STK11	*Serine/threonine kinase 11* gene
STS	Soft tissue sarcomas
TP53	*Tumor protein p53* gene
TP63	*Tumor protein p63* gene

V. Scientific Background

Li-Fraumeni syndrome (LFS) is a rare cancer predisposition syndrome associated with a germline mutation in the tumor suppressor gene TP53 (tumor protein p53) on chromosome 17p13.1 (Correa, 2016). This genetic mutation has an autosomal dominant pattern of inheritance with high penetrance. TP53 encodes for a ubiquitous transcription factor that is responsible for a complex set of critical regulatory functions that promote DNA repair and tumor suppression during episodes of cellular stress and DNA damage (Sorrell et al., 2013). Most TP53 mutations are clustered in the DNA-binding domain within specific codons, such as 175 and 248 (Villani et al., 2016). TP53 mutations are often missense alterations (Petitjean et al., 2007) that cause a change in one nucleotide and encode for a different amino acid than the one

typically found in that location within the protein. Missense mutations are usually transcriptionally inactive leading to downstream events permissive for development of various malignancies throughout life; however, some reports have shown gain of function oncogenic effects in TP53 (Brosh & Rotter, 2009; Sigal & Rotter, 2000).

Li-Fraumeni syndrome is characterized clinically by the development of cancers arising in multiple organ systems, often at a young age (Birch et al., 1998; Birch et al., 1994; Garber et al., 1991; Li et al., 1988; Mai et al., 2012; Malkin et al., 1990; Olivier et al., 2010; Ruijs et al., 2010). These patients have a very high lifetime cumulative risk of developing malignancies and early-onset malignancies; around 50% of the individuals carrying mutations in TP53 will develop cancer by the age of 30 years (Hwang et al., 2003; Lustbader et al., 1992; Schneider et al., 2013). While many tumor types can be seen in patients with LFS, four cancers (breast, sarcoma, brain, and adrenocortical carcinoma) comprise about 80% of LFS associated tumors (Gonzalez et al., 2009; Li et al., 1988; Lynch et al., 1978; Olivier et al., 2003).

Breast Cancer accounts for about 30% of all LFS-associated tumors (Gonzalez et al., 2009; Olivier et al., 2003). Individuals with LFS-associated breast cancer tend to present at an earlier age (in the 20s or early 30s) with more advanced stage disease at the time of initial diagnosis (NCCN, 2024). The ability to distinguish between a germline TP53 mutation (LFS) and a somatic TP53 pathogenic variant (TP53 mosaicism or clonal hematopoiesis) is very important for breast cancer patients and relatives and may help to determine the best method of treatment; "For PV [pathogenic variant] carriers in high-penetrance genes like BRCA1, BRCA2, and TP53, prophylactic mastectomy is often recommended and radiation therapy avoided when possible" (Batalini et al., 2019).

Sarcomas account for about 30% of all LFS-associated tumors (Gonzalez et al., 2009; Ognjanovic et al., 2012; Olivier et al., 2003; Palmero et al., 2010). Multiple types of soft tissue sarcomas and osteosarcoma are associated with LFS; but Ewing's sarcoma, gastrointestinal stromal cell tumors (GIST), desmoid tumors, and angiosarcomas have not been reported in LFS patients (Olivier et al., 2003).

Brain Tumors occur in approximately 14% of individuals with TP53 mutations (Gonzalez et al., 2009; Olivier et al., 2003; Palmero et al., 2010; Ruijs et al., 2010). Glioblastomas/astrocytomas are the most common, but medulloblastoma, ependymoma, supratentorial primitive neuroectodermal tumors, and choroid plexus tumors may also be seen (Farrell & Plotkin, 2007; Ruijs et al., 2010).

Adrenocortical Carcinoma (ACC) accounts for about seven percent of cancers in TP53 mutation carriers overall (Gonzalez et al., 2009; Palmero et al., 2010). While ACC has been diagnosed in individuals with LFS at a wide range of ages, it is

considered a hallmark of LFS when diagnosed in childhood (Gonzalez et al., 2009; Herrmann et al., 2012; Palmero et al., 2010).

Other LFS Cancers

Beyond the four core LFS cancers, the next most frequently associated cancers include leukemia, lung, colorectal, skin, gastric, and ovarian (Gonzalez et al., 2009; Masciari et al., 2011; Olivier et al., 2003; Palmero et al., 2010; Walsh et al., 2011; Wong et al., 2006). All cancer types are diagnosed at younger than average ages.

Over the years, several types of classifying systems have been developed for LFS diagnostic purposes (shown below in table 1). The classic LFS phenotype was clinically defined before the identification of germline mutations in TP53; these criteria are the most stringent and are the ones used to make a clinical diagnosis of LFS (with or without the identification of a deleterious germline TP53 mutation) (Li et al., 1988). Further studies revealed that, although highly specific for TP53 germline mutations, these criteria fail to include many mutation-positive families. Broader criteria were developed by Birch and Eeles to identify families which are Li-Fraumeni-like (LFL) (Birch et al., 1994; Eeles, 1995). The most robust analysis of TP53 mutation carriers to date was performed in France by Bougeard et al. (2008); these analyses helped to develop the most recent version of the Chompret criteria which can better identify families with milder phenotypes (Bougeard et al., 2001; Bougeard, Renaux-Petel, Flaman, Charbonnier, Fermey, Belotti, Gauthier-Villars, Stoppa-Lyonnet, Consolino, Brugières, et al., 2015; Chompret et al., 2001; Gonzalez et al., 2009). The Chompret criteria for clinical diagnoses of LFS was shown to provide the highest positive predictive value and, when combined with the classic LFS criteria, provided the highest sensitivity for identifying individuals with LFS (Bougeard, Renaux-Petel, Flaman, Charbonnier, Fermey, Belotti, Gauthier-Villars, Stoppa-Lyonnet, Consolino, Brugieres, et al., 2015; Bougeard et al., 2008; Tinat et al., 2009).

Table 1: Types of LFS classifying systems

Clinical criteria	Description
Classical LFS (Li et al., 1988)	**I-**sarcoma diagnosed in childhood/young adulthood (≤ 45 years) **and**
	II-first-degree relative with any cancer in young adulthood (≤ 45 years) **and**
	III-first- or second-degree relative with any cancer diagnosed in young adulthood (≤ 45 years) **or** sarcoma diagnosed at any age.
LFL – Birch (Birch et al., 1994)	**I-**childhood cancer (at any age) or sarcoma, CNS tumor, or ACC in young adulthood (≤ 45 years) **and**

Clinical criteria	Description
	II-first- or second-degree relative with LFS-spectrum cancer (sarcoma, BC, CNS tumor, ACC, leukemia) at any age **and**
	III-first- or second-degree relative with any cancer diagnosed at age < 60 years.
LFL – Eeles 1 and 2 (Eeles, 1995)	**I-**at least two first- or second-degree relatives with LFS-spectrum cancer (sarcoma, BC, CNS tumor, ACC, leukemia, melanoma, prostate cancer, pancreatic cancer) diagnosed at any age **I-** sarcoma diagnosed at any age **and II-**at least two other tumors diagnosed in one or more first- or second-degree relatives: BC at age < 50 years; CNS tumor, leukemia, ACC, melanoma, prostate cancer, pancreatic cancer at age < 60 years; **or** sarcoma at any age.
LFL – Chompret (Chompret et al., 2001)	**I-**diagnosis of sarcoma, CNS tumor, BC, ACC at age < 36 years **and II-**first- or second-degree relative with any of the above cancers (except BC if proband had BC) **or** relative with multiple primary tumors at any age **or III-**multiple primary tumors, including two of the following: sarcoma, CNS tumor, BC, or ACC, with the first tumor diagnosed at age < 36 years regardless of family history; **or IV-**ACC at any age, regardless of family history.
LFL – Modified Chompret (Bougeard et al., 2008; Tinat et al., 2009)	**I-**index case with LFS-spectrum cancer (sarcoma, BC, CNS tumor, ACC, leukemia, bronchioloalveolar carcinoma) occurring at age < 46 years **and II-**a first- or second-degree relative with LFS-spectrum cancer occurring at age < 56 years (except BC if the index case has BC as well), **or** multiple tumors; **or III-**index patient with multiple tumors, at least two of which are in the LFS spectrum, the first occurring at age < 46 years; **or IV-**ACC or choroid plexus carcinoma occurring at any age or BC occurring at age < 36 years without *BRCA1* or *BRCA2* mutations.
LFL – Revisiting Li-Fraumeni Syndrome from TP53 Mutation Carriers (Bougeard, Renaux-Petel, Flaman, Charbonnier, Fermey, Belotti, Gauthier-Villars,	**Familial presentation:** proband with tumor belonging to LFS tumor spectrum (eg, premenopausal breast cancer, soft tissue sarcoma, osteosarcoma, CNS tumor, adrenocortical carcinoma) before age 46 yr, AND at least on first or second-degree relative with LFS tumor (except breast cancer if proband has breast cancer) before age 56 yr or with multiple tumors; **Multiple primitive tumors:** Proband with multiple tumors (except multiple breast tumors), two of which belong to LFS tumor spectrum and first of which occurred before age 46 yr. **Rare tumors:** patient with adrenocortical carcinoma, choroid plexus tumor or

Clinical criteria	Description
Stoppa-Lyonnet, Consolino, Brugières, et al., 2015)	rhabdomyosarcoma of embryonal anaplastic subtype, irrespective of family history; **Early-onset breast cancer:** Breast cancer before age 31 yr

ACC: adrenocortical carcinoma; BC: breast cancer; CNS: central nervous system; LFS: Li-Fraumeni syndrome; LFL: Li-Fraumeni-like syndrome.

As noted above, the TP53 gene has an autosomal dominant pattern of inheritance (Evans, 2024). Mutations such as this can be studied with a pedigree, which is a genetic based family tree. Pedigrees begin with the "proband," which is the subject being studied or tested. If one of the proband's parents carries the TP53 mutation, each sibling has a 50% risk of having the mutation. If neither parent is found to carry the mutation, the risk to siblings is low, but they should be tested due to the possibility of germline mosaicism. Offspring of a proband have a 50% risk of carrying the mutation. Phenotypes of families carrying TP53 mutations can be highly variable (Malkin, 2011; McBride et al., 2014).

Additionally, mutations in TP53 can lead to different consequences on gene function. A locus is a fixed position on a chromosome where a gene is located. The possibility of a second locus involved in LFS is an additional issue in the etiology of the syndrome since approximately 20% of LFS and up to 80% of LFL families do not exhibit TP53 mutations (Malkin, 2011; McBride et al., 2014). However, no association was found with p53 partners in tumor suppressor pathways, including BAX (BCL2 Associated X) (Barlow et al., 2004), CDKN2A (Cyclin Dependent Kinase Inhibitor 2A) (Portwine et al., 2000), TP63 (tumor protein p63) (Bougeard et al., 2001), CHEK2 (Checkpoint kinase 2) (Bougeard et al., 2001), BCL10 (BCL10 Immune Signaling Adaptor) (Stone et al., 1999), or PTEN (Phosphatase and tensin homolog) (Brown et al., 2000) in TP53-negative families. Although a few studies have linked other loci to LFS (Aury-Landas et al., 2013; Bachinski et al., 2005), TP53 remains the only gene conclusively associated to the syndrome (Evans, 2024).

Large panels or single gene tests can be used to identify a TP53 pathogenic variant. For example, Invitae has developed a test which analyzes only the TP53 gene with a three mL whole blood sample; this test has a turnaround time of 10-21 days (Invitae, 2024). Blueprint Genetics has developed a similar one gene panel test which also analyzes the TP53 gene in three to four weeks (BluePrint, 2022).

It is common to find TP53 mutations during tumor profiling, but germline mutations are very rare. Germline testing is not recommended if somatic mutations in TP53 are

found unless there is a defined personal or family history indicative of Li-Fraumeni syndrome (Peshkin, 2024).

Clinical Utility and Validity

The reported percentage of LFS due to TP53 mutation varies between studies and criteria used. According to (Schneider et al., 2013), approximately 80 percent of individuals with features of LFS will have an identifiable TP53 mutation. Families that have clinical features of LFS without TP53 mutation are more likely to have a different hereditary cancer syndrome (Schneider et al., 2013). Some studies have reported that 70% to 80% of families meeting the classic LFS criteria have the TP53 mutation (Nagy et al., 2004; Varley, 2003). However, Gonzalez et al. (2009) reported that a slightly lower positive predictive value for the p53 mutation rate using the classic criteria among 341 patients (56%), with high specificity of 91% but low sensitivity (40%). Chompret et al. (2001) reported TP53 mutations can be found in 20% of cases using the Chompret criteria. Gonzalez et al. (2009) reported a higher positive predictive value for LFL syndrome using Chompret criteria (35%) than Birch (16%) or Eeles (14%).

Gonzalez et al. (2009) used a clinical testing cohort to understand the spectrum of tumors associated with germline p53 mutations. Mutations were identified in 17% (91 of 525) of patients submitted for testing. All families with a p53 mutation had at least one family member with a sarcoma, breast, brain, or adrenocortical carcinoma. Overall, 75 patients with a p53 mutation had an adequate family history, and out of these 75, 71 fulfilled the classic LFS or Chompret criteria. When the classic LFS and Chompret criteria were used together, the testing sensitivity was 95%, and the specificity was 52% (Gonzalez et al., 2009).

Villani et al. (2011) assessed the feasibility and clinical impact of a comprehensive surveillance protocol in asymptomatic TP53 mutation carriers in eight families with LFS. A total of 33 TP53 mutation carriers were identified, 18 of whom underwent surveillance. In the surveillance group, 10 tumors developed in seven patients, and all seven patients were alive after a median follow-up of 24 months. In the non-surveillance group, 12 tumors developed in 10 patients, and only two were alive after 24 months. The authors reported a three-year overall survival of 100% in the surveillance group compared to 21% in the non-surveillance group (Villani et al., 2011).

Bougeard, Renaux-Petel, Flaman, Charbonnier, Fermey, Belotti, Gauthier-Villars, Stoppa-Lyonnet, Consolino, Brugieres, et al. (2015) evaluated the genetic spectrum of LFS. The authors identified 415 TP53 mutation carriers with 133 different TP53 mutations. A total of 322 of these carriers were affected and eventually developed 552 tumors. In childhood, the LFS tumor spectrum was as follows: “osteosarcomas, adrenocortical carcinomas, central nervous system (CNS) tumors, and soft tissue

sarcomas (STS) observed in 30%, 27%, 26%, and 23% of the patients, respectively." Adults presented with breast carcinomas in 79% of females and with soft tissue sarcomas in 27% of overall patients. Age of onset varied according to type of mutation; carriers with dominant-negative missense mutations had a mean onset of 21.3 years, carriers with loss of function mutations had a mean onset of 28.5 years, and carriers with genomic rearrangements had a mean onset of 35.8 years. The authors suggested that stratifying clinical management of LFS by class of mutation may be useful (Bougeard, Renaux-Petel, Flaman, Charbonnier, Fermey, Belotti, Gauthier-Villars, Stoppa-Lyonnet, Consolino, Brugieres, et al., 2015).

In 2016, Villani et al. (2016) updated their assessment of a prospective observational study and modified the surveillance protocol. Out of the 89 carriers of TP53 pathogenic variants in 39 unrelated families, 40 (45%) agreed to surveillance and 49 (55%) declined surveillance. The authors reported a five year overall survival was 88.8% in the surveillance group and 59.6% in the non-surveillance group (Villani et al., 2016).

Rana et al. (2018) compared the histories of patients whose TP53 mutations (TP53+) were identified by panel testing to those whose mutations were identified by single-gene testing. A total of 126 TP53+ patients were identified with panel testing, and 96 were identified with single-gene testing. The patients who were identified with panel testing were older at "first cancer identification" and at cancer diagnosis. Established LFS testing criteria were met less often in patients in the panel testing cohort, and phenotypes of the panel testing cohort were often different from those in the single-gene cohort (Rana et al., 2018).

Bakhuizen et al. (2019) completed a nation-wide analysis in the Netherlands which measured TP53 germline mutations in early-onset breast cancer cases. This study included data from 370 individuals diagnosed with breast cancer between 2005 and 2016 who were younger than 30 years at the time of diagnosis. All individuals included in the study were tested for TP53 genetic mutations. A total of eight of these individuals were found to carry a likely pathogenic TP53 sequence (less than one percent), showing the rarity of a TP53 mutation in breast cancer cases. However, the researchers note that TP53 mutation prevalence was similar or greater in other studies which included patients with an older age of onset, questioning whether an early age of onset is necessary as a TP53 genetic testing criterion (Bakhuizen et al., 2019).

Lincoln et al. (2020) studied the yield and utility of germline genetic testing following tumor DNA sequencing in patients with cancer. Germline testing was performed on 2023 patients and the prevalence of pathogenic germline variants (PGVs) was calculated. PGVs were found in 617 of the 2023 patients associated with cancers of the breast, colorectal, renal, lung, and bladder. About 82% of the patients identified with a

PGV met the criteria for follow-up testing and 8.1% of PGVs were missed by tumor sequencing. Only four percent of pathogenic TP53 variants were germline, but 64% of the germline TP53 carriers did not meet the Chompret criteria for germline TP53 testing. It was found that genes which frequently acquire somatic mutations were a challenge because clinicians assumed TP53 to be somatic, so TP53 variants identified by tumor germline sequencing were underreported. The authors conclude that although the yield of germline findings of the TP53 gene is relatively low, the clinical impact can be substantial. Therefore, they recommend broader germline testing for these genes despite the low yield (Lincoln et al., 2020).

Terradas et al. (2021) studied TP53 variants that were detected in colorectal cancer patients without a LFS phenotype. A total of 473 patients with colorectal cancer were assessed for TP53 pathogenic variants. Pathogenic variants were identified in 0.05% of the control and 0.26% of the colorectal cancer patients, none of whom fulfilled the clinical criteria for TP53 testing. The authors conclude that "TP53 pathogenic variants should not be unequivocally associated with LFS. Prospective follow-up of carriers of germline TP53 pathogenic variants in the absence of LFS phenotypes will define how surveillance and clinical management of these individuals should be performed" (Terradas et al., 2021).

Yamamoto et al. (2020) performed an analysis of 194 patients with advanced cancer who had undergone next-generation sequencing (NGS) performed only on tumor specimens. The goal was to identify variants that had the potential to be secondary findings. Out of 194 patients, 120 were identified with possible secondary findings. The gene with the highest incidence was TP53, with a total of 97 variants identified among 91 patients. Of these patients, nine had additional germline testing performed, with 14 variant genes involved (BRCA1, n = 1; BRCA2, n = 2; PTEN, n = 2; RB1, n = 1; SMAD4, n = 1; STK11, n = 1; TP53, n = 6). Those persons identified with TP53 variants were confirmed as somatic variants, as opposed to germline. The authors concluded, "We analyzed 24 patients with TP53 variants who underwent a paired tumor–normal NGS assay. As expected, all TP53 variants were confirmed to be somatic variants. A total of 30 patients were tested for germline variants in TP53, but none of them resulted in true SFs, suggesting the low prevalence of SFs in this gene" (Yamamoto et al., 2020).

Patel et al. (2022) studied the clinical utility of widespread germline testing of cancer patients outside of guidelines. The goal of the study was to diversify the research area, as past studies on cancer screening have "skewed towards wealthier socioeconomic populations that lack ethnic diversity." The authors conducted a single center prospective study on germline testing, aiming to "understudied real-world populations." The study included 67 patients, reported as "African American (14), White (50), or Asian American (3)." All patients were outside of guideline concordance for

genetic testing due to either young age, rare tumors, or recurrent or multiple malignancies. There were 11 pathogenic or likely pathogenic variants that increased susceptibility to cancer, including rare germline findings and LFS. The authors concluded that "current practice of guidelines and genetic counselor recommended testing should be re-examined for broader testing for all incoming cancer patients in a more inclusive manner" (Patel et al., 2022).

VI. Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

The NCCN maintains guidelines for the diagnosis and management of Li-Fraumeni Syndrome (NCCN, 2024).

NCCN recommends testing for Li-Fraumeni Syndrome in the following situations:

- Individual from a family with a known *TP53* pathogenic/likely pathogenic variant
- Classic Li-Fraumeni syndrome criteria
- Chompret criteria
- Personal or family history of pediatric hypodiploid acute lymphoblastic leukemia
- In individuals with cancer with a pathogenic/likely pathogenic TP53 variant identified on tumor only genomic testing, germline testing should be considered for:
    1. Those meeting one or more of the other LFS testing criterion above after reevaluation of personal and family history
    2. Those diagnosed age <30 with any cancer
    3. Those with clinical scenario not meeting these criteria but warranting germline evaluation per clinician discretion

The classic Li-Fraumeni syndrome criteria are as follows:

- "Combination of an individual diagnosed age <45 y with a sarcoma AND
- A first-degree relative diagnosed age <45 y with cancer AND
- An additional first- or second-degree relative in the same lineage with cancer diagnosed <45 y, or a sarcoma at any age" (NCCN, 2024).

The Chompret criteria are as follows:

- "Individual with a tumor from LFS tumor spectrum (eg, soft tissue sarcoma, osteosarcoma, CNS tumor, breast cancer, adrenocortical carcinoma [ACC]) before 46 y of age, AND at least one first- or second-degree relative with any of the aforementioned cancers (other than breast cancer if the proband has breast cancer) before the age of 56 y or with multiple primaries at any age OR

- Individual with multiple tumors (except multiple breast tumors), two of which belong to LFS tumor spectrum with the initial cancer occurring before the age of 46 y OR
- Individual with adrenocortical carcinoma, or choroid plexus carcinoma or rhabdomyosarcoma of embryonal anaplastic subtype, at any age of onset, regardless of family history OR
- Breast cancer before age 31 years" (NCCN, 2024)

If these criteria are fulfilled, the *TP53* gene may be tested. If the familial pathogenic variant of *TP53* is known, that variant may be tested for. If it is unknown, a comprehensive *TP53* test may be done.

Reproductive options:

- "For patients of reproductive age, advise about options for prenatal diagnosis and assisted reproduction including pre-implantation genetic diagnosis and donor gametes. Discussion should include known risks, limitations, and benefits of these technologies" (NCCN, 2024).

For relatives:

- "Advise about possible inherited cancer risk to relatives, and options for risk assessment, testing, and management.
- Counselor should discuss the importance of genetic counseling and testing for relatives who also may be at increased risk " (NCCN, 2024).

Children (under 18 years):

- "Genetic testing is generally not recommended when results would not impact medical management"(NCCN, 2024).

American College of Medical Genetics and Genomics (ACMG)

The ACMG has noted *TP53* as a gene whose secondary findings should be reported if found (Kalia et al., 2017). In 2021, a joint ACMG/AMP variant interpretation guideline was published for germline *TP53* variants; the authors note that "intense" cancer surveillance for those patients with *TP53* germline pathogenic variants can lead to "reduced cancer-related mortality" (Fortuno et al., 2021).

Li-Fraumeni Syndrome Association (LFSA)

The LFSA notes certain criteria that can be used to determine if genetic testing should be performed. The classic LFS criteria, Chrompret criteria, Birch definition of Li-

Fraumeni-like syndrome, and Eeles definition of Li-Fraumeni-syndrome may all be fulfilled to consider genetic testing (LFSA, 2024). The association goes on to add that the potential of genetic testing (and the implications of the results) should always involve discussions with a genetic counselor, medical providers, and family.

National Organization of Rare Diseases (NORD)

The NORD states that "Li-Fraumeni syndrome is diagnosed based on the presence of a so called pathogenic or likely pathogenic variant in the *TP53* gene."; further, "The potential of genetic testing (and the implications of the results) should always involve discussions with a genetic counselor, medical providers, and family" (NORD, 2021). The NORD also notes that genetic testing can be considered based on classic LFS criteria, Chrompret criteria, the Birch definition of Li-Fraumeni-like syndrome, and the Eeles definition of Li-Fraumeni-syndrome.

American Society of Breast Surgeons (ASBrS)

The ASBrS have published consensus guidelines on genetic testing for hereditary breast cancer. These guidelines state that "Increased access to testing would likely lead to more patients pursuing testing and improving rates of identification of gene carriers. Breast surgeons are well positioned to be a resource for patients who may benefit from testing. Breast surgeons can identify individuals who are suitable for testing, inform patients of the risks and benefits, provide access to genetic testing, and also discuss risk management strategies for those patients who test positive. For patients with less common mutations, strong consideration should be given to consultation with cancer genetics specialists. Hereditary mutations to be considered include *BRCA 1&2*, *PALB2*, and other hereditary breast cancer syndromes, which include but are not limited to Li-Fraumeni syndrome (*TP53* pathogenic variant), Cowden syndrome (*PTEN* pathogenic variant), hereditary diffuse gastric cancer syndrome (*CDH1* pathogenic variant), and Peutz-Jeghers syndrome (*STK11* pathogenic variant)" (Manahan et al., 2019).

European Reference Network (ERN) GENTURIS

The ERN provides recommendations on cancer patients who should be tested for *TP53* germline mutations. They recommend testing the following patients (Frebourg et al., 2020):

- Patients who meet the Chompret Criteria. These include those with familial presentation, multiple primitive tumors, rare tumors such as adrenocortical carcinoma, choroid plexus carcinoma, or rhabdomyosarcoma, or very early-onset breast cancer (Breast cancer before 31 years, irrespective of family history)

- Patients who are children or adolescents presenting with hypodiploid acute lymphoblastic leukemia, unexplained sonic hedgehog-driven medulloblastoma, or Jaw osteosarcoma.
- Patients who develop a second primary tumour, within the radiotherapy field of a first core *TP53* tumor which occurred before 46 years, should be tested for germline *TP53* variants
- Children with any cancer from southern and south-eastern Brazilian families should be tested for the p.R337H Brazilian founder germline *TP53* variant.

The ERN does not recommend testing "patients older than 46 years presenting with breast cancer without personal or familial history fulfilling the 'Chompret Criteria'." If a patient with isolated breast cancer does not fulfill the Chompret Criteria but has a *TP53* variant, the patient should be referred to an expert multidisciplinary team for discussion (Frebourg et al., 2020).

The ERN also provides testing recommendations for pre-symptomatic individuals. They recommend that:

- "Adult first-degree relatives of individuals with germline disease causing TP53 variants should be offered testing for the same germline TP53 variant.
- Testing in childhood of first-degree relatives of individuals with germline disease-causing TP53 variants should be systematically offered, if database shows that the variant can be considered as a high cancer risk TP53 variant conferring a high cancer risk in childhood" (Frebourg et al., 2020).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by

the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81351	TP53 (tumor protein 53) (eg, Li-Fraumeni syndrome) gene analysis; full gene sequence
81352	TP53 (tumor protein 53) (eg, Li-Fraumeni syndrome) gene analysis; targeted sequence analysis (eg, 4 oncology)
81353	TP53 (tumor protein 53) (eg, Li-Fraumeni syndrome) gene analysis; known familial variant

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Aury-Landas, J., Bougeard, G., Castel, H., Hernandez-Vargas, H., Drouet, A., Latouche, J. B., Schouft, M. T., Ferec, C., Leroux, D., Lasset, C., Coupier, I., Caron, O., Herceg, Z., Frebourg, T., & Flaman, J. M. (2013). Germline copy number variation of genes involved in chromatin remodelling in families suggestive of Li-Fraumeni syndrome with brain tumours. *Eur J Hum Genet*, *21*(12), 1369-1376. https://doi.org/10.1038/ejhg.2013.68

Bachinski, L. L., Olufemi, S. E., Zhou, X., Wu, C. C., Yip, L., Shete, S., Lozano, G., Amos, C. I., Strong, L. C., & Krahe, R. (2005). Genetic mapping of a third Li-Fraumeni syndrome predisposition locus to human chromosome 1q23. *Cancer Res*, *65*(2), 427-431. https://pubmed.ncbi.nlm.nih.gov/15695383/

Bakhuizen, J. J., Hogervorst, F. B., Velthuizen, M. E., Ruijs, M. W., van Engelen, K., van Os, T. A., Gille, J. J., Collee, M., van den Ouweland, A. M., van Asperen, C. J., Kets, C. M., Mensenkamp, A. R., Leter, E. M., Blok, M. J., de Jong, M. M., & Ausems, M. G. (2019). TP53 germline mutation testing in early-onset breast cancer: findings from a nationwide cohort. *Fam Cancer*, *18*(2), 273-280. https://doi.org/10.1007/s10689-018-00118-0

Barlow, J. W., Mous, M., Wiley, J. C., Varley, J. M., Lozano, G., Strong, L. C., & Malkin, D. (2004). Germ line BAX alterations are infrequent in Li-Fraumeni syndrome. *Cancer Epidemiol Biomarkers Prev*, *13*(8), 1403-1406. https://pubmed.ncbi.nlm.nih.gov/15298965/

Batalini, F., Peacock, E. G., Stobie, L., Robertson, A., Garber, J., Weitzel, J. N., & Tung, N. M. (2019). Li-Fraumeni syndrome: not a straightforward diagnosis anymore-the interpretation of pathogenic variants of low allele frequency and the differences between germline PVs, mosaicism, and clonal hematopoiesis. *Breast Cancer Res*, *21*(1), 107. https://doi.org/10.1186/s13058-019-1193-1

Birch, J. M., Blair, V., Kelsey, A. M., Evans, D. G., Harris, M., Tricker, K. J., & Varley, J. M. (1998). Cancer phenotype correlates with constitutional TP53 genotype in families with the Li-Fraumeni syndrome. *Oncogene*, *17*(9), 1061-1068. https://doi.org/10.1038/sj.onc.1202033

Birch, J. M., Hartley, A. L., Tricker, K. J., Prosser, J., Condie, A., Kelsey, A. M., Harris, M., Jones, P. H., Binchy, A., Crowther, D., & et al. (1994). Prevalence and diversity of constitutional mutations in the p53 gene among 21 Li-Fraumeni families. *Cancer Res*, *54*(5), 1298-1304. http://cancerres.aacrjournals.org/content/54/5/1298.long

BluePrint. (2022). *TP53 single gene test*. https://blueprintgenetics.com/tests/single-gene-tests/tp53-single-gene-test-2/

Bougeard, G., Limacher, J. M., Martin, C., Charbonnier, F., Killian, A., Delattre, O., Longy, M., Jonveaux, P., Fricker, J. P., Stoppa-Lyonnet, D., Flaman, J. M., & Frebourg, T. (2001). Detection of 11 germline inactivating TP53 mutations and absence of TP63 and HCHK2 mutations in 17 French families with Li-Fraumeni or Li-Fraumeni-like syndrome. *J Med Genet*, *38*(4), 253-257. https://doi.org/10.1136/jmg.38.4.253

Bougeard, G., Renaux-Petel, M., Flaman, J. M., Charbonnier, C., Fermey, P., Belotti, M., Gauthier-Villars, M., Stoppa-Lyonnet, D., Consolino, E., Brugieres, L., Caron, O., Benusiglio, P. R., Bressac-de Paillerets, B., Bonadona, V., Bonaiti-Pellie, C., Tinat, J., Baert-Desurmont, S., & Frebourg, T. (2015). Revisiting Li-Fraumeni Syndrome From TP53 Mutation Carriers. *J Clin Oncol*, *33*(21), 2345-2352. https://doi.org/10.1200/jco.2014.59.5728

Bougeard, G., Renaux-Petel, M., Flaman, J. M., Charbonnier, C., Fermey, P., Belotti, M., Gauthier-Villars, M., Stoppa-Lyonnet, D., Consolino, E., Brugières, L., Caron, O., Benusiglio, P. R., Bressac-de Paillerets, B., Bonadona, V., Bonaïti-Pellié, C., Tinat, J., Baert-Desurmont, S., & Frebourg, T. (2015). Revisiting Li-Fraumeni Syndrome From TP53 Mutation Carriers. *J Clin Oncol*, *33*(21), 2345-2352. https://doi.org/10.1200/jco.2014.59.5728

Bougeard, G., Sesboue, R., Baert-Desurmont, S., Vasseur, S., Martin, C., Tinat, J., Brugieres, L., Chompret, A., de Paillerets, B. B., Stoppa-Lyonnet, D., Bonaiti-Pellie, C., Frebourg, T., & French, L. F. S. w. g. (2008). Molecular basis of the Li-Fraumeni syndrome: an update from the French LFS families. *J Med Genet*, *45*(8), 535-538. https://doi.org/10.1136/jmg.2008.057570

Brosh, R., & Rotter, V. (2009). When mutants gain new powers: news from the mutant p53 field. *Nat Rev Cancer*, *9*(10), 701-713. https://doi.org/10.1038/nrc2693

Brown, L. T., Sexsmith, E., & Malkin, D. (2000). Identification of a novel PTEN intronic deletion in Li-Fraumeni syndrome and its effect on RNA processing. *Cancer Genet Cytogenet*, *123*(1), 65-68. https://doi.org/10.1016/S0165-4608(00)00303-4

Chompret, A., Abel, A., Stoppa-Lyonnet, D., Brugieres, L., Pages, S., Feunteun, J., & Bonaiti-Pellie, C. (2001). Sensitivity and predictive value of criteria for p53 germline mutation screening. *J Med Genet*, *38*(1), 43-47. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1734716/pdf/v038p00043.pdf

Correa, H. (2016). Li-Fraumeni Syndrome. *J Pediatr Genet*, *5*(2), 84-88. https://doi.org/10.1055/s-0036-1579759

Eeles, R. A. (1995). Germline mutations in the TP53 gene. *Cancer Surv*, *25*, 101-124.

Evans, D. G. (2024, March 13). *Li-Fraumeni syndrome*. https://www.uptodate.com/contents/li-fraumeni-syndrome

Farrell, C. J., & Plotkin, S. R. (2007). Genetic causes of brain tumors: neurofibromatosis, tuberous sclerosis, von Hippel-Lindau, and other syndromes. *Neurol Clin*, *25*(4), 925-946, viii. https://doi.org/10.1016/j.ncl.2007.07.008

Fortuno, C., Lee, K., Olivier, M., Pesaran, T., Mai, P. L., de Andrade, K. C., Attardi, L. D., Crowley, S., Evans, D. G., Feng, B. J., Foreman, A. K. M., Frone, M. N., Huether, R., James, P. A., McGoldrick, K., Mester, J., Seifert, B. A., Slavin, T. P., Witkowski, L., . . . Savage, S. A. (2021). Specifications of the ACMG/AMP variant interpretation guidelines for germline TP53 variants. *Hum Mutat*, *42*(3), 223-236. https://doi.org/10.1002/humu.24152

Frebourg, T., Bajalica Lagercrantz, S., Oliveira, C., Magenheim, R., & Evans, D. G. (2020). Guidelines for the Li-Fraumeni and heritable TP53-related cancer syndromes. *Eur J Hum Genet*, *28*(10), 1379-1386. https://doi.org/10.1038/s41431-020-0638-4

Garber, J. E., Goldstein, A. M., Kantor, A. F., Dreyfus, M. G., Fraumeni, J. F., Jr., & Li, F. P. (1991). Follow-up study of twenty-four families with Li-Fraumeni syndrome. *Cancer Res*, *51*(22), 6094-6097. https://pubmed.ncbi.nlm.nih.gov/1933872/

Gonzalez, K. D., Noltner, K. A., Buzin, C. H., Gu, D., Wen-Fong, C. Y., Nguyen, V. Q., Han, J. H., Lowstuter, K., Longmate, J., Sommer, S. S., & Weitzel, J. N. (2009). Beyond Li Fraumeni Syndrome: clinical characteristics of families with p53 germline mutations. *J Clin Oncol*, *27*(8), 1250-1256. https://doi.org/10.1200/jco.2008.16.6959

Herrmann, L. J., Heinze, B., Fassnacht, M., Willenberg, H. S., Quinkler, M., Reisch, N., Zink, M., Allolio, B., & Hahner, S. (2012). TP53 germline mutations in adult patients with adrenocortical carcinoma. *J Clin Endocrinol Metab*, *97*(3), E476-485. https://doi.org/10.1210/jc.2011-1982

Hwang, S. J., Lozano, G., Amos, C. I., & Strong, L. C. (2003). Germline p53 mutations in a cohort with childhood sarcoma: sex differences in cancer risk. *Am J Hum Genet*, *72*(4), 975-983. https://doi.org/10.1086/374567

Invitae. (2024). *Invitae Li-Fraumeni Syndrome Test*. https://www.invitae.com/us/providers/test-catalog/gene-20572

Kalia, S. S., Adelman, K., Bale, S. J., Chung, W. K., Eng, C., Evans, J. P., Herman, G. E., Hufnagel, S. B., Klein, T. E., Korf, B. R., McKelvey, K. D., Ormond, K. E., Richards, C. S., Vlangos, C. N., Watson, M., Martin, C. L., & Miller, D. T. (2017). Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. *Genet Med*, *19*(2), 249-255. https://doi.org/10.1038/gim.2016.190

LFSA. (2024). *CRITERIA FOR LFS | LI-FRAUMENI SYNDROME ASSOCIATION*. Li-Fraumeni Syndrome Association. Retrieved 05/15/2019 from https://www.lfsassociation.org/what-is-lfs/lfs-critieria/

Li, F. P., Fraumeni, J. F., Jr., Mulvihill, J. J., Blattner, W. A., Dreyfus, M. G., Tucker, M. A., & Miller, R. W. (1988). A cancer family syndrome in twenty-four kindreds. *Cancer Res*, *48*(18), 5358-5362. http://cancerres.aacrjournals.org/content/48/18/5358.long

Lincoln, S. E., Nussbaum, R. L., Kurian, A. W., Nielsen, S. M., Das, K., Michalski, S., Yang, S., Ngo, N., Blanco, A., & Esplin, E. D. (2020). Yield and Utility of Germline Testing Following Tumor Sequencing in Patients With Cancer. *JAMA Netw Open*, *3*(10), e2019452. https://doi.org/10.1001/jamanetworkopen.2020.19452

Lustbader, E. D., Williams, W. R., Bondy, M. L., Strom, S., & Strong, L. C. (1992). Segregation analysis of cancer in families of childhood soft-tissue-sarcoma patients. *Am J Hum Genet*, *51*(2), 344-356. https://pubmed.ncbi.nlm.nih.gov/1642235/

Lynch, H. T., Mulcahy, G. M., Harris, R. E., Guirgis, H. A., & Lynch, J. F. (1978). Genetic and pathologic findings in a kindred with hereditary sarcoma, breast cancer, brain tumors, leukemia, lung, laryngeal, and adrenal cortical carcinoma. *Cancer*, *41*(5), 2055-2064. https://doi.org/10.1002/1097-0142(197805)41:5%3C2055::AID-CNCR2820410554%3E3.0.CO;2-X

Mai, P. L., Best, A. F., Peters, J. A., DeCastro, R. M., Khincha, P. P., Loud, J. T., Bremer, R. C., Rosenberg, P. S., & Savage, S. A. (2016). Risks of first and subsequent cancers among TP53 mutation carriers in the National Cancer Institute Li-Fraumeni syndrome cohort. *Cancer*, *122*(23), 3673-3681. https://doi.org/10.1002/cncr.30248

Mai, P. L., Malkin, D., Garber, J. E., Schiffman, J. D., Weitzel, J. N., Strong, L. C., Wyss, O., Locke, L., Means, V., Achatz, M. I., Hainaut, P., Frebourg, T., Evans, D. G., Bleiker, E., Patenaude, A., Schneider, K., Wilfond, B., Peters, J. A., Hwang, P. M., . . . Savage, S. A. (2012). Li-Fraumeni syndrome: report of a clinical research workshop and creation of a research consortium. *Cancer Genet*, *205*(10), 479-487. https://doi.org/10.1016/j.cancergen.2012.06.008

Malkin, D. (2011). Li-fraumeni syndrome. *Genes Cancer*, *2*(4), 475-484. https://doi.org/10.1177/1947601911413466

Malkin, D., Li, F. P., Strong, L. C., Fraumeni, J. F., Jr., Nelson, C. E., Kim, D. H., Kassel, J., Gryka, M. A., Bischoff, F. Z., Tainsky, M. A., & et al. (1990). Germ line p53 mutations in

a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science*, *250*(4985), 1233-1238. https://doi.org/10.1126/science.1978757

Manahan, E. R., Kuerer, H. M., Sebastian, M., Hughes, K. S., Boughey, J. C., Euhus, D. M., Boolbol, S. K., & Taylor, W. A. (2019). Consensus Guidelines on Genetic` Testing for Hereditary Breast Cancer from the American Society of Breast Surgeons. *Ann Surg Oncol*, *26*(10), 3025-3031. https://doi.org/10.1245/s10434-019-07549-8

Masciari, S., Dewanwala, A., Stoffel, E. M., Lauwers, G. Y., Zheng, H., Achatz, M. I., Riegert-Johnson, D., Foretova, L., Silva, E. M., Digianni, L., Verselis, S. J., Schneider, K., Li, F. P., Fraumeni, J., Garber, J. E., & Syngal, S. (2011). Gastric cancer in individuals with Li-Fraumeni syndrome. *Genet Med*, *13*(7), 651-657. https://doi.org/10.1097/GIM.0b013e31821628b6

McBride, K. A., Ballinger, M. L., Killick, E., Kirk, J., Tattersall, M. H., Eeles, R. A., Thomas, D. M., & Mitchell, G. (2014). Li-Fraumeni syndrome: cancer risk assessment and clinical management. *Nat Rev Clin Oncol*, *11*(5), 260-271. https://doi.org/10.1038/nrclinonc.2014.41

Nagy, R., Sweet, K., & Eng, C. (2004). Highly penetrant hereditary cancer syndromes. *Oncogene*, *23*(38), 6445-6470. https://doi.org/10.1038/sj.onc.1207714

NCCN. (2024, February 12, 2024). *Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic Version 3.2024*. https://www.nccn.org/professionals/physician_gls/pdf/genetics_bop.pdf

NORD. (2021). *Li-Fraumeni Syndrome*. https://rarediseases.org/rare-diseases/li-fraumeni-syndrome/

Ognjanovic, S., Olivier, M., Bergemann, T. L., & Hainaut, P. (2012). Sarcomas in TP53 germline mutation carriers: a review of the IARC TP53 database. *Cancer*, *118*(5), 1387-1396. https://doi.org/10.1002/cncr.26390

Olivier, M., Goldgar, D. E., Sodha, N., Ohgaki, H., Kleihues, P., Hainaut, P., & Eeles, R. A. (2003). Li-Fraumeni and related syndromes: correlation between tumor type, family structure, and TP53 genotype. *Cancer Res*, *63*(20), 6643-6650. https://pubmed.ncbi.nlm.nih.gov/14583457/

Olivier, M., Hollstein, M., & Hainaut, P. (2010). TP53 mutations in human cancers: origins, consequences, and clinical use. *Cold Spring Harb Perspect Biol*, *2*(1), a001008. https://doi.org/10.1101/cshperspect.a001008

Palmero, E. I., Achatz, M. I., Ashton-Prolla, P., Olivier, M., & Hainaut, P. (2010). Tumor protein 53 mutations and inherited cancer: beyond Li-Fraumeni syndrome. *Curr Opin Oncol*, *22*(1), 64-69. https://doi.org/10.1097/CCO.0b013e328333bf00

Patel, K. B., Oh, W. K., Jun, T., Naidu, S., Nathwani, N., Hansen, G., Hubert, A., Mukhi, H., Chhadwa, S., Zabala, V., Gill, S., Branchcomb, R., Mehta, D., Nixon, S., Kodali, A., & Zimmerman, M. (2022). Clinical utility of widespread germline testing of cancer patients in a diverse community cancer clinic. *Journal of Clinical Oncology*, *40*(16_suppl), e18537-e18537. https://doi.org/10.1200/JCO.2022.40.16_suppl.e18537

Peshkin, B. N. I., Claudine,. (2024, March 18). *Genetic testing and management of individuals at risk of hereditary breast and ovarian cancer syndromes*. https://www.uptodate.com/contents/genetic-testing-and-management-of-individuals-at-risk-of-hereditary-breast-and-ovarian-cancer-syndromes#H15

Petitjean, A., Mathe, E., Kato, S., Ishioka, C., Tavtigian, S. V., Hainaut, P., & Olivier, M. (2007). Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database. *Hum Mutat*, *28*(6), 622-629. https://doi.org/10.1002/humu.20495

Portwine, C., Lees, J., Verselis, S., Li, F. P., & Malkin, D. (2000). Absence of germline p16(INK4a) alterations in p53 wild type Li-Fraumeni syndrome families. *J Med Genet*, *37*(8), E13. https://doi.org/10.1136/jmg.37.8.e13

Rana, H. Q., Gelman, R., LaDuca, H., McFarland, R., Dalton, E., Thompson, J., Speare, V., Dolinsky, J. S., Chao, E. C., & Garber, J. E. (2018). Differences in TP53 Mutation Carrier Phenotypes Emerge From Panel-Based Testing. *J Natl Cancer Inst*. https://doi.org/10.1093/jnci/djy001

Ruijs, M. W., Verhoef, S., Rookus, M. A., Pruntel, R., van der Hout, A. H., Hogervorst, F. B., Kluijt, I., Sijmons, R. H., Aalfs, C. M., Wagner, A., Ausems, M. G., Hoogerbrugge, N., van Asperen, C. J., Gomez Garcia, E. B., Meijers-Heijboer, H., Ten Kate, L. P., Menko, F. H., & van 't Veer, L. J. (2010). TP53 germline mutation testing in 180 families suspected of Li-Fraumeni syndrome: mutation detection rate and relative frequency of cancers in different familial phenotypes. In *J Med Genet* (Vol. 47, pp. 421-428). https://doi.org/10.1136/jmg.2009.073429

Schneider, K., Zelley, K., Nichols, K. E., & Garber, J. (2013). Li-Fraumeni Syndrome. In R. A. Pagon, M. P. Adam, H. H. Ardinger, S. E. Wallace, A. Amemiya, L. J. Bean, T. D. Bird, N. Ledbetter, H. C. Mefford, R. J. Smith, & K. Stephens (Eds.), *GeneReviews*. University of Washington, Seattle. https://www.ncbi.nlm.nih.gov/books/NBK1311/

Sigal, A., & Rotter, V. (2000). Oncogenic mutations of the p53 tumor suppressor: the demons of the guardian of the genome. *Cancer Res*, *60*(24), 6788-6793. https://aacrjournals.org/cancerres/article/60/24/6788/507141/Oncogenic-Mutations-of-the-p53-Tumor-Suppressor

Sorrell, A. D., Espenschied, C. R., Culver, J. O., & Weitzel, J. N. (2013). TP53 Testing and Li-Fraumeni Syndrome: Current Status of Clinical Applications and Future Directions. *Mol Diagn Ther*, *17*(1), 31-47. https://doi.org/10.1007/s40291-013-0020-0

Stone, J. G., Eeles, R. A., Sodha, N., Murday, V., Sheriden, E., & Houlston, R. S. (1999). Analysis of Li-Fraumeni syndrome and Li-Fraumeni-like families for germline mutations in Bcl10. *Cancer Lett*, *147*(1-2), 181-185. https://doi.org/10.1016/S0304-3835(99)00291-8

Terradas, M., Mur, P., Belhadj, S., Woodward, E. R., Burghel, G. J., Munoz-Torres, P. M., Quintana, I., Navarro, M., Brunet, J., Lazaro, C., Pineda, M., Moreno, V., Capella, G., Evans, D. G. R., & Valle, L. (2021). TP53, a gene for colorectal cancer

predisposition in the absence of Li-Fraumeni-associated phenotypes. *Gut*, *70*(6), 1139-1146. https://doi.org/10.1136/gutjnl-2020-321825

Tinat, J., Bougeard, G., Baert-Desurmont, S., Vasseur, S., Martin, C., Bouvignies, E., Caron, O., Bressac-de Paillerets, B., Berthet, P., Dugast, C., Bonaiti-Pellie, C., Stoppa-Lyonnet, D., & Frebourg, T. (2009). 2009 version of the Chompret criteria for Li Fraumeni syndrome. *J Clin Oncol*, *27*(26), e108-109; author reply e110. https://doi.org/10.1200/jco.2009.22.7967

Varley, J. M. (2003). Germline TP53 mutations and Li-Fraumeni syndrome. *Hum Mutat*, *21*(3), 313-320. https://doi.org/10.1002/humu.10185

Villani, A., Shore, A., Wasserman, J. D., Stephens, D., Kim, R. H., Druker, H., Gallinger, B., Naumer, A., Kohlmann, W., Novokmet, A., Tabori, U., Tijerin, M., Greer, M. L., Finlay, J. L., Schiffman, J. D., & Malkin, D. (2016). Biochemical and imaging surveillance in germline TP53 mutation carriers with Li-Fraumeni syndrome: 11 year follow-up of a prospective observational study. *Lancet Oncol*, *17*(9), 1295-1305. https://doi.org/10.1016/s1470-2045(16)30249-2

Villani, A., Tabori, U., Schiffman, J., Shlien, A., Beyene, J., Druker, H., Novokmet, A., Finlay, J., & Malkin, D. (2011). Biochemical and imaging surveillance in germline TP53 mutation carriers with Li-Fraumeni syndrome: a prospective observational study. *Lancet Oncol*, *12*(6), 559-567. https://doi.org/10.1016/s1470-2045(11)70119-x

Walsh, T., Casadei, S., Lee, M. K., Pennil, C. C., Nord, A. S., Thornton, A. M., Roeb, W., Agnew, K. J., Stray, S. M., Wickramanayake, A., Norquist, B., Pennington, K. P., Garcia, R. L., King, M. C., & Swisher, E. M. (2011). Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. *Proc Natl Acad Sci U S A*, *108*(44), 18032-18037. https://doi.org/10.1073/pnas.1115052108

Wong, P., Verselis, S. J., Garber, J. E., Schneider, K., DiGianni, L., Stockwell, D. H., Li, F. P., & Syngal, S. (2006). Prevalence of early onset colorectal cancer in 397 patients with classic Li-Fraumeni syndrome. *Gastroenterology*, *130*(1), 73-79. https://doi.org/10.1053/j.gastro.2005.10.014

Yamamoto, Y., Kanai, M., Kou, T., Sugiyama, A., Nakamura, E., Miyake, H., Yamada, T., Nishigaki, M., Kondo, T., Murakami, H., Torishima, M., Matsumoto, S., Kosugi, S., & Muto, M. (2020). Clinical significance of TP53 variants as possible secondary findings in tumor-only next-generation sequencing. *Journal of Human Genetics*, *65*(2), 125-132. https://doi.org/10.1038/s10038-019-0681-6

Genetic Testing for Lipoprotein(a) Variant(s) as a Decision Aid for Aspirin Treatment and/or CVD Risk Assessment

Policy Number: AHS – M2082 – Genetic Testing for Lipoprotein(a) Variant(s) as a Decision Aid for Aspirin Treatment and/or CVD Risk Assessment	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Lipoprotein(a) (Lp(a)) is a type of low-density lipoprotein (LDL) that consists of a cholesterol bearing LDL – like particle (apolipoprotein B-100) bound to the plasminogen-like glycoprotein apolipoprotein(a) (apo(a)) (Lu et al., 2015; Schmidt et al., 2016) and has been associated with increased risk for cardiovascular disease (CVD) (Tsimikas et al., 2018). Genetic variants of the Lp(a) gene, *LPA*, (rs3798220 and rs10455872) have been significantly associated with Lp(a) levels (Lu et al., 2015) and could serve as indicators of CVD risk (Lee et al., 2017). The genetic variant rs3798220 was found to have a higher risk for thrombosis and therefore may derive more benefit from the anti-thrombotic properties of aspirin (Chasman et al., 2009). As a result, testing for the rs3798220 variant has been proposed as a method of stratifying benefit from aspirin treatment (Shiffman et al., 2012).

This policy only addresses the detection of specific genetic variants of Lp(a) as a decision aid for aspirin therapy or CVD risk.

For information on serum measurement of Lp(a) levels see AHS-G2050-Cardiovascular Disease Risk Assessment.

II. Related Policies

Policy Number	Policy Title
AHS-M2180	Genetic Markers for Assessing Risk of Cardiovascular Disease

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

1) Genetic testing for *LPA* variant(s) (e.g., LPA-Aspirin Check® and Cardio IQ® LPA Aspirin Genotype) **DOES NOT MEET COVERAGE CRITERIA**.

IV. Table of Terminology

Term	Definition
AACE	American Association of Clinical Endocrinologists
ACC	American College of Cardiology
ACE	American College of Endocrinology
AHA	American Heart Association
apo(a)	Apolipoprotein(A)
APOE	*Apolipoprotein E*
AS	Aortic stenosis
ASCP	American Society for Clinical Pathology
ASCVD	Atherosclerotic cardiovascular disease
BMI	Body mass index
CAC	Coronary artery calcification
CAVD	Calcific aortic stenosis
CAVD	Calcific aortic valve disease
CHD	Coronary heart disease
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid
CNVs	Copy number variants
CV	Cardiovascular
CVD	Cardiovascular Disease
DNA	Deoxyribonucleic acid
EAS	European Atherosclerosis Society
ESC	The European Society for Cardiology
FDA	Food and Drug Administration

Confidential and Proprietary Information of Avalon Health Services, LLC, d/b/a Avalon Healthcare Solutions. All Rights Reserved.

FH	Familial hypercholesterolemia
HDL	High-density lipoprotein
KIV	Kringle (five cysteine-rich domains) IV
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol
LDT	Laboratory developed test
LDTs	Laboratory-developed tests
LPA	Lipoprotein(a)
Lp(a)	Lipoprotein(a)
MI	Myocardial infarctions
NHLBI	National Heart, Lung, and Blood Institute
NLA	The National Lipid Association
PCI	Percutaneous coronary intervention
PCSK9	Proprotein convertase subtilisin/kexin type 9
PSA	Prostate specific antigen
SNP	Single nucleotide polymorphism
SNPs	Single nucleotide polymorphisms
USPSTF	United States Preventive Services Task Force

V. Scientific Background

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality; with over 11.5% of American adults (27.6 million) diagnosed with heart disease, it claims more lives each year than cancer and chronic lower respiratory disease combined (Benjamin et al., 2018). While progression of CVD is multifactorial, pathophysiological, and epidemiological, genetic studies have provided substantial evidence that Lp(a) is a causal risk factor contributing to CVD (Rosenson et al., 2024). Lp(a) is also elevated in heterozygous familial hypercholesterolemia, further increasing atherosclerotic CVD risk in that disease setting (Rosenson et al., 2024). The physiological role of Lp(a) is to bind and transport proinflammatory oxidized phospholipids in plasma, but its key relation to CVD has been the involvement in atherothrombosis, from the formation of an atherosclerotic plaque through inducing expression of inflammatory mediators and increasing foam formation, to thrombosis following plaque rupture (Fras, 2020). Independent of any other risk factors, Lp(a) was positively associated with increased risk of myocardial infarctions (MI) as well (Paré et al., 2019).

Since first described by Berg (1963) as a genetic trait increased in patients with coronary heart disease (Berg et al., 1974), Lp(a) has been characterized as a type of LDL consisting of apolipoprotein B-100 covalently bound to apolipoprotein(a) (Steyrer et al., 1994). Lipoprotein(a) levels are 75% to 95% heritable and predominately

Confidential and Proprietary Information of Avalon Health Services, LLC, d/b/a Avalon Healthcare Solutions. All Rights Reserved.

determined by single-nucleotide variants at the LPA gene and copy number variants (CNVs) in the kringle IV type 2 domain (Trinder et al., 2021). The plasma level and size of Lp(a) are regulated through strict genetic control by the apo(a) gene (*LPA*) on chromosome 6q26-27 with some influence from the *APOE* locus (Erhart et al., 2018; Lu et al., 2015; Moriarty et al., 2017). The *LPA* gene is highly polymorphic based on the number of kringle (five cysteine-rich domains) IV (KIV) repeats, therefore encoding >40 apo(a) isoforms (Marcovina et al., 1996) of varying molecular weights (Rosenson et al., 2024).

As it is genetically controlled, the concentration of Lp(a) is generally stable, correlates inversely with molecular size (smaller size correlating with higher serum levels), and is minimally influenced by age, weight, and diet (Enkhmaa et al., 2016; Tregouet et al., 2009). Genetic variants of apo(a) have been found to have predictive value in coronary heart disease (CHD) (Anderson et al., 2013; Cairns et al., 2017; Helgadottir et al., 2012; Lee et al., 2017; Zekavat et al., 2018; Zewinger et al., 2017). Beyond CHD, genetically lowered Lp(a) is also associated with a lower risk of peripheral vascular disease, stroke, heart failure, and aortic stenosis (Emdin et al., 2016).

The prevalence and association of these genetic variants with apo(a) size and Lp(a) levels are highly variable and ethnicity-specific. Out of 118 single nucleotide polymorphisms (SNPs) identified, rs3798220 is most prevalent in Hispanics (42.38%), rs10455872 in Whites (14.27%), and rs9457951 in Blacks (32.92%). In Hispanics, the rs3798220 variant was associated with large isoforms and lower Lp(a) levels, but in Whites, this variant was associated with very small isoforms and higher Lp(a) levels (Lee et al., 2017). In a separate study that analyzed the relationship between Lp(a) concentration and risk of MI, Paré et al. (2019) found that the clinical use of Lp(a) concentrations for interventions to reduce MI risk would be useful among diverse populations, especially South Asians and Latin Americans, but not Africans or Arabs since there was an insignificant association between high Lp(a) concentration and MI risk in these populations (Paré et al., 2019).

Although its biology and pathophysiology are still incompletely understood (Tsimikas et al., 2018), Lp(a) is recognized as both atherogenic (Grainger et al., 1993; Hajjar et al., 1989; Helgadottir et al., 2012) and thrombogenic (Caplice et al., 2001; Marcovina & Koschinsky, 2003), possibly due to its structural homology with plasminogen (Hancock et al., 2003; McLean et al., 1987). It is thought that Lp(a) could compete with plasminogen for fibrin binding, ultimately resulting in impaired fibrinolysis (Hervio et al., 1995).

A specific SNP in the *LPA* gene (rs3798220) results in an isoleucine-to-methionine substitution within the inactive protease domain, triggering a smaller number of

Confidential and Proprietary Information of Avalon Health Services, LLC, d/b/a Avalon Healthcare Solutions. All Rights Reserved.

kringle IV repeats, elevated Lp(a) levels, and a greater risk for CVD (Clarke et al., 2009; Helgadottir et al., 2012; Luke et al., 2007). This amino acid substitution (I4399M) has been studied for its effects on coagulation, fibrinolysis, and overall fibrin cloth structure (Scipione et al., 2017).

Carriers of either the rs3798220 or rs10455872 variant were found to have no difference in plasminogen concentration or clot lysis time (Wang et al., 2016). The I4399M variant was found to accelerate the coagulation of plasma clots *in vitro*, therefore suggesting that those with this variant may benefit from the anti-thrombotic properties of aspirin (Scipione et al., 2017). Further, a difference in phenotypic expression between different ethnic groups has been found. Among non-Caucasians, carriers of the rs3798220 variant had increased clot permeability and shorter lysis time, whereas among Caucasians, the trend was for decreased permeability and longer lysis time (Rowland et al., 2014). A correlation was identified between the I4399M variant and both elevated plasma Lp(a) levels and an increased risk of CHD; carriers of this variant in population studies also showed an increased benefit of aspirin therapy (Scipione et al., 2017).

Clinical Utility and Validity

The additional information obtained from the testing for Lp(a) genotype may aid physicians in better estimating the benefit/risk of aspirin therapy and therefore aid in deciding whether to prescribe aspirin for individual patients. *LPA* genotyping in the context of the aspirin use guidelines for primary prevention of CVD was found to be potentially cost-effective (Shiffman et al., 2012). However, traditional plasma-based hemostasis-thrombosis laboratory testing may be more effective at managing venous thrombotic disease than a single DNA variant with a small effect size and no established mechanism linking aspirin with Lp(a) (Nagalla & Bray, 2016).

After a randomized trial of low-dose aspirin, the authors found that rs3798220 was associated with elevated Lp(a) and doubled CVD risk that could be attenuated by aspirin; carriers appeared to benefit more from aspirin than non-carriers (Chasman et al., 2009).

Ozkan et al. (2019) have recently shown that Lp(a) gene polymorphisms play a role in the development of calcific aortic stenosis or calcific aortic valve disease (CAVD). Blood samples were taken from 75 patients previously diagnosed with CAVD and 77 healthy controls, and results showed that "A significant association among smoking, elevated LDL level and creatinine, low albumin levels, Lp(a) level, rs10455872, and rs3798220 polymorphisms may be considered genetic risk factors for the development of calcific aortic stenosis" (Ozkan et al., 2019). However, even with a strong statistically significant relationship between the Lp(a) gene polymorphisms (rs10455872 and rs3798220) and

Confidential and Proprietary Information of Avalon Health Services, LLC, d/b/a Avalon Healthcare Solutions. All Rights Reserved.

CAVD, this study contained a relatively small sample size, suggesting that more research needs to be completed to validate these results. This research has been corroborated by Pechlivanis et al. (2020) who demonstrated that the rs10455872 SNP has a statistically significant association with coronary artery calcification, a predictor of coronary artery disease (Pechlivanis et al., 2020).

A large-scale study with 44,703 participants of European descent was completed, and a relationship was identified between two Lp(a) variants (rs10455872 and rs3798220) and aortic stenosis (AS) development (Chen et al., 2018). While a relationship between both of these Lp(a) variants has already been established in regard to circulating Lp(a) plasma levels and a high Lp(a) risk score, these data seem to confirm the association between these Lp(a) variants and valvular or cardiac disease events. Final results from this study showed that the participants with these two high-risk alleles had a twice or greater chance of developing AS; however, it must be noted that participants with AS were on average older than the controls, meaning that some controls could still develop AS (Chen et al., 2018).

Mu-Han-Ha-Li et al. (2018) conducted a study with 1,863 Chinese patients with very high CVD risk (as identified on coronary angiography) to analyze the connection between Lp(a) levels and the risks of CVD and diabetes. Researchers concluded that a high number of *LPA* KIV type 2 repeats, and therefore lower serum Lp(a) levels, is associated with an increased risk of type 2 diabetes in a Chinese population with high CVD risk. This data suggests that a large Lp(a) isoform size, and thus low Lp(a) concentration, can have a causal effect on type 2 diabetes (Mu-Han-Ha-Li et al., 2018). With this novel association, it becomes essential for genetic testing of *LPA* gene variants to not only follow up on CVD risk to assess benefit from aspirin therapy, but for the possible latter development of comorbidities like type two diabetes.

Additional researchers have identified a potential relationship between Lp(a) SNPs and a high inflammatory response that may result in an increased CVD risk in pregnant individuals. Tuten et al. (2019) analyzed data from 200 pregnant Turkish individuals, evaluating 14 different Lp(a) SNPs. Results found that two of the Lp(a) SNPs, rs9355296 and rs3798220, were identified as risk factors for preeclampsia, and that rs9355296 carriers reported higher vascular inflammatory rates (Tuten et al., 2019). These results suggest that specific Lp(a) variants may possibly be used as biomarkers for future cardiovascular events and inflammation.

Moreover, Wang and Zhang (2019) showed that high Lp(a) levels are associated with adverse clinicopathological features in prostate cancer patients. Patients with a prostate specific antigen (PSA) level ≥ 100 ng/ml had significantly higher Lp(a) levels; this was believed to be a result of compensatory mechanisms to chronic inflammation

Confidential and Proprietary Information of Avalon Health Services, LLC, d/b/a Avalon Healthcare Solutions. All Rights Reserved.

caused by tumor aggressiveness and invasion. The researchers also found that the percentage of metastases increased with elevation in Lp(a) level, while body mass index (BMI) decreased with the Lp(a) elevation. The increased metastasis in the setting of high Lp(a) levels was believed to be due to facilitated formations of fibrin networks (apo(a), a part of Lp(a), has structural homologues to kringle IV in plasminogen, which normally induces fibrinolysis) and thrombus formation that allowed for cancer cell adhesion (Wang & Zhang, 2019). Genetic testing for Lp(a) may not only benefit CVD risk assessment with aspirin therapy considerations, but also may have implications for cancer development and treatment.

Pechlivanis et al. (2020) studied the association of *LPA* gene variants (rs10455872 and rs3798220) and IL1F9 (rs13415097) with coronary artery calcification (CAC). LPA levels from 3799 patients were analyzed using linear regression models to explore the association between the variants and CAC. The LPA SNP rs10455872 showed a statistically significant association with CAC. The results of this study show that "rs10455872, mediated by Lp(a) levels, might play a role in promoting the development of atherosclerosis leading to cardiovascular disease events" (Pechlivanis et al., 2020).

In a prospective study, Yoon et al. (2021) studied the association of LPA with recurrent ischemic events after percutaneous coronary intervention (PCI). Baseline LPA levels from 12,064 patients who underwent PCI were studied. A total of 3,747 (31.1%) patients had high Lp(a) (>30 mg/dL) and 8,317 (68.9%) patients had low Lp(a) (≤30 mg/dL). After a seven-year follow-up, 2.0 per 100 person-years in the high-LPA group experienced CV death, spontaneous myocardial infarction, and ischemic stroke compared to 1.6 per 100 person-years in the low-Lp(a) group. Overall, the authors conclude that "Elevated levels of Lp(a) were significantly associated with the recurrent ischemic events in patients who underwent PCI" which provides a rationale to test LPA lowering therapy for secondary prevention in patients undergoing PCI (Yoon et al., 2021).

Murdock et al. (2021) developed a panel test for genes associated with CVD that included an evaluation of *LPA* polymorphisms. The authors studied 709 patients from cardiology clinics. In total, "32% of patients had a genetic finding with clinical management implications." *LPA* polymorphisms were found in 20% of patients, which lead to "diet, lifestyle, and other changes." The authors concluded that the results "support the use of genetic information in routine cardiovascular health management" (Murdock et al., 2021). Familial hypercholesterolemia (FH) and elevated plasma Lp(a) are both inherited conditions associated with ASCVD. Chakraborty et al. (2022) studied the detection of elevated Lp(a) during cascade testing of relatives of people diagnosed with FH. The authors used an immunoassay to test for FH and Lp(a) in 162 people. The

Confidential and Proprietary Information of Avalon Health Services, LLC, d/b/a Avalon Healthcare Solutions. All Rights Reserved.

prevalence of FH and elevated Lp(a) was 60.5% in adults (n=136) and 41.1% in children (n=26). The proportion of relatives with elevated Lp(a) was higher when they had relatives with Lp(a) ≥100 mg/dL than relatives with Lp(a) between 50 and 99 mg/dL. The authors concluded that dual testing of families for FH and high Lp(a) from appropriate relatives can detect new cases of FH, and elevated Lp(a) with or without FH. Lastly, the authors note that "the findings accord with the co-dominant and independent heritability of FH and Lp(a)" (Chakraborty et al., 2022).

Gu et al. (2022) investigated whether elevated Lp(a) and two corresponding *LPA* polymorphisms played a role in the prediction of cardiovascular events. The authors enrolled 2766 individuals from Peking University People's Hospital. The study included a cohort with coronary heart disease and a separate control group. The authors demonstrated that Lp(a) level and the amount of *LPA* SNPs were associated with the risk and severity of coronary heart disease. The rs6415084 and rs12194138 polymorphisms were particularly associated with serum Lp(a) levels. The authors concluded via univariate logistic regression analysis and multiple logistic regression analysis that, "These data together indicated that the level of Lp(a) and the prevalence of LPA SNPs rs6415084 (CT/TT) and rs12194138 (AT/TT) is positively correlated with the severity of CHD" (Gu et al., 2022).

VI. Guidelines and Recommendations

American Association of Clinical Endocrinologists (AACE) and American College of Endocrinology (ACE)

The AACE/ACE published guidelines for the management of Dyslipidemia and Prevention of Cardiovascular Disease (Jellinger et al., 2017) which state:

"Testing for lipoprotein(a) is therefore not generally recommended, although it may provide useful information to ascribe risk in Caucasians with ASCVD, those with an unexplained family history of early ASCVD, or those with unknown family history such as adopted individuals."

In 2020, the AACE and ACE released a consensus statement for the management of Dyslipidemia and Prevention of Cardiovascular Disease and provided recommendations for the assessment and management of elevated Lipoprotein (a). Within this statement, they recommend measuring Lp(a) in patients with a family history of premature ASCVD and/or increased Lp(a) and all patients with premature or recurrent ASCVD despite LDL-C lowering (Handelsman et al., 2020).

Genetic screening for Lp(a) variants is not mentioned.

Confidential and Proprietary Information of Avalon Health Services, LLC, d/b/a Avalon Healthcare Solutions. All Rights Reserved.

National Heart, Lung, and Blood Institute (NHLBI)

The NHLBI published Working Group Recommendations to Reduce Lipoprotein(a)-Mediated Risk of Cardiovascular Disease and Aortic Stenosis (Tsimikas et al., 2018) which endorsed the European Society of Cardiology/European Atherosclerosis Society, Canadian Cardiovascular Society, and National Lipid Association Guidelines while making additional specific recommendations to facilitate basic, mechanistic, preclinical, and clinical research on Lp(a).

Genetic screening for Lp(a) variants is not mentioned (Tsimikas et al., 2018).

American College of Cardiology (ACC)/American Heart Association (AHH)

In 2019, the ACC and AHA released guidelines on the primary prevention of CVD. The guidelines list "Risk-Enhancing Factors for Clinician–Patient Risk Discussion" which include Lp(a) as a "lipid/biomarker associated with increased atherosclerotic cardiovascular disease risk." The guidelines note, "elevated Lp(a): A relative indication for its measurement is family history of premature ASCVD. An Lp(a) ≥50 mg/dL or ≥125 nmol/L constitutes a risk-enhancing factor, especially at higher levels of Lp(a)" (Arnett et al., 2019).

In 2022, the AHA released a scientific statement about Lp(a) that supports the ACC/AHH joint guidelines. The statement also includes recommendations on how and when Lp(a) testing should be performed. "At present, the evidence in favor of screening for Lp(a) is the strongest for those with a family or personal history of ASCVD, with consideration of cascade screening in appropriate individuals" (Reyes-Soffer et al., 2022).

Genetic screening for Lp(a) variants is not mentioned (Reyes-Soffer et al., 2022).

The National Lipid Association (NLA)

The NLA considers Lp(a) to be an important clinical biomarker and risk factor for atherosclerotic cardiovascular disease. It is stated that a main obstacle towards the clinical use of Lp(a) is that measurements and various other targeted levels have not yet been standardized in the industry; for example, several of the available assays are reporting results in differing units, such as in mass instead of concentration (Wilson et al., 2019). Based on current data, Wilson et al. (2019) has stated that Lp(a) testing in clinical practice is reasonable for select individuals with the qualifications listed below:

- Adults older than 20 years with a family history of premature atherosclerotic cardiovascular disease (ASCVD)

Confidential and Proprietary Information of Avalon Health Services, LLC, d/b/a Avalon Healthcare Solutions. All Rights Reserved.

- "Individuals with premature ASCVD (55y of age in men; 65y of age in women), particularly in the absence of traditional risk factors
- Individuals with primary severe hypercholesterolemia (LDL-C ≥ 190 mg/dL) or suspected FH
- Individuals at very-high-risk of ASCVD to better define those who are more likely to benefit from PCSK9 inhibitor therapy"

Wilson et al. (2019) also stated that Lp(a) testing may be reasonable in patients with:

- "Intermediate (7.5%–19.9%) 10-y ASCVD risk when the decision to use a statin is uncertain, to improve risk stratification in primary prevention
- Borderline (5%–7.4%) 10-y ASCVD risk when the decision to use a statin is uncertain, to improve risk stratification in primary prevention
- Less-than-anticipated LDL-C lowering, despite good adherence to LDL-C lowering therapy
- A family history of elevated Lp(a)
- Calcific valvular aortic stenosis
- Recurrent or progressive ASCVD, despite optimal lipid-lowering therapy"

In 2022 scientific statement, the NLA writes:

- "Lp(a) testing is reasonable to refine risk assessment for ASCVD events in adults with:
  - First-degree relatives with premature ASCVD (<55 y of age in men; <65 y of age in women).
  - A personal history of premature ASCVD.
  - Primary severe hypercholesterolemia (LDL-C ≥190 mg/dL) or suspected FH.
- Lp(a) testing may be reasonable in adults:
  - To aid in the clinician-patient discussion about whether to prescribe a statin in those aged 40-75 y with borderline (5%-7.4%) 10-y ASCVD risk.
  - To identify a possible cause for a less-than-anticipated LDL-C lowering to evidence-based LDL-C-lowering therapy.
  - To use in cascade screening of family members with severe hypercholesterolemia.
  - To identify those at risk for progressive [valvular aortic stenosis]" (Wilson et al., 2022)

In 2024, the NLA released a focused update to the 2019 NLA scientific statement that included recommendations for Lp(a) testing in clinical practice (Marlys L. Koschinsky et al., 2024):

Confidential and Proprietary Information of Avalon Health Services, LLC, d/b/a Avalon Healthcare Solutions. All Rights Reserved.

1. "Adults (aged ≥18 y): Measurement of Lp(a) in all adults is reasonable to refine risk assessment for ASCVD events
2. Youth (aged <18 y): Selective screening of Lp(a) is recommended in high-risk patients (e.g., clinically suspected or genetically confirmed FH, ischemic stroke of unknown cause, first-degree relatives with a history of premature ASCVD [age <55 years in men, <65 years in women], or first-degree relatives with elevated Lp(a))
3. When Lp(a) levels are used for ASCVD risk assessment, it is reasonable to use measurements ≥125 nmol/L (≥50 mg/dL) as levels suggesting high risk, levels <75 nmol/L (<30 mg/dL) as low risk, and levels between as intermediate risk" (Marlys L. Koschinsky et al., 2024)

Genetic screening for Lp(a) variants was mentioned in the most recent NLA guideline update (Marlys L. Koschinsky et al., 2024). "While genetic risk scores incorporating genetic variants at or near the LPA locus are increasingly accurate for identifying individuals likely to have elevated Lp(a) and to develop CVD, Lp(a) genetic risk score appears to add no incremental value for CVD risk classification compared with Lp(a) concentrations alone. Additionally, the generalizability of genetic risk scores to groups with non-White ancestry remains uncertain. Therefore, at this time, Lp(a) measurement is preferable for risk ascertainment; all the necessary information for appraisal of Lp(a)-attributable cardiovascular risk is embodied in the measurement of plasma Lp(a) concentration itself, mandating increased screening to identify individuals with elevated Lp(a) and high risk for CVD" (Marlys L. Koschinsky et al., 2024).

The European Atherosclerosis Society (EAS)

In a 2022 update, the European Atherosclerosis Society recommends testing Lp(a) concentration at least once per lifetime in adults. The EAS also suggests measurement of Lp(a) in youth with a (1) history of ischemic stroke or a (2) family history of premature ASCVD or (3) elevated Lp(a) level and no other known risk factors.

The consensus statement suggests other testing may have value under certain conditions. "Cascade testing has potential value in familial hypercholesterolaemia, or with family or personal history of (very) high Lp(a) or premature ASCVD. Without specific Lp(a)-lowering therapies, early intensive risk factor management is recommended, targeted according to global cardiovascular risk and Lp(a) level" (Kronenberg et al., 2022).

Under a subtitle posing the question "Is there a role for genetic testing?" the authors write: "Measurement of Lp(a) concentration is sufficient for Lp(a)-related risk estimation without the need for genotyping, polygenic risk scores, or investigation of expressed apo(a) isoform sizes" (Kronenberg et al., 2022).

Confidential and Proprietary Information of Avalon Health Services, LLC, d/b/a Avalon Healthcare Solutions. All Rights Reserved.

The European Society for Cardiology (ESC) and European Atherosclerosis Society (EAS) Joint Guideline

The ESC and EAS published a joint guideline for the Management of Dyslipidemias which recommends:

- "Lp(a) measurement should be considered at least once in each adult person's lifetime to identify those with very high inherited Lp(a) levels > 180mg/dL (>430 nmol/L) who may have a lifetime risk of ASCVD equivalent to the risk associated with heterozygous familial hypercholesterolaemia (Level of Recommendation: C)
- Lp(a) should be considered in selected patients with a family history of premature CVD, and for reclassification in people who are borderline between moderate and high-risk (Level of Recommendation: C)" (Mach et al., 2019).

Genetic screening for Lp(a) variants is not mentioned (Mach et al., 2019).

HEART UK Medical, Scientific, and Research Committee

HEART UK published guidelines for Lp(a) measurement in specific adult populations. HEART UK recommends that Lp(a) is measured in adults as follows: "1) those with a personal or family history of premature atherosclerotic CVD; 2) those with first-degree relatives who have Lp(a) levels >200 nmol/l; 3) patients with familial hypercholesterolemia; 4) patients with calcific aortic valve stenosis and 5) those with borderline (but <15%) 10-year risk of a cardiovascular event" (Cegla et al., 2019).

On genetic testing for Lp(a) levels, the guideline also noted, "Genetic testing for SNPs associated with serum Lp(a) levels is not currently advocated for in routine clinical practice" (Cegla et al., 2019).

U.S. Preventive Services Task Force (USPSTF)

The USPSTF 2022 guidelines on aspirin use to prevent cardiovascular disease do not mention Lp(a) or genetic screening for Lp(a) (Guirguis-Blake et al., 2016; USPSTF, 2022).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-

Confidential and Proprietary Information of Avalon Health Services, LLC, d/b/a Avalon Healthcare Solutions. All Rights Reserved.

database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

The LPA-Aspirin Check® detects the presence of the rs3798220 allele and is considered a laboratory developed test (LDT); this test is developed, validated, and performed by individual laboratories.

The Cardio IQ® LPA Aspirin Genotype test is able to detect individuals who are at risk of high plasma Lp(a) levels, which may suggest an increased risk of cardiovascular events; this assay may also assist in determining if the patient's cardiovascular disease risk may be lowered by low-dose aspirin therapy (Quest Diagnostics, 2019). This test has not been cleared or approved by the FDA.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81479	Unlisted molecular pathology procedure

Current Procedural Terminology© American Medical Association. All Rights Reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Anderson, J. L., Knight, S., May, H. T., Horne, B. D., Bair, T. L., Huntinghouse, J. A., Rollo, J. S., Muhlestein, J. B., & Carlquist, J. F. (2013). Validation and quantification of genetic determinants of lipoprotein-a levels and predictive value for angiographic coronary artery disease. *Am J Cardiol*, *112*(6), 799-804. https://doi.org/10.1016/j.amjcard.2013.05.009

Arnett, D. K., Blumenthal, R. S., Albert, M. A., Buroker, A. B., Goldberger, Z. D., Hahn, E. J., Himmelfarb, C. D., Khera, A., Lloyd-Jones, D., McEvoy, J. W., Michos, E. D., Miedema, M. D., Muñoz, D., Smith, S. C., Jr., Virani, S. S., Williams, K. A., Sr., Yeboah, J., & Ziaeian,

B. (2019). 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation*, *140*(11), e596-e646. https://doi.org/10.1161/cir.0000000000000678

Benjamin, E. J., Virani, S. S., Callaway, C. W., Chamberlain, A. M., Chang, A. R., Cheng, S., Chiuve, S. E., Cushman, M., Delling, F. N., Deo, R., de Ferranti, S. D., Ferguson, J. F., Fornage, M., Gillespie, C., Isasi, C. R., Jimenez, M. C., Jordan, L. C., Judd, S. E., Lackland, D., . . . Stroke Statistics, S. (2018). Heart Disease and Stroke Statistics-2018 Update: A Report From the American Heart Association. *Circulation*, *137*(12), e67-e492. https://doi.org/10.1161/CIR.0000000000000558

Berg, K. (1963). A New Serum Type System in Man--the Lp System. *Acta Pathol Microbiol Scand*, *59*, 369-382. https://doi.org/10.1111/j.1699-0463.1963.tb01808.x

Berg, K., Dahlen, G., & Frick, M. H. (1974). Lp(a) lipoprotein and pre-beta1-lipoprotein in patients with coronary heart disease. *Clin Genet*, *6*(3), 230-235. https://doi.org/10.1111/j.1399-0004.1974.tb00657.x

Cairns, B. J., Coffey, S., Travis, R. C., Prendergast, B., Green, J., Engert, J. C., Lathrop, M., Thanassoulis, G., & Clarke, R. (2017). A Replicated, Genome-Wide Significant Association of Aortic Stenosis With a Genetic Variant for Lipoprotein(a): Meta-Analysis of Published and Novel Data. *Circulation*, *135*(12), 1181-1183. https://doi.org/10.1161/CIRCULATIONAHA.116.026103

Caplice, N. M., Panetta, C., Peterson, T. E., Kleppe, L. S., Mueske, C. S., Kostner, G. M., Broze, G. J., Jr., & Simari, R. D. (2001). Lipoprotein (a) binds and inactivates tissue factor pathway inhibitor: a novel link between lipoproteins and thrombosis. *Blood*, *98*(10), 2980-2987. https://doi.org/10.1182/blood.V98.10.2980

Cegla, J., Neely, R. D. G., France, M., Ferns, G., Byrne, C. D., Halcox, J., Datta, D., Capps, N., Shoulders, C., Qureshi, N., Rees, A., Main, L., Cramb, R., Viljoen, A., Payne, J., & Soran, H. (2019). HEART UK consensus statement on Lipoprotein(a): A call to action. *Atherosclerosis*, *291*, 62-70. https://doi.org/10.1016/j.atherosclerosis.2019.10.011

Chakraborty, A., Pang, J., Chan, D. C., Ellis, K. L., Hooper, A. J., Bell, D. A., Burnett, J. R., Moses, E. K., & Watts, G. F. (2022). Cascade testing for elevated lipoprotein(a) in relatives of probands with familial hypercholesterolaemia and elevated lipoprotein(a). *Atherosclerosis*, *349*, 219-226. https://doi.org/10.1016/j.atherosclerosis.2021.11.004

Chasman, D. I., Shiffman, D., Zee, R. Y., Louie, J. Z., Luke, M. M., Rowland, C. M., Catanese, J. J., Buring, J. E., Devlin, J. J., & Ridker, P. M. (2009). Polymorphism in the apolipoprotein(a) gene, plasma lipoprotein(a), cardiovascular disease, and low-dose aspirin therapy. *Atherosclerosis*, *203*(2), 371-376. https://doi.org/10.1016/j.atherosclerosis.2008.07.019

Chen, H. Y., Dufresne, L., Burr, H., Ambikkumar, A., Yasui, N., Luk, K., Ranatunga, D. K., Whitmer, R. A., Lathrop, M., Engert, J. C., & Thanassoulis, G. (2018). Association of

Confidential and Proprietary Information of Avalon Health Services, LLC, d/b/a Avalon Healthcare Solutions. All Rights Reserved.

LPA Variants With Aortic Stenosis: A Large-Scale Study Using Diagnostic and Procedural Codes From Electronic Health Records. *JAMA Cardiol*, *3*(1), 18-23. https://doi.org/10.1001/jamacardio.2017.4266

Clarke, R., Peden, J. F., Hopewell, J. C., Kyriakou, T., Goel, A., Heath, S. C., Parish, S., Barlera, S., Franzosi, M. G., Rust, S., Bennett, D., Silveira, A., Malarstig, A., Green, F. R., Lathrop, M., Gigante, B., Leander, K., de Faire, U., Seedorf, U., . . . Consortium, P. (2009). Genetic variants associated with Lp(a) lipoprotein level and coronary disease. *N Engl J Med*, *361*(26), 2518-2528. https://doi.org/10.1056/NEJMoa0902604

Emdin, C. A., Khera, A. V., Natarajan, P., Klarin, D., Won, H. H., Peloso, G. M., Stitziel, N. O., Nomura, A., Zekavat, S. M., Bick, A. G., Gupta, N., Asselta, R., Duga, S., Merlini, P. A., Correa, A., Kessler, T., Wilson, J. G., Bown, M. J., Hall, A. S., . . . Consortium, C. A. E. (2016). Phenotypic Characterization of Genetically Lowered Human Lipoprotein(a) Levels. *J Am Coll Cardiol*, *68*(25), 2761-2772. https://doi.org/10.1016/j.jacc.2016.10.033

Enkhmaa, B., Anuurad, E., & Berglund, L. (2016). Lipoprotein (a): impact by ethnicity and environmental and medical conditions. *J Lipid Res*, *57*(7), 1111-1125. https://doi.org/10.1194/jlr.R051904

Erhart, G., Lamina, C., Lehtimaki, T., Marques-Vidal, P., Kahonen, M., Vollenweider, P., Raitakari, O. T., Waeber, G., Thorand, B., Strauch, K., Gieger, C., Meitinger, T., Peters, A., Kronenberg, F., & Coassin, S. (2018). Genetic Factors Explain a Major Fraction of the 50% Lower Lipoprotein(a) Concentrations in Finns. *Arterioscler Thromb Vasc Biol*, *38*(5), 1230-1241. https://doi.org/10.1161/ATVBAHA.118.310865

Fras, Z. (2020). Increased cardiovascular risk associated with hyperlipoproteinemia (a) and the challenges of current and future therapeutic possibilities. *Anatol J Cardiol*, *23*(2), 60-69. https://doi.org/10.14744/AnatolJCardiol.2019.56068

Grainger, D. J., Kirschenlohr, H. L., Metcalfe, J. C., Weissberg, P. L., Wade, D. P., & Lawn, R. M. (1993). Proliferation of human smooth muscle cells promoted by lipoprotein(a). *Science*, *260*(5114), 1655-1658. https://doi.org/10.1126/science.8503012

Gu, J.-X., Huang, J., Li, S.-S., Zhou, L.-H., Yang, M., Li, Y., Zhang, A.-M., Yin, Y., Zhang, N., Jia, M., & Su, M. (2022). Elevated lipoprotein(a) and genetic polymorphisms in the LPA gene may predict cardiovascular events. *Scientific Reports*, *12*(1), 3588. https://doi.org/10.1038/s41598-022-07596-4

Guirguis-Blake, J. M., Evans, C. V., Senger, C. A., O'Connor, E. A., & Whitlock, E. P. (2016). Aspirin for the Primary Prevention of Cardiovascular Events: A Systematic Evidence Review for the U.S. Preventive Services Task Force. *Ann Intern Med*, *164*(12), 804-813. https://doi.org/10.7326/m15-2113

Hajjar, K. A., Gavish, D., Breslow, J. L., & Nachman, R. L. (1989). Lipoprotein(a) modulation of endothelial cell surface fibrinolysis and its potential role in atherosclerosis. *Nature*, *339*(6222), 303-305. https://doi.org/10.1038/339303a0

Confidential and Proprietary Information of Avalon Health Services, LLC, d/b/a Avalon Healthcare Solutions. All Rights Reserved.

Hancock, M. A., Boffa, M. B., Marcovina, S. M., Nesheim, M. E., & Koschinsky, M. L. (2003). Inhibition of plasminogen activation by lipoprotein(a): critical domains in apolipoprotein(a) and mechanism of inhibition on fibrin and degraded fibrin surfaces. *J Biol Chem*, *278*(26), 23260-23269. https://doi.org/10.1074/jbc.M302780200

Handelsman, Y., Jellinger, P. S., Guerin, C. K., Bloomgarden, Z. T., Brinton, E. A., Budoff, M. J., Davidson, M. H., Einhorn, D., Fazio, S., Fonseca, V. A., Garber, A. J., Grunberger, G., Krauss, R. M., Mechanick, J. I., Rosenblit, P. D., Smith, D. A., & Wyne, K. L. (2020). Consensus Statement by the American Association of Clinical Endocrinologists and American College of Endocrinology on the Management of Dyslipidemia and Prevention of Cardiovascular Disease Algorithm – 2020 Executive Summary. *Endocrine Practice*, *26*(10), 1196-1224. https://doi.org/10.4158/CS-2020-0490

Helgadottir, A., Gretarsdottir, S., Thorleifsson, G., Holm, H., Patel, R. S., Gudnason, T., Jones, G. T., van Rij, A. M., Eapen, D. J., Baas, A. F., Tregouet, D. A., Morange, P. E., Emmerich, J., Lindblad, B., Gottsater, A., Kiemeny, L. A., Lindholt, J. S., Sakalihasan, N., Ferrell, R. E., . . . Stefansson, K. (2012). Apolipoprotein(a) genetic sequence variants associated with systemic atherosclerosis and coronary atherosclerotic burden but not with venous thromboembolism. *J Am Coll Cardiol*, *60*(8), 722-729. https://doi.org/10.1016/j.jacc.2012.01.078

Hervio, L., Durlach, V., Girard-Globa, A., & Angles-Cano, E. (1995). Multiple binding with identical linkage: a mechanism that explains the effect of lipoprotein(a) on fibrinolysis. *Biochemistry*, *34*(41), 13353-13358. https://doi.org/10.1021/bi00041a011

Jellinger, P. S., Handelsman, Y., Rosenblit, P. D., Bloomgarden, Z. T., Fonseca, V. A., Garber, A. J., Grunberger, G., Guerin, C. K., Bell, D. S. H., Mechanick, J. I., Pessah-Pollack, R., Wyne, K., Smith, D., Brinton, E. A., Fazio, S., & Davidson, M. (2017). AMERICAN ASSOCIATION OF CLINICAL ENDOCRINOLOGISTS AND AMERICAN COLLEGE OF ENDOCRINOLOGY GUIDELINES FOR MANAGEMENT OF DYSLIPIDEMIA AND PREVENTION OF CARDIOVASCULAR DISEASE. *Endocr Pract*, *23*(Suppl 2), 1-87. https://doi.org/10.4158/ep171764.appgl

Kronenberg, F., Mora, S., Stroes, E. S. G., Ference, B. A., Arsenault, B. J., Berglund, L., Dweck, M. R., Koschinsky, M., Lambert, G., Mach, F., McNeal, C. J., Moriarty, P. M., Natarajan, P., Nordestgaard, B. G., Parhofer, K. G., Virani, S. S., von Eckardstein, A., Watts, G. F., Stock, J. K., . . . Catapano, A. L. (2022). Lipoprotein(a) in atherosclerotic cardiovascular disease and aortic stenosis: a European Atherosclerosis Society consensus statement *European Heart Journal*, *43*(39), 3925-3946. https://doi.org/10.1093/eurheartj/ehac361

Lee, S. R., Prasad, A., Choi, Y. S., Xing, C., Clopton, P., Witztum, J. L., & Tsimikas, S. (2017). LPA Gene, Ethnicity, and Cardiovascular Events. *Circulation*, *135*(3), 251-263. https://doi.org/10.1161/CIRCULATIONAHA.116.024611

Confidential and Proprietary Information of Avalon Health Services, LLC, d/b/a Avalon Healthcare Solutions. All Rights Reserved.

Lu, W., Cheng, Y. C., Chen, K., Wang, H., Gerhard, G. S., Still, C. D., Chu, X., Yang, R., Parihar, A., O'Connell, J. R., Pollin, T. I., Angles-Cano, E., Quon, M. J., Mitchell, B. D., Shuldiner, A. R., & Fu, M. (2015). Evidence for several independent genetic variants affecting lipoprotein (a) cholesterol levels. *Hum Mol Genet*, *24*(8), 2390-2400. https://doi.org/10.1093/hmg/ddu731

Luke, M. M., Kane, J. P., Liu, D. M., Rowland, C. M., Shiffman, D., Cassano, J., Catanese, J. J., Pullinger, C. R., Leong, D. U., Arellano, A. R., Tong, C. H., Movsesyan, I., Naya-Vigne, J., Noordhof, C., Feric, N. T., Malloy, M. J., Topol, E. J., Koschinsky, M. L., Devlin, J. J., & Ellis, S. G. (2007). A polymorphism in the protease-like domain of apolipoprotein(a) is associated with severe coronary artery disease. *Arterioscler Thromb Vasc Biol*, *27*(9), 2030-2036. https://doi.org/10.1161/ATVBAHA.107.141291

Mach, F., Baigent, C., Catapano, A. L., Koskinas, K. C., Casula, M., Badimon, L., Chapman, M. J., De Backer, G. G., Delgado, V., Ference, B. A., Graham, I. M., Halliday, A., Landmesser, U., Mihaylova, B., Pedersen, T. R., Riccardi, G., Richter, D. J., Sabatine, M. S., Taskinen, M.-R., . . . Group, E. S. D. (2019). 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk: The Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS). *European Heart Journal*, *41*(1), 111-188. https://doi.org/10.1093/eurheartj/ehz455

Marcovina, S. M., Hobbs, H. H., & Albers, J. J. (1996). Relation between number of apolipoprotein(a) kringle 4 repeats and mobility of isoforms in agarose gel: basis for a standardized isoform nomenclature. *Clin Chem*, *42*(3), 436-439. https://www.ncbi.nlm.nih.gov/pubmed/8598109

Marcovina, S. M., & Koschinsky, M. L. (2003). Evaluation of lipoprotein(a) as a prothrombotic factor: progress from bench to bedside. *Curr Opin Lipidol*, *14*(4), 361-366. https://doi.org/10.1097/00041433-200308000-00004

Marlys L. Koschinsky, P., Archna Bajaj, MD, MSCE, Michael B. Boffa, PhD,, Dave L. Dixon, P., Keith C. Ferdinand, MD, Samuel S. Gidding, MD,, Edward A. Gill, M., Terry A. Jacobson, MD, Erin D. Michos, MD, MHS,, Maya S. Safarova, M., PhD, Daniel E. Soffer, MD, Pam R. Taub, MD,, & Michael J. Wilkinson, M., Don P. Wilson, MD, Christie M. Ballantyne, MD. (2024). A focused update to the 2019 NLA scientific statement on use of lipoprotein(a) in clinical practice. https://doi.org/10.1016/j.jacl.2024.03.001

McLean, J. W., Tomlinson, J. E., Kuang, W. J., Eaton, D. L., Chen, E. Y., Fless, G. M., Scanu, A. M., & Lawn, R. M. (1987). cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. *Nature*, *330*(6144), 132-137. https://doi.org/10.1038/330132a0

Moriarty, P. M., Varvel, S. A., Gordts, P. L., McConnell, J. P., & Tsimikas, S. (2017). Lipoprotein(a) Mass Levels Increase Significantly According to APOE Genotype: An Analysis of 431 239 Patients. *Arterioscler Thromb Vasc Biol*, *37*(3), 580-588. https://doi.org/10.1161/ATVBAHA.116.308704

Confidential and Proprietary Information of Avalon Health Services, LLC, d/b/a Avalon Healthcare Solutions. All Rights Reserved.

Mu-Han-Ha-Li, D. L., Zhai, T. Y., Ling, Y., & Gao, X. (2018). LPA kringle IV type 2 is associated with type 2 diabetes in a Chinese population with very high cardiovascular risk. *J Lipid Res*, *59*(5), 884-891. https://doi.org/10.1194/jlr.P082792

Murdock, D. R., Venner, E., Muzny, D. M., Metcalf, G. A., Murugan, M., Hadley, T. D., Chander, V., de Vries, P. S., Jia, X., Hussain, A., Agha, A. M., Sabo, A., Li, S., Meng, Q., Hu, J., Tian, X., Cohen, M., Yi, V., Kovar, C. L., . . . Gibbs, R. A. (2021). Genetic testing in ambulatory cardiology clinics reveals high rate of findings with clinical management implications. *Genet Med*, *23*(12), 2404-2414. https://doi.org/10.1038/s41436-021-01294-8

Nagalla, S., & Bray, P. F. (2016). Personalized medicine in thrombosis: back to the future. *Blood*, *127*(22), 2665-2671. https://doi.org/10.1182/blood-2015-11-634832

Ozkan, U., Ozcelik, F., Yildiz, M., & Budak, M. (2019). Lipoprotein(a) Gene Polymorphism Increases a Risk Factor for Aortic Valve Calcification. *J Cardiovasc Dev Dis*, *6*(3). https://doi.org/10.3390/jcdd6030031

Paré, G., Çaku, A., McQueen, M., Anand Sonia, S., Enas, E., Clarke, R., Boffa Michael, B., Koschinsky, M., Wang, X., Yusuf, S., & null, n. (2019). Lipoprotein(a) Levels and the Risk of Myocardial Infarction Among 7 Ethnic Groups. *Circulation*, *139*(12), 1472-1482. https://doi.org/10.1161/CIRCULATIONAHA.118.034311

Pechlivanis, S., Mahabadi, A. A., Hoffmann, P., Nöthen, M. M., Broecker-Preuss, M., Erbel, R., Moebus, S., Stang, A., & Jöckel, K.-H. (2020). Association between lipoprotein(a) (Lp(a)) levels and Lp(a) genetic variants with coronary artery calcification. *BMC Medical Genetics*, *21*(1), 62. https://doi.org/10.1186/s12881-020-01003-3

Quest Diagnostics. (2019). *Cardio IQ® LPA Aspirin Genotype*. https://testdirectory.questdiagnostics.com/test/test-detail/90553/cardio-iq-lpa-aspirin-genotype?cc=MASTER

Reyes-Soffer, G., Ginsberg, H. N., Berglund, L., Duell, P. B., Heffron, S. P., Kamstrup, P. R., Lloyd-Jones, D. M., Marcovina, S. M., Yeang, C., & Koschinsky, M. L. (2022). Lipoprotein(a): A Genetically Determined, Causal, and Prevalent Risk Factor for Atherosclerotic Cardiovascular Disease: A Scientific Statement From the American Heart Association. *Arterioscler Thromb Vasc Biol*, *42*(1), e48-e60. https://doi.org/10.1161/atv.0000000000000147

Rosenson, R., Stein, J., & Durrington, P. (2024, Oct 09, 2024). *Lipoprotein(a)*. https://www.uptodate.com/contents/lipoprotein-a

Rowland, C. M., Pullinger, C. R., Luke, M. M., Shiffman, D., Green, L., Movsesyan, I., Devlin, J. J., Malloy, M. J., Kane, J. P., & Undas, A. (2014). Lipoprotein (a), LPA Ile4399Met, and fibrin clot properties. *Thromb Res*, *133*(5), 863-867. https://doi.org/10.1016/j.thromres.2014.01.024

Schmidt, K., Noureen, A., Kronenberg, F., & Utermann, G. (2016). Structure, function, and genetics of lipoprotein (a). *J Lipid Res*, *57*(8), 1339-1359. https://doi.org/10.1194/jlr.R067314

Confidential and Proprietary Information of Avalon Health Services, LLC, d/b/a Avalon Healthcare Solutions. All Rights Reserved.

Scipione, C. A., McAiney, J. T., Simard, D. J., Bazzi, Z. A., Gemin, M., Romagnuolo, R., Macrae, F. L., Ariens, R. A., Hegele, R. A., Auld, J., Gauld, J. W., Boffa, M. B., & Koschinsky, M. L. (2017). Characterization of the I4399M variant of apolipoprotein(a): implications for altered prothrombotic properties of lipoprotein(a). *J Thromb Haemost*, *15*(9), 1834-1844. https://doi.org/10.1111/jth.13759

Shiffman, D., Slawsky, K., Fusfeld, L., Devlin, J. J., & Goss, T. F. (2012). Cost-effectiveness model of use of genetic testing as an aid in assessing the likely benefit of aspirin therapy for primary prevention of cardiovascular disease. *Clin Ther*, *34*(6), 1387-1394. https://doi.org/10.1016/j.clinthera.2012.04.004

Steyrer, E., Durovic, S., Frank, S., Giessauf, W., Burger, A., Dieplinger, H., Zechner, R., & Kostner, G. M. (1994). The role of lecithin: cholesterol acyltransferase for lipoprotein (a) assembly. Structural integrity of low density lipoproteins is a prerequisite for Lp(a) formation in human plasma. *J Clin Invest*, *94*(6), 2330-2340. https://doi.org/10.1172/JCI117598

Tregouet, D. A., Konig, I. R., Erdmann, J., Munteanu, A., Braund, P. S., Hall, A. S., Grosshennig, A., Linsel-Nitschke, P., Perret, C., DeSuremain, M., Meitinger, T., Wright, B. J., Preuss, M., Balmforth, A. J., Ball, S. G., Meisinger, C., Germain, C., Evans, A., Arveiler, D., . . . Samani, N. J. (2009). Genome-wide haplotype association study identifies the SLC22A3-LPAL2-LPA gene cluster as a risk locus for coronary artery disease. *Nat Genet*, *41*(3), 283-285. https://doi.org/10.1038/ng.314

Trinder, M., Uddin, M. M., Finneran, P., Aragam, K. G., & Natarajan, P. (2021). Clinical Utility of Lipoprotein(a) and LPA Genetic Risk Score in Risk Prediction of Incident Atherosclerotic Cardiovascular Disease. *JAMA Cardiology*, *6*(3), 287-295. https://doi.org/10.1001/jamacardio.2020.5398

Tsimikas, S., Fazio, S., Ferdinand, K. C., Ginsberg, H. N., Koschinsky, M. L., Marcovina, S. M., Moriarty, P. M., Rader, D. J., Remaley, A. T., Reyes-Soffer, G., Santos, R. D., Thanassoulis, G., Witztum, J. L., Danthi, S., Olive, M., & Liu, L. (2018). NHLBI Working Group Recommendations to Reduce Lipoprotein(a)-Mediated Risk of Cardiovascular Disease and Aortic Stenosis. *J Am Coll Cardiol*, *71*(2), 177-192. https://doi.org/10.1016/j.jacc.2017.11.014

Tuten, A., Gungor, Z., Ekmekci, H., Ekmekci, O. B., Kucur, M., Yilmaz, N., Donma, O., Sonmez, H., Acikgoz, A., & Madazli, R. (2019). Relationship between LPA SNPs and inflammatory burden in patients with preeclampsia to address future cardiovascular risk. *J Matern Fetal Neonatal Med*, 1-9. https://doi.org/10.1080/14767058.2019.1622667

USPSTF. (2022). *Aspirin Use to Prevent Cardiovascular Disease: Preventive Medication*. https://www.uspreventiveservicestaskforce.org/uspstf/recommendation/aspirin-to-prevent-cardiovascular-disease-preventive-medication

Confidential and Proprietary Information of Avalon Health Services, LLC, d/b/a Avalon Healthcare Solutions. All Rights Reserved.

Wang, F. M., & Zhang, Y. (2019). High Lipoprotein(a) Level Is Independently Associated with Adverse Clinicopathological Features in Patients with Prostate Cancer. *Dis Markers*, *2019*, 9483935. https://doi.org/10.1155/2019/9483935

Wang, H., Hong, C. E., Lewis, J. P., Zhu, Y., Wang, X., Chu, X., Backman, J., Hu, Z., Yang, P., Still, C. D., Gerhard, G. S., & Fu, M. (2016). Effect of Two Lipoprotein (a)-Associated Genetic Variants on Plasminogen Levels and Fibrinolysis. *G3 (Bethesda)*, *6*(11), 3525-3532. https://doi.org/10.1534/g3.116.034702

Wilson, D. P., Jacobson, T. A., Jones, P. H., Koschinsky, M. L., McNeal, C. J., Nordestgaard, B. G., & Orringer, C. E. (2019). Use of Lipoprotein(a) in clinical practice: A biomarker whose time has come. A scientific statement from the National Lipid Association. *J Clin Lipidol*, *13*(3), 374-392. https://doi.org/10.1016/j.jacl.2019.04.010

Wilson, D. P., Jacobson, T. A., Jones, P. H., Koschinsky, M. L., McNeal, C. J., Nordestgaard, B. G., & Orringer, C. E. (2022). Use of Lipoprotein(a) in clinical practice: A biomarker whose time has come. A scientific statement from the National Lipid Association. *J Clin Lipidol*. https://doi.org/10.1016/j.jacl.2022.08.007

Yoon, Y. H., Ahn, J. M., Kang, D. Y., Lee, P. H., Kang, S. J., Park, D. W., Lee, S. W., Kim, Y. H., Han, K. H., Lee, C. W., Park, S. W., & Park, S. J. (2021). Association of Lipoprotein(a) With Recurrent Ischemic Events Following Percutaneous Coronary Intervention. *JACC Cardiovasc Interv*, *14*(18), 2059-2068. https://doi.org/10.1016/j.jcin.2021.07.042

Zekavat, S. M., Ruotsalainen, S., Handsaker, R. E., Alver, M., Bloom, J., Poterba, T., Seed, C., Ernst, J., Chaffin, M., Engreitz, J., Peloso, G. M., Manichaikul, A., Yang, C., Ryan, K. A., Fu, M., Johnson, W. C., Tsai, M., Budoff, M., Vasan, R. S., . . . Group, N. T. L. W. (2018). Deep coverage whole genome sequences and plasma lipoprotein(a) in individuals of European and African ancestries. *Nat Commun*, *9*(1), 2606. https://doi.org/10.1038/s41467-018-04668-w

Zewinger, S., Kleber, M. E., Tragante, V., McCubrey, R. O., Schmidt, A. F., Direk, K., Laufs, U., Werner, C., Koenig, W., Rothenbacher, D., Mons, U., Breitling, L. P., Brenner, H., Jennings, R. T., Petrakis, I., Triem, S., Klug, M., Filips, A., Blankenberg, S., . . . Speer, T. (2017). Relations between lipoprotein(a) concentrations, LPA genetic variants, and the risk of mortality in patients with established coronary heart disease: a molecular and genetic association study. *Lancet Diabetes Endocrinol*, *5*(7), 534-543. https://doi.org/10.1016/S2213-8587(17)30096-7

Confidential and Proprietary Information of Avalon Health Services, LLC, d/b/a Avalon Healthcare Solutions. All Rights Reserved.

Genetic Testing for Neurodegenerative Disorders

Policy Number: AHS – M2167 – Genetic Testing for Neurodegenerative Disorders	Prior Policy Name and Number, as applicable:
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Neurodegenerative diseases are characterized by progressive loss of neurons along with deposition of misfolded proteins throughout the body, leading to clinical symptoms such as cognitive decline and movement problems. Conditions that fall within this classification include Parkinson Disease, dystonia, ataxia, and more (Kovacs, 2016).

This policy does not address Alzheimer Disease or ataxia due to mitochondrial disorders. For information on these conditions, please see AHS-M2038 Genetic Testing for Alzheimer Disease or AHS-M2085 Genetic Testing of Mitochondrial Disorders.

For guidance on preconception screening for spinal muscular atrophies, please refer to AHS-M2179-Prenatal Screening (Genetic).

II. Related Policies

Policy Number	Policy Title
AHS-	Genetic Testing for *FMR1* Mutations
AHS-	Genetic Testing for Familial Alzheimer Disease
AHS-	Genetic Testing for Diagnosis of Inherited Peripheral Neuropathies
AHS-	Genetic Testing of Mitochondrial Disorders
AHS-	General Genetic Testing, Germline Disorders
AHS-	Prenatal Screening (Genetic)

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Genetic counseling **IS REQUIRED** for individuals prior to and after undergoing genetic testing for diagnostic, carrier, and/or risk assessment purposes.

Amyotrophic Lateral Sclerosis (ALS)

2) For diagnosis in individuals with suspected ALS **and** a first-degree **or** second-degree relative (see Note 1) with ALS or frontotemporal dementia, genetic testing for ALS, including the genes *C9ORF72, SOD1, TARDBP,* and *FUS*, **MEETS COVERAGE CRITERIA**.

Ataxias, including Friedreich ataxia

3) For individuals with sporadic ataxia **or** a family history compatible with an inherited cerebellar ataxia with no known deleterious familial mutation, single gene testing (when a specific ataxia is suspected) or multi-gene panel testing (when a specific ataxia is not suspected) **MEETS COVERAGE CRITERIA.**

4) To confirm a diagnosis in individuals with suspected ataxia-telangiectasia, genetic testing for *ATM* **MEETS COVERAGE CRITERIA**.

Dystonias

5) Genetic testing of *TOR1A* (formerly *DYT1)* **MEETS COVERAGE CRITERIA** in the following situations:
   a) In individuals with limb-onset, primary dystonia before the age of 30 years.
   b) In individuals with limb-onset, primary dystonia with onset after age 30 when there is a family history compatible with early-onset dystonia.

6) Genetic testing of *THAP1* (formerly *DYT6)* **MEETS COVERAGE CRITERIA** in the following situations:
   a) In individuals with an early-onset dystonia or familial dystonia with cranio-cervical predominance.
   b) In individuals with early-onset dystonia after exclusion of *TOR1A*-associated dystonia.

7) To aid in the diagnosis of symptomatic individuals with familial paroxysmal nonkinesigenic dyskinesia (PNKD), genetic testing of *PNKD* **MEETS COVERAGE CRITERIA.**

8) In individuals with paroxysmal exercise-induced dyskinesia, genetic testing of *SLC2A1* (formerly *GLUT1)* **MEETS COVERAGE CRITERIA** if the individual has at least one of the following:

   a) A history of epileptic seizures.

   b) Hemolytic anemia.

   c) A low CSF/serum glucose ratio.

9) In asymptomatic individuals, genetic testing of *TOR1A* **DOES NOT MEET COVERAGE CRITERIA**.

Hereditary Spastic Paraplegia (HSP)

10) To confirm clinical diagnosis and to determine the genetic type of Hereditary Spastic Paraplegia (HSP), genetic testing for HSP **MEETS COVERAGE CRITERIA**.

Huntington disease (HD)

11) Genetic testing for Huntington disease **MEETS COVERAGE CRITERIA** in the following situations:

   a) When an adult patient presents with an otherwise unexplained clinical syndrome of a progressive choreatic movement disorder and neuropsychiatric disturbances.

   b) In an adult patient with a positive family history of the disease.

   c) In a juvenile patient with the following:

      i) A known familial history of HD.

      ii) Presenting with two or more of the following:

         (a) Declining school performance
         (b) Seizures
         (c) Oral motor dysfunction
         (d) Rigidity
         (e) Gait disturbance

Parkinsonism, including Parkinson disease

12) Genetic testing of *SNCA* **MEETS COVERAGE CRITERIA** for an individual only if there is a family history with multiple affected members in more than one generation suggestive of dominant inheritance.

13) Genetic testing of *LRRK2* **MEETS COVERAGE CRITERIA** in the following situations:

    a) In symptomatic individuals with a positive family history suggestive of dominant inheritance.

    b) In symptomatic individuals belonging to a population with known high mutation frequencies of the *LRRK2* gene (i.e., Ashkenazi Jews, Imazighen, and Euskaldunak).

14) Genetic testing of the *PRKN* (formerly *PARK2* or *parkin*) *PINK1*, and *PARK7* (formerly *DJ-1)* genes **MEETS COVERAGE CRITERIA** in the following situations:

    a) In individuals with an onset of disease by the age of 50 years with a positive family history suggestive of recessive inheritance.

    b) In individuals with an onset of disease by the age of 40 years regardless of family history.

15) Genetic testing of the *ATP13A2, PLA2G6,* and *FBXO7* genes **MEETS COVERAGE CRITERIA** only when **all** of the following conditions are met:

    a) With onset of disease by the age of 40 years

    b) Prior testing of *PRKN*, *PINK1,* and *PARK7* genes was negative for known pathogenic variants.

Spinal Muscular Atrophies (SMA)

16) To diagnose individuals suspected of having SMA, genetic testing for SMA (*SMN1* deletion/mutation and *SMN2* copy number) **MEETS COVERAGE CRITERIA**.

Wilson disease (WD)

17) Genetic testing of *ATP7B* **MEETS COVERAGE CRITERIA** in the following situations:

    a) To confirm a diagnosis of Wilson disease in a symptomatic individual.

    b) In a first-degree relative (see Note 1) of an individual with known *ATP7B* mutation to guide potential therapy.

NOTES:

Note 1: First-degree relatives include parents, full siblings, and children of the individual. Second-degree relatives include grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings of the individual.

Note 2: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
ACOG	American College of Obstetricians and Gynecologists
ACP33	*SPG21 abhydrolase domain containing, maspardin*
ADAR	*Adenosine deaminase RNA specific*
ADCK3	AARF domain containing kinase 3
ADCY5	Adenylate cyclase 5
ADHSP	Autosomal dominant hereditary spastic paraplegia
AFG3L2	*AFG3 like matrix AAA peptidase subunit 2*
ALDH18A1	*Aldehyde dehydrogenase 18 family member A1*
ALDH3A2	*Aldehyde dehydrogenase 3 family member A2*
ALS	Amyotrophic lateral sclerosis
ALSA	Amyotrophic Lateral Sclerosis Association
AMPD2	*Adenosine monophosphate deaminase 2*
ANO3	*Anoctamin 3*
AP4B1	*Adaptor related protein complex 4 subunit beta 1*
AP4M1	*Adaptor related protein complex 4 subunit mu 1*
AP4S1	*Adaptor related protein complex 4 subunit sigma 1*
AP5Z1	*Adaptor related protein complex 5 subunit zeta 1*
APTX	Ataxia with oculomotor apraxia
ARHSP	Autosomal recessive hereditary spastic paraplegia
AT	Ataxia-telangiectasia
ATAD3A	*ATPase family AAA domain containing 3A*
ATL1	*Atlastin GTPase 1*
ATLD	Ataxia telangiectasia-like disorder
ATM	Ataxia-telangiectasia mutated
ATN1	*Atrophin 1*
ATP	Adenosine triphosphate
ATP13A2	*ATPase cation-transporting 13A2*
ATP2B4	*ATPase plasma membrane Ca2+ transporting 4*

ATP7B	*ATPase copper transporting beta*
ATXN1/2/3/7/8/10	*Ataxin 1/2/3/7/8/10*
ATXN8OS	*Ataxin 8 opposite strand lncRNA*
B4GALNT1	*Beta-1,4-N-acetyl-galactosaminyltransferase 1*
BEAN1	*Brain expressed associated with NEDD4 1*
BICD2	*BICD cargo adaptor 2*
BSCL2	*BSCL2 lipid droplet biogenesis associated, seipin*
C12orf65	*Mitochondrial translation release factor in rescue*
C19orf12	*Chromosome 19 open reading frame 12*
C9ORF72	*Chromosome 9 Open Reading Frame 72*
CACNA1A	*Calcium Voltage – Gated Channel Subunit Alpha 1 A*
CCDC88C	*Coiled-coil domain containing 88C*
CLIA	Clinical Laboratory Improvement Amendments
CMS	Centres for Medicare and Medicaid Services
CMT	Charcot Marie-Tooth Neuropathy
CPT1C	*Carnitine palmitoyltransferase 1C*
CSF	Cerebrospinal fluid
CYP2U1	*Cytochrome P450 family 2 subfamily U member 1*
CYP7B1	*Cytochrome P450 family 7 subfamily B member 1*
DD	Developmental delay
DDHD1/2	*DDHD domain containing ½*
DI-CMT	Dominant intermediate Charcot Marie-Tooth neuropathy
DJ-1	*Parkinsonism associated deglycase*
DNA	Deoxyribonucleic acid
DNM2	*Dynamin 2*
DRPLA	Dentatorubral-pallidoluysian atrophy
DYT1	*Dystonia 1/6/8/11*
EEF2	*Eukaryotic translation elongation factor 2*
EFNS	European Federation of Neurological Societies
EHDN	Working Group on Genetic Counselling and Testing of the European Huntington's Disease Network
ELOVL5	*Elongation of very long chain fatty acid elongase 5*
ENMC	European Neuromuscular Center
ENS	European Neurological Society
ENTPD1	*Ectonucleoside triphosphate diphosphohydrolase 1*
ERLIN1/2	*ER lipid raft associated ½*
ESPGHAN	Hepatology Committee of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition
FA	Friedreich's ataxia

FA2H	*Fatty acid 2-hydroxylase*
FALSA	Familial amyotrophic lateral sclerosis
FARA	Friedreich's Ataxia Research Alliance
FGF14	*Fibroblast growth factor 14*
FMR1	*Fragile X mental retardation 1*
FRDA	*Frataxin*
FUS	*FUS RNA binding protein*
FXN	*Frataxin*
FXTAS	Fragile X Associated Tremor and Ataxia Syndrome
GAA	*Alpha glucosidase*
GAD1	*Glutamate decarboxylase 1*
GAK- DGKQ	*Cyclin G associated kinase-diacylglycerol kinase theta*
GBA	*Glucocerebrosidase*
GBA2	*Glucocerebrosidase beta 2*
GCH1	*GTP cyclohydrolase 1*
GJC2	*Gap junction protein gamma 2*
GLUT1	*Glucose transporter protein type 1*
GRID2	*Glutamate ionotropic receptor delta type subunit 2*
H2O2	Hydrogen peroxide
HD	Huntington disease/Huntington's disease
HDL4	Huntington disease like 4
HLA	*Human leukocyte antigen*
HMN	Hereditary motor neuropathy
HSP	Hereditary spastic paraplegia
HSPD1	*Heat shock protein family D (Hsp60) member 1*
HTT	*Huntingtin*
ICARS	International cooperative ataxia rating scale
ID	Intellectual disability
ITALSGEN	Italian Amyotrophic Lateral Sclerosis Genetic consortium
ITPR1	*Inositol 1,4,5-trisphosphate receptor type 1*
KCND3	*Potassium voltage-gated channel subfamily D member 3*
KIAA1096	*Proline-rich coiled-coil 2C*
KIF1A/1C/5A	*Kinesin family member 1A/1C/5A*
KLC2/4	*Kinesin light chain 2/4*
L1CAM	*L1 cell adhesion molecule*
LDTs	Laboratory developed tests
LRRK2	Leucine rich repeat kinase-2
MARS1	*Methionyl-tRNA synthetase 1*

MDS	International Parkinson and Movement Disorder Society
MDS-ES	Movement Disorder Society – European Section
MELAS	Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes
MERRF	Myoclonus epilepsy, ragged-red-fibers
MHC	*Major histocompatibility complex*
MR1	*Major histocompatibility complex, class I-related*
MRE11A	*MRE11 homolog, double strand break repair nuclease*
mRNA	Messenger ribonucleic acid
MT-ATP6	*Mitochondrially encoded ATP synthase membrane subunit 6*
NAF	National Ataxia Foundation
NARP	Neuropathy, ataxia, and retinitis pigmentosa
NIPA1	*NIPA magnesium transporter 1*
NOP56	*NOP56 ribonucleoprotein*
NORD	National Organization for Rare Disorders
NT5C2	*5'-nucleotidase, cytosolic II*
O2	Oxygen
PARK1/2/4/7/8	*Parkinson disease ½/4/7/8*
PD	Parkinson disease
PDYN	*Prodynorphin*
PGAP1	*Post-GPI attachment to proteins inositol deacylase 1*
PINK1	*PTEN induced kinase 1*
PKND	Paroxysmal dyskinesia with dystonia
PLA2G6	*Phospholipase A2 group VI*
PLP1	*Proteolipid protein 1*
PNKD	Paroxysmal nonkinesigenic dyskinesia
PNPLA6	*Patatin like phospholipase domain containing 6*
POLG	*DNA polymerase gamma, catalytic subunit*
PPP2R2B	*Protein phosphatase 2 regulatory subunit B beta*
PRKCG	*Protein kinase C gamma*
REEP1/2	*Receptor accessory protein ½*
RTN2	*Reticulon 2*
SACS	*Sacsin molecular chaperone*
SARA	Scale for the assessment and rating of ataxia
SCA	Spinocerebellar ataxia
SCAR9	Spinocerebellar ataxia 9
SGCE	*Sarcoglycan epsilon*
SLC2A1	*Solute carrier family 2, member 1*
SLC16A2	*Solute carrier family 16, member 2*

SLC33A1	*Solute carrier family 33, member 1*
SMA	Spinal muscular atrophies
SMN1/2	*Survival of motor neuron ½*
SNCA	*Alpha-synuclein*
SNP	Single nucleotide variant
SOD1	*Superoxide dismutase 1*
SPAST	Spastin
SPG1 – 74	*Spastic paraplegia 1 – 74*
SPTBN2	*Spectrin beta, non-erythrocytic 2*
STR	Short tandem repeat
TARDBP	*TAR DNA binding protein*
TBP	*TATA-binding protein*
TECPR2	*Tectonin beta-propeller repeat containing 2*
TFG	*Trafficking from ER to golgi regulator*
TGM6	*Transglutaminase 6*
THAP1	*THAP domain containing 1*
TOR1A	*Torsin family 1 member A*
TRPC3	*Transient receptor potential cation channel subfamily C member 3*
TTBK2	*Tau tubulin kinase 2*
TTPA	*Alpha tocopherol transfer protein*
TUBB4A	*Tubulin beta 4A class Iva*
UBQLN2	*Ubiquilin 2*
UPDRS	Unified Parkinson's Disease Rating Scale
USP8	*Ubiquitin specific peptidase 8*
WASHC5	*WASH complex subunit 5*
WD	Wilson Disease/Wilson's Disease
WDR48	*WD repeat domain 48*
ZFYVE26/27	*Zinc finger FYVE-type containing 26/27*

V. Scientific Background

Neurodegenerative diseases are characterized by progressive loss of neurons along with deposition of misfolded proteins throughout the body. These misfolded proteins have altered biochemical properties, causing dysfunction. Clinical symptoms may include cognitive decline (primarily dementia) and movement problems (cerebellar dysfunction, hyper- or hypo-kinesia, etc.). The molecular spectrum of these disorders may vary, but typically involve oxidative or neuroinflammatory damage (Kovacs, 2016).

Ataxias (including Friedreich ataxia)

Ataxias encompass the set of conditions that are characterized by "motor incoordination resulting from dysfunction of the cerebellum and its connections" (Opal & Zoghbi, 2024b). This policy focuses on progressive and degenerative ataxias, which are further subdivided into autosomal dominant, autosomal recessive, and X-linked forms.

Friedreich ataxia is the most common hereditary ataxia and is inherited in an autosomal recessive manner. Most cases are caused by mutations in the frataxin gene (*FXN*), which is responsible for transport and management of iron. The frataxin mutation is typically an expanded trinucleotide (van de Warrenburg et al.) repeat in the first intron of the frataxin gene, which reduces expression of frataxin. Severity of phenotype varies with the number of repeats; larger repeats are generally more severe. Impaired iron management leads to a variety of clinical symptoms, such as neurological problems (progressive ataxia, dysphagia, motor weakness, loss of tendon reflexes, etc.), cardiomyopathy, diabetes mellitus, and skeletal deformities. Clinical findings may suggest Friedreich ataxia, but diagnosis is generally confirmed through genetic testing (Opal & Zoghbi, 2023).

Another autosomal recessive ataxia is ataxia-telangiectasia (AT). AT is caused by a defective gene on chromosome 11q22.3, leading to faulty DNA repair mechanisms. This gene (designated AT "M" for mutated) primarily regulates the cell cycle and prevents the cell cycle from progressing if there is DNA damage. When this gene fails, somatic mutations may accumulate. Symptoms such as immune deficiency, cerebellar ataxia, unusual eye movements, and other neurologic abnormalities are characteristic of ataxia-telangiectasia. Ataxia is one of the first clinical symptoms of patients with AT, but other organ systems are usually affected, such as the skin and circulatory system. Similarly, ataxia-telangiectasia-like disorder (ATLD) can affect individuals similarly to AT; however, ATLD is due to mutations within the *MRE11A* gene involved in double-strand DNA break recognition and repair. The rate of neurodegeneration in ATLD is typically slower than AT. ATLD is more rare than AT; however, "it is estimated that as many as 5 percent of AT cases may be incorrectly diagnosed and actually have ATLD, given the similarity in clinical manifestations and coding sizes of the two affected genes" (Opal, 2024).

Spinocerebellar ataxias (SCAs) are the most common autosomal dominant ataxias. At least 30 types of SCAs with varying phenotypes occur, although, cerebellar ataxia is a primary feature of each type. For example, SCA1 is characterized by dysarthria and bulbar dysfunction whereas SCA2 is characterized by "slow saccadic eye movements." Several SCA types have a signature CAG repeat beyond what is present in the wildtype; this expansion is pathogenic. As with Friedreich ataxia, larger number of repeats usually lead to more severe symptoms. The four most common SCAs are SCA1, 2, 3,

and 6, and each type is caused by a different pathogenic mutation. Below is a table displaying each SCA, its distinguishing features, and its primary associated gene (Opal & Zoghbi, 2024a).

Disorder	Distinguishing features	Gene
SCA1	Pyramidal signs, peripheral neuropathy	*ATXN1*
SCA2	Slow saccades; less often myoclonus, areflexia	*ATXN2*
SCA3 (MJD)	Slow saccades, persistent stare, extrapyramidal signs, peripheral neuropathy	*ATXN3*
SCA4	Sensory neuropathy	*16q22.1*
SCA5	Early onset but slow progression	*SPTBN2*
SCA6	May have very late onset, mild, may lack family history, nystagmus	*CACNA1A*
SCA7	Macular degeneration	*ATXN7*
SCA8	Mild disease	*ATXN8, ATXN8OS*
SCA9	Not assigned	
SCA10	Generalized or complex partial seizures	*ATXN10*
SCA11	Mild disease	*TTBK2*
SCA12	Tremor, dementia	*PPP2R2B*
SCA13	Mental retardation	*KCNC3*
SCA14	Intermittent myoclonus with early onset disease	*PRKCG*
SCA15/16	Slowly progressive	*ITPR1*
SCA17 (or HDL4)[1]	Gait ataxia, dementia	*TBP*
SCA18	Pyramidal signs, weakness, sensory axonal neuropathy	*7q22-q32*
SCA19/22	Predominantly cerebellar syndrome, sometimes with cognitive impairment or myoclonus	*KCND3* gene
SCA20	Palatal tremor and dysphonia	*11q12*
SCA21	Mild to severe cognitive impairment	*TMEM240*
SCA23	Distal sensory deficits	*PDYN*
SCA24	Recessive inheritance; redesignated as SCAR4	*1p36*
SCA25	Sensory neuropathy, facial tics, gastrointestinal symptoms	*2p21-p13*
SCA26	Pure cerebellar ataxia	*EEF2*
SCA27	Cognitive impairment	*FGF14*
SCA28	Ophthalmoparesis and ptosis	*AFG3L2*

Disorder	Distinguishing features	Gene
SCA29	Early onset, nonprogressive ataxia; may be an allelic variant of SCA15	*3p26*
SCA30	Slowly progressive, relatively pure ataxia	*4q34.3-q35.1*
SCA31	Decreased muscle tone	*BEAN*
SCA32	Cognitive impairment; affected individuals with azoospermia and testicular atrophy	*7q32-q33*
SCA33	Not assigned	
SCA34	Skin lesions consisting of papulosquamous erythematous ichthyosiform plaques	*ELOVL4*
SCA35	Late onset, slowly progressive gait and limb ataxia	*TGM6*
SCA36	Late onset, truncal ataxia, dysarthria, variable motor neuron disease and sensorineural hearing loss	*NOP56*
SCA37	Late onset, falls, dysarthria, clumsiness, abnormal vertical eye movements	*1p32*
SCA38	Slowly progressive pure cerebellar phenotype	*ELOVL5*
SCA39	Not assigned	
SCA40	Hyperreflexia and spasticity	*CCDC88*
DRPLA	Chorea, seizures, myoclonus, dementia	*ATN1*
[1]SCA17 is synonymous with HDL4 (Huntington disease-like 4) (Toyoshima et al., 2012).		

Jacobi et al. (2015) described the disease progression of SCAs 1, 2, 3, and 6. A total of 462 patients were evaluated on the Scale for the Assessment and Rating of Ataxia (SARA). Annual SARA score increase was 2.11 for SCA1 patients, 1.49 for SCA2, 1.56 for SCA3, and 0.80 for SCA6. The increase of non-ataxia signs plateaued in types 1, 2, and 3. SCA6 symptoms were found to increase more slowly than the other three types. Factors associated with a faster increase of SARA score across all types were short duration of follow-up, older age at inclusion (per additional year), and longer repeat expansions (per additional repeat unit) (Jacobi et al., 2015).

Reetz et al. (2015) examined the effect of the number of GAA repeats in the *FXN* gene on clinical symptoms of Friedreich's Ataxia (FA). A total of 592 patients with FA were sequenced and evaluated. The authors found that with every 100 GAA repeats, the age of onset was 2-3 years earlier. Disease progression was also found to be faster in patients with more repeats; the annual worsening of the Scale for the Assessment and Rating of Ataxia (SARA) score was 1.04 points per year and 1.37 points per year for early and intermediate onset (≤14 and 15-24 years, respectively), compared to 0.56 points per year for late-onset patients (≥25 years) (Reetz et al., 2015).

Leotti et al. (2021) examined the contribution that the expanded CAG repeat length has on the rate of disease progression in spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD). Expanded CAG repeat in ATXN3 is the mutation that causes SCA3/MJD, and the length of CAG repeat can determine the age of onset of clinical symptoms. The authors studied 82 patients with SCA3/MJD over 15 years using the International Cooperative Ataxia Rating Scale (ICARS) and found that "The length of the CAG repeat was positively correlated with a more rapid ICARS progression, explaining 30% of the differences between patients." The authors concluded that the length of CAG repeat in ATXN3 has major influence over clinical symptoms (Leotti et al., 2021).

Schuermans et al. (2023) performed an observational study to assess the diagnostic value of exome sequencing and multigene panel analysis for adult-onset neurologic disorders, including ataxias. In 2019, 6 diagnostic gene panels were introduced at the center for Medical Genetics of the Ghent University Hospital to diagnose patients with neurologic disorders. One of these panels was for ataxia and spasticity and included 390 genes. While the most common ataxia genes were not included in this panel, only 33% of the diagnosed patients had first been tested for SCAs, Friedrich ataxia, or fragile X tremor/ataxia syndrome. The panels included single nucleotide variants, small indels, and copy number variants. Of the panels and targeted patient populations examined by the authors, the multi-gene panel for ataxia and spasticity had the highest diagnostic yield, identifying the causal pathogenic variant (s) in 19% of the assessed patients (70 of 365) (Schuermans et al., 2023).

Dystonias

Dystonias are a class of movement disorders characterized by "sustained or intermittent muscle contractions causing abnormal, often repetitive movements, postures, or both" (Deik & Comelia, 2023). Movements are typically twisting or patterned and are often worsened by voluntary action. The basic neurochemistry of dystonia is unknown (and without consistent findings), and cell degeneration is typically not seen. However, some types of dystonia (particularly early-onset versions) have clear associations with certain genes. For example, *TOR1A* and *THAP1* both carry pathogenic mutations for early-onset dystonia. *TOR1A* (*DYT1*) encodes a protein that binds to ATP (torsin A) while *THAP1* (*DYT6*) encodes a transcription regulator for torsin A (Deik & Comelia, 2023).

Dystonias are divided into classes or types. They can be focal (involving a single site), multifocal segmental (involving region(s) of the body), generalized (involving the trunk and at least two additional sites), or hemidystonia (affecting only one side of the body). The etiology of the disorder can be either idiopathic or of known causation. Dystonias

can be due to trauma or may be inherited. Those forms of proven genetic origin can be inherited in different inheritance patterns, including autosomal dominant, autosomal recessive, X-linked recessive, or even mitochondrial inheritance. The most common inherited form of dystonia is DYT-TOR1A (or DYT1) dystonia. DYT-TOR1A accounts for approximately 40-65% of early-onset generalized dystonia in populations other than the Ashkenazi Jewish population. Within the latter population, DYT-TOR1A is estimated to account for 90% of these cases (Deik & Comelia, 2023). Even though DYT-TOR1A dystonia is inherited in an autosomal dominant pattern, the penetrance is only 30% (Bressman, 2004; Deik & Comelia, 2023; Ozelius & Lubarr, 2016). Paroxysmal dyskinesia with dystonia (PKND) is a special class of dystonia that involves spontaneous episodes of dystonia. Several environmental factors have been proposed to precipitate these episodes, such as stress, caffeine, and fatigue. The primary gene associated with PKND is *MR1*, or *DYT8*. Another special class of dystonia is myoclonus-dystonia, which is characterized by short, involuntary movements of the neck or arms in addition to normal dystonia symptoms. The main established type of myoclonus-dystonia is caused by mutations in the *SGCE* (*DYT11*) gene (Deik & Comelia, 2023).

Zech et al. (2017) performed whole exome sequencing on 16 patients with "genetically undefined early-onset generalized dystonia." Six patients had mutations of known dystonia-related genes. The mutated genes were *GCH1, THAP1, TOR1A, ANO3*, and *ADCY5*. The authors noted *GCH1, THAP1*, and *TOR1A* as associated with isolated, generalized dystonia and *ANO3* and *ADCY5* associated with a combined myoclonus-dystonia phenotype (Zech et al., 2017).

Parkinsonism (Parkinson Disease, PD)

Parkinsonism is a constellation of symptoms with "any combination of bradykinesia, rest tremor, rigidity, and postural instability." The most common form of parkinsonism is Parkinson disease (PD), a progressive neurodegenerative disorder characterized by degeneration of dopaminergic neurons in the brain (Chou, 2024b). The pathogenesis of PD is driven by loss of dopamine from the basal ganglia in the brain; though a number of compensatory mechanisms may mitigate this loss of dopamine, the progression of disease eventually leads to clinical symptoms (Jankovic, 2024). The "cardinal" features of PD are "tremor, bradykinesia, and rigidity"; postural instability is commonly considered a defining feature, yet it typically manifests late in the course of disease. Other motor symptoms such as dysphagia, blurred vision, shuffling, are common; these secondary motor symptoms are commonly derived from the cardinal features. Nonmotor symptoms include cognitive deterioration, dementia, and other mood disorders (Chou, 2024a).

The exact cause of PD is unknown, but several genetic factors have been Identified. These genes do not imply a particular phenotype, and each mutation vary in severity of symptomology. Genes associated with PD are *SNCA (PARK1/4), LRRK2 (PARK8), PINK1, PARK2, DJ-1 (PARK7)*, and *GBA* (Jankovic, 2024).

GBA- (glucocerebrosidase) associated PD is coupled with the lysosomal storage condition known as Gaucher disease, which is commonly seen in Ashkenazi Jews (Jankovic, 2024). Sidransky et al. (2009) compared PD patients with a *GBA* mutation to those with PD but without a *GBA* mutation, and they found that the patients with a *GBA* mutation had an earlier age of onset and greater chance of cognitive impairment, albeit with less pronounced cardinal features (Sidransky et al., 2009).

SNCA encodes alpha-synuclein. Although its exact role is not well understood, it is thought to function in synaptic plasticity and makes up as much as one percent of total central nervous system protein. Observations suggest a role for mutated alpha-synuclein in the pathogenesis of PD; for example, Lewy bodies, the primary pathologic hallmark of PD, have insoluble, aggregated alpha-synuclein as a major component. It may also be possible for misfolded alpha-synuclein to be transmitted from diseased neurons to healthy ones. *PARK1* refers to a missense mutation in *SNCA* whereas *PARK4* refers to a multiplication (Jankovic, 2024).

LRRK2 (*leucine-rich repeat kinase-2*) encodes a protein called dardarin. Dardarin is thought to function as a kinase for phosphorylation of certain proteins, such as alpha-synuclein and microtubule-associated protein tau. Dardarin may also be implicated in membrane and protein transport. The phenotype of *LRRK2* mutations is noted to be less severe than other genotypes of PD; patients have been observed to respond to levodopa, have a later age of onset, and less severe cognitive deterioration (Jankovic, 2024).

PARK2 encodes a protein called parkin. This protein is associated with degradation of certain proteins in wild-type genes; the mutated version of parkin cannot clear proteins, allowing them to aggregate in the neuron. This mutation typically leads to an early-age onset of PD and clinical symptoms, although the severity of these early symptoms does not appear to be significantly worse than other genotypes (Jankovic, 2024).

DJ-1 and *PINK1* are both associated with autosomal recessive inheritance and early age of onset (under 50 for *PINK1* mutations, under 40 for *DJ-1* mutations). *PINK1* mutations are possibly associated with mitochondrial dysfunction whereas *DJ-1* mutations may lead to increased neuro-oxidative stress (Jankovic, 2024).

Nalls et al. (2014) performed a meta-analysis of genome-wide association studies on PD. A common set of 7893274 variants with 13708 cases and 95282 controls were evaluated. Thirty-two loci were identified as having genome-wide significant association. These 32 loci were re-tested in an independent set of 5353 cases and 5551 controls, and 24 of these loci replicated their significance. Four loci (*GBA, GAK-DGKQ, SNCA, HLA* region) were considered to have a "secondary independent risk variant." The authors noted that the effect of each individual loci was small, but cumulative risk was "substantial" (Nalls et al., 2014).

Maple-Grødem et al. (2021) studied the significance of *glucocerebrosidase* gene (*GBA*) carrier status on motor impairment in patients with incident PD. The authors studied 528 patients with PD, using genomic DNA assessment and the Unified Parkinson's Disease Rating Scale (UPDRS). *GBA* carriers had a faster annual increase in UPDRS score than non-carriers. The authors conclude that "*GBA* variants are linked to a more aggressive motor disease course over 7 years from diagnosis in patients with PD" (Maple-Grødem et al., 2021).

Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder that causes significant motor neuron degeneration all over the body. This causes a variety of neuromuscular problems, such as spasticity, weakness, atrophy, hyperreflexia, cognitive impairment, and eventual death. ALS is divided into two categories: sporadic (90% of cases) and familial (10%) (Elman et al., 2023).

The primary genes tested in ALS cases are superoxide dismutase (*SOD1*) and chromosome 9 open reading frame 72 (*C9ORF72*), both of which lead to familial ALS. The enzyme SOD1 catalyzes toxic superoxide radicals to O2 and H2O2. The mutation thought to be the primary cause of *SOD1*-mediated toxicity is a gain-of-function mutation, creating many reactive oxygen species. Other hypotheses of *SOD1*-mediated toxicity include misfolded proteins caused by *SOD1* mutations and production of protein aggregates that damage motor neurons (Maragakis, 2023).

C9ORF72 expansions are another common cause of familial ALS. This mutation is a hexanucleotide repeat (GGGGCC) that forms a structure called the G-quadruplex. The exact pathogenic mechanism is unknown, but some hypotheses include creation of defective RNA transcripts and creation of toxic dipeptide proteins that cause RNA processing to falter (Maragakis, 2023).

Chiò et al. (2012) evaluated the genetic landscape of ALS in an Italian cohort. A total of 475 patients were examined, and 51 were noted to carry a mutation associated with ALS. Familial ALS was found in 46 of these patients, and 31 of these 46 were found to

have a genetic mutation (leaving 20 mutations in the remaining 429 sporadic cases). After performing a logistic regression, the authors found that the chance to carry a genetic mutation was related to the presence of comorbid frontotemporal dementia by an odds ratio of 3.5 (Chiò et al., 2012).

Vajda et al. (2017) evaluated clinician opinion on genetic testing in ALS. Responses from 167 clinicians in 21 countries were analyzed. Approximately 90.2% of respondents were found to have offered genetic testing to patients they defined as having familial ALS and 49.4% to patients with sporadic ALS. The four main genes tested were *SOD1, C9ORF72, TARDBP*, and *FUS*. Further, 42% of respondents did not offer genetic testing to asymptomatic family members of patients with familial ALS (Vajda et al., 2017).

Bandres-Ciga et al. (2019) used publicly available genome-wide association studies to identify shared polygenic risk genetic factors and casual associations in 20,806 ALS cases and 59,804 controls. Positive associations were found with smoking and moderate physical activity levels, and negative associations were found with higher education, cognitive performance, and light physical activity levels. Further, the authors report that "hyperlipidemia is a causal risk factor for ALS and localized putative functional signals within loci of interest" (Bandres-Ciga et al., 2019).

Wilson Disease (WD)

Wilson disease (WD) is a condition caused by defective copper transport. This leads to accumulation of copper in several organs, such as the brain, eyes, and liver. Eventually, the liver becomes cirrhotic, while other neurological conditions may develop. The primary gene handling hepatocyte copper transport is *ATP7B*. Normally, this gene mediates the transport of copper into apoceruloplasmin, which is then secreted into the bloodstream. Mutations in this gene cause impaired binding of copper to the protein, causing copper accumulation in the hepatocyte and eventually the bloodstream (Schilsky, 2024).

Dong et al. (2016) evaluated the genetic spectrum of WD in Chinese patients. A total of 632 patients with WD were compared against 503 controls. Further, 161 variants were found in the WD patents, and 142 were considered pathogenic or "likely pathogenic." The authors concluded that 569 of the 632 patients (90%) could be diagnosed with two or more "likely pathogenic" or worse variants. Finally, the 14 most common variants were found at least once in 537 of the 569 (94%) genetically diagnosed patients (Dong et al., 2016).

Huntington Disease (HD)

Huntington disease (HD) is a progressive, neurodegenerative disorder characterized by choreiform (brief, abrupt, and involuntary) movements, psychiatric disorders, and eventual dementia. During the early stages of the disease, patients may be able to function day-to-day and perform typical tasks; however, as the disease progresses, patients lose their ability to function independently and require assistance. In the late stages of the disease, patients often become bedridden as cognitive and motor ability continues to decline, with death occurring 10 to 40 years after onset. Currently there is no cure, and the disorder is inherited in an autosomal dominant fashion (Suchowersky, 2023).

Huntington disease is primarily caused by a trinucleotide repeat expansion. A cytosine-adenine-guanine "repeat" encodes for polyglutamine tracts in the *huntingtin* (*HTT*) gene, and the "expansion" refers to additional repeats of this trinucleotide side. Approximately 6-26 CAG repeats is considered wild-type, 27-35 repeats is considered intermediate (i.e., typically do not cause disease but may expand in future generations), and ≥36 repeats is considered diagnostic of HD. CAG repeat length is considered to correlate with both rate of disease progression and severity of neurological changes. The CAG repeat expansion leads to a toxic "gain-of-function" of the *HTT* protein, and although the exact function of this huntingtin protein is unknown, it interacts with several different proteins, implying that it has a function in several cellular events. Mutant huntingtin is seen to disrupt transcription, activation of proteases, synaptic transmission, and more (Zoghbi & Orr, 2023).

Baig et al. (2016) reviewed 22 years of predictive testing performed by the UK's Huntington Consortium. A total of 9407 predictive tests were performed over 23 testing centers, with 8441 tests on individuals considered at 50% predictive risk. Of these 8441, 4629 were mutation negative and 3790 were mutation positive (with 22 tests as "uninterpretable"). A prevalence figure of 12.3×10^{-5} was used to evaluate the "cumulative uptake" of predictive testing at the 50% risk level; this amount was calculated to be 17.4% (the number of individuals at 50% risk that had undergone predictive testing). The authors concluded that the majority of individuals at risk for HD had not undergone predictive testing (Baig et al., 2016).

Spinal Muscular Atrophies (SMA)

Spinal muscular atrophy (SMA) disorders encompass the set of disorders that are characterized by the degeneration of anterior "horn" cells in the spinal cord and motor nuclei in the lower brainstem. This leads to muscle weakness and atrophy, although cognition is unaffected. There are currently five main types of SMA, types 0 to 4. These types are organized by age of onset and clinical presentation, with types 0 and 1 presenting earliest and with the most severe symptoms and type 4 as the least severe

phenotype. For example, type 0 presents prenatally and death occurs by six months, whereas type 4 patients usually remain ambulatory and have a normal lifespan (Bodamer, 2023).

The primary gene mutation occurs in the survival motor neuron 1 (*SMN1*) gene. This gene encodes a protein that appears to play a role in mRNA synthesis. The most common mutation in *SMN1* is a deletion of exon 7, representing up to 94% of SMA patients. Another gene, *SMN2*, may cause phenotypic changes in *SMN1* due to its effect as a gene modifier. *SMN2* encodes an extremely similar protein to *SMN1* (only one nucleotide difference), and it may compensate for *SMN1* loss. Severity of SMA correlates inversely with amount of *SMN2* gene copy numbers, which varies from 0 to 8 (Bodamer, 2023).

Zarkov et al. (2015) evaluated the association between clinical symptoms and *SMN2* gene copy numbers. Forty-three patients with SMA were examined, and 37 of them had homozygous deletions of *SMN1* exon 7. The genetic characterization of these 37 patients were as follows: "One had SMA type I with 3 SMN2 copies, 11 had SMA type II with 3.1 +/- 0.7 copies, 17 had SMA type III with 3.7 +/- 0.9 copies, while 8 had SMA type IV with 4.2 +/- 0.9 copies." The authors concluded that "a higher SMN2 gene copy number correlated with less severe disease phenotype," but they noted that potential other phenotype modifiers could not be ignored (Zarkov et al., 2015).

Hereditary Spastic Paraplegia (HSP)

Hereditary spastic paraplegia (HSP) represents a group of genetic neurodegenerative diseases characterized by increased spasticity of the lower limbs over time (Shribman et al., 2019). Spastic gait is often the only, or main, feature of the syndrome; bladder dysfunction is a common clinical finding as well. More than 70 types of HSP have been identified, and are often due to axon degeneration, leading to progressive degeneration of the corticospinal tracts (Fink, 2014; Opal & Ajroud-Driss, 2022). The classification of HSP may be based on age of onset, rate of progression, degree of spasticity, and genetics with more than 55 loci related to the disease (Opal & Ajroud-Driss, 2022). Some of the more common autosomal dominant forms of HSP may be caused by mutations in the *ATL1, SPAST, KIAA1096, KIF5A,* and *REEP1* genes; additional genes are associated with autosomal recessive forms, x-linked forms, and mitochondrial forms of HSP (Opal & Ajroud-Driss, 2022).

Dong et al. (2018) performed next-generation sequencing on 149 genes associated with HSP in a cohort of 99 individuals. A retrospective study on other patients with HSP was also completed. Different genetic mutations cause different subtypes of HSP such as SPG4, SPG3A, and SPG6. The researchers note that "In ADHSP [autosomal dominant

HSP], we found that SPG4 (79%) was the most prevalent [subtype], followed by SPG3A (11%), SPG6 (4%) and SPG33 (2%)… In ARHSP [autosomal recessive HSP], the most common subtype was SPG11 (53%), followed by SPG5 (32%), SPG35 (6%) and SPG46 (3%)" (Dong et al., 2018).

In 2018, GeneReview published an updated overview of HSP. This overview was last updated in 2021. This document states the following regarding genetic testing:

- "Concurrent or serial single-gene testing can be considered if clinical findings and/or family history indicate that involvement of a particular gene or small subset of genes is most likely (see Tables 1, 2, 3, and 4)"
- "A multigene panel that includes some or all of the genes listed in Table 1 is most likely to identify the genetic cause of the condition at the most reasonable cost while limiting identification of variants of uncertain significance and pathogenic variants in genes that do not explain the underlying phenotype"
- "Comprehensive genomic testing (which does not require the clinician to determine which gene[s] are likely involved) may be considered. Exome sequencing is most commonly used; genome sequencing is also possible. Exome array (when clinically available) may be considered if exome sequencing is not diagnostic"
- "Recommendations for the evaluation of parents of a proband with an apparent *de novo* pathogenic variant include molecular genetic testing of both parents for the pathogenic variant identified in the proband" (Hedera, 2021).

GeneReviews has published four tables (below) which show the genes associated with autosomal dominant HSP, autosomal recessive HSP, X-linked HSP, and maternal (mitochondrial) HSP.

Table 1: Hereditary Spastic Paraplegia: Genes and Distinguishing Clinical Features – Autosomal Dominant Inheritance (Hedera, 2021)

Gene[1]	HSP	Type of HSP	Onset	Distinguishing Clinical
ADAR	Not assigned	Uncomplicated	Early childhood	Abnormal pattern of interferon expression determined by reverse transcription PCR assay
ALDH18A1	SPG9A	Complicated	Adolescence to adulthood (1 subject w/infantile onset)	Cataracts Gastroesophageal reflux Motor neuronopathy Variably present: Dysarthria

Gene[1]	HSP	Type of HSP	Onset	Distinguishing Clinical
				Ataxia Cognitive impairment
ATAD3A	Not assigned	Complicated	Early onset	Amyotrophy Hyperkinetic movements May be confused w/hyperkinetic cerebral palsy
ATL1	SPG3A	Uncomplicated	Infantile to childhood (rarely adult onset)	Progression may be minimal w/static course. May present as spastic diplegic cerebral palsy Complicated phenotype w/peripheral neuropathy or autonomic failure reported
BICD2	Not assigned	Complicated	Childhood or adult	Infantile onset associated w/SMA w/variable upper motor signs & contractures Adult onset associated w/mild amyotrophy
BSCL2 [2]	SPG17	Complicated	Adulthood	Distal amyotrophy affecting hands & feet Motor neuropathy Can be indistinguishable from ALS
CPT1C	SPG73	Uncomplicated	Early adulthood	Foot deformity may be present.
DNM2 [3]	Not assigned	Complicated	Before age 20 years	Axonal polyneuropathy may be present. Mild distal amyotrophy in feet
ERLIN2	SPG18 [4]	Uncomplicated	Juvenile to adulthood	None
HSPD1	SPG13	Uncomplicated	Adulthood	Mild distal amyotrophy
KIF5A	SPG30	Uncomplicated (for AD inheritance)	Juvenile to adulthood	Some individuals have mild ID.

Gene[1]	HSP	Type of HSP	Onset	Distinguishing Clinical
				Optic nerve atrophy, epilepsy can be rarely seen in AD SPG30.
KIF5A [4]	SPG10	Complicated	Juvenile or adulthood	Polyneuropathy Pes cavus
NIPA1	SPG6	Uncomplicated	Adulthood (infantile onset rare)	Severe weakness & spasticity Rapidly progressive Rarely, complicated by epilepsy or variable peripheral neuropathy
***ATP2B4* (*PMCA4*)**	Not assigned	Uncomplicated	Adulthood	None
REEP1	SPG31	Uncomplicated	Variable from 2nd to 7th decades	Mild amyotrophy variably present.
REEP2	SPG72	Uncomplicated	Very early, average age 4 years	Musculoskeletal problems Mild postural tremor
RTN2	SPG12	Uncomplicated	Before age 20 years	None
SLC33A1	SPG42	Uncomplicated	Early adulthood	Slowly progressive Mild *pes cavus*
SPAST	SPG4	Uncomplicated	Variable from infancy to 7th decade	Cognitive decline & dementia common Distal amyotrophy variably present Complicated phenotype w/ataxia variably present
SPG7	SPG7	Uncomplicated or complicated	Juvenile or adulthood	Dysarthria Ataxia Optic atrophy Supranuclear palsy Mitochondrial abnormalities on skeletal muscle biopsy
WASHC5	SPG8	Uncomplicated	Adulthood (rare infantile onset reported)	Severe motor deficit in some individuals

Gene[1]	HSP	Type of HSP	Onset	Distinguishing Clinical
TUBB4A [5]	Not assigned	Complicated	Juvenile	Cerebellar ataxia MRI evidence of hypomyelination
ZFYVE27	SPG33	Uncomplicated	Adulthood	Mild pes cavus

AD = autosomal dominant; AR = autosomal recessive; ALS = amyotrophic lateral sclerosis; CMT = Charcot-Marie-Tooth neuropathy; DI-CMT = dominant intermediate Charcot-Marie-Tooth neuropathy; HMN = hereditary motor neuropathy; HSP = hereditary spastic paraplegia; SMA = spinal muscular atrophy

Table 2: Hereditary Spastic Paraplegia: Genes and Distinguishing Clinical Features – Autosomal Recessive Inheritance (Hedera, 2021)

Gene [1]	HSP Designation	Type of HSP	Onset	Distinguishing Clinical Features	Other
SPG21 (ACP33)	SPG21	Complicated	Childhood	Ataxia Adult-onset dementia & parkinsonism Polyneuropathy Akinetic mutism seen in advanced cases	Rare, first described in Old Order Amish population (later identified in various ethnic groups) Also known as Mast syndrome
ALDH18A1	SPG9B	Complicated	Adolescence to adulthood (one subject w/infantile onset)	Cataracts Gastroesophageal reflux Motor neuronopathy Variably present: Dysarthria Ataxia Cognitive impairment	Rare Allelic w/AD HSP (SPG9A)

Gene [1]	HSP Designation	Type of HSP	Onset	Distinguishing Clinical Features	Other
ALDH3A2	Not assigned	Complicated	Childhood	Congenital ichthyosis Macular dystrophy Leukodystrophy Seizures in ~40% of patients	Rare Most common in people of Swedish ancestry Known as Sjögren-Larsson syndrome
AMPD2 [2]	SPG63	Complicated	Infancy	Short stature Thin corpus callosum White matter changes	Rare
AP4B1	SPG47	Complicated	Infancy	Severe ID Facial dysmorphism Seizures Stereotypic laughter w/tongue protrusion	Rare
AP4E1	SPG51	Complicated	Infancy	Severe ID Facial dysmorphism Seizures Stereotypic laughter w/tongue protrusion	Rare
AP4M1	SPG50	Complicated	Infancy	Severe ID Facial dysmorphism Seizures Stereotypic laughter w/tongue protrusion	Rare
AP4S1	SPG52	Complicated	Infancy	Severe ID Facial dysmorphism Seizures Stereotypic laughter w/tongue protrusion	Rare

Gene [1]	HSP Designation	Type of HSP	Onset	Distinguishing Clinical Features	Other
AP5Z1	SPG48	Uncomplicated	Typically adulthood; rarely infancy	Urinary incontinence Parkinsonism Dystonia Thin corpus callosum Leukodystrophy Severe DD in infantile onset	Single family
ATL1	SPG3A	Uncomplicated	Infantile to childhood (rarely adult onset)	Progression may be minimal w/static course May present as spastic diplegic cerebral palsy Complicated phenotype w/peripheral neuropathy or autonomic failure reported	AR inheritance is very rare.
B4GALNT1	SPG26	Complicated	Juvenile	Amyotrophy Dysarthria Ataxia DD Dystonia	Rare
BICD2	Not assigned	Complicated	Childhood	Amyotrophy Contractures	Rare
C12orf65	SPG55	Complicated	Childhood	DD Visual loss Polyneuropathy Arthrogryposis Signs of mitochondrial encephalomyopathy, some classified as Leigh syndrome	Rare

Gene [1]	HSP Designation	Type of HSP	Onset	Distinguishing Clinical Features	Other
C19orf12	SPG43	Complicated	Childhood	Amyotrophy Dysarthria Multiple contractures Neurodegeneration w/brain iron accumulation in some	Rare
CYP2U1	SPG56	Complicated	Infancy	Severe DD Dystonia Polyneuropathy Calcification of basal ganglia	Rare
CYP7B1	SPG5A	Uncomplicated or complicated	Juvenile to early adulthood	Ataxia Polyneuropathy Extrapyramidal signs MRI signs of leukodystrophy	SPG5A was diagnosed in 9 of 172 families w/histories consistent w/AR inheritance of HSP. [3]
DDHD1	SPG28	Uncomplicated	Childhood	Scoliosis	Rare
DDHD2	SPG54	Complicated	Infancy	Severe DD Optic atrophy Thin corpus callosum Leukodystrophy	Rare
ENTPD1	SPG64	Complicated	Infancy	Mild cognitive disability Behavioral disturbances White matter changes	Rare

Gene [1]	HSP Designation	Type of HSP	Onset	Distinguishing Clinical Features	Other
ERLIN1	SPG62	Complicated	Childhood	Amyotrophy Ataxia Phenotype consistent w/juvenile onset of ALS reported	Rare
ERLIN2	SPG18	Complicated (rarely pure AR HSP reported)	Childhood	DD Seizures Contractures Juvenile primary lateral sclerosis phenotype reported Allelic w/AD pure HSP	Rare
FA2H [4]	SPG35	Complicated	Childhood	Seizures Dystonia Parkinsonism w/iron accumulation in basal ganglia	Rare
GAD1	Not assigned	Complicated	Childhood	Moderate to severe ID Single reported family was described as having AR cerebral palsy	Rare (single family reported)
GBA2	SPG46	Complicated	Childhood	DD Ataxia Hearing loss Polyneuropathy	Rare
GJC2 [5]	SPG44	Complicated	Childhood	Febrile seizures Deafness Episodic spasms Variable degree of leukodystrophy	Rare
GRID2 [6]	Not assigned	Complicated	Childhood	Amyotrophy Ataxia	Rare

Gene [1]	HSP Designation	Type of HSP	Onset	Distinguishing Clinical Features	Other
IBA57 [7]	SPG74	Complicated	Childhood	Optic atrophy Peripheral neuropathy	Rare
KIF1A [8]	SPG30	Complicated	Childhood	Spastic ataxia Polyneuropathy	Rare
KIF1C	SPG58	Complicated	Childhood	Spastic ataxia Dystonia	Rare
KLC2	Not assigned	Complicated	Childhood	Optic atrophy Neuropathy Contractures in later stages Cognition remains intact	Rare Also known as spastic paraplegia optic atrophy, & neuropathy (SPOAN)
KLC4	Not assigned	Complicated	Childhood	Ataxia Multiple contractures Variable degree of leukodystrophy	Rare
MARS1 [9]	SPG70	Complicated	Infancy	Nephrotic syndrome, polyneuropathy Mild ID Late onset of CMT2 (axonal) type also reported	Rare
NT5C2	SPG45	Complicated	Childhood	Optic atrophy Nystagmus Strabismus ID Hypoplastic corpus callosum	Rare
PGAP1 [10]	SPG67	Complicated	Infancy	Severe DD Tremor Agenesis of corpus callosum Hypomyelination	Rare

Gene [1]	HSP Designation	Type of HSP	Onset	Distinguishing Clinical Features	Other
PNPLA6 [11]	SPG39	Complicated	Childhood	Amyotrophy Endocrine abnormalities w/short stature or hypogonadotropic hypogonadism Chorioretinal dystrophy	Rare
REEP2	SPG72	Uncomplicated	Early childhood	Musculoskeletal problems Mild postural tremor	Rare Inheritance can be dominant or recessive.
SPART	SPG20	Complicated	Juvenile	Distal amyotrophy Short stature Kyphoscoliosis Multiple limb contractures	Rare Mostly seen among Old Order Amish
SPG7	SPG7	Uncomplicated or complicated	Juvenile or adulthood	Dysarthria Ataxia Optic atrophy Supranuclear palsy Mitochondrial abnormalities on skeletal muscle biopsy	5%-12% of AR HSP AD inheritance suggested for some pathogenic variants; this remains controversial
SPG11	SPG11	Complicated	Childhood or early adulthood	DD Optic atrophy Ataxia Pseudobulbar signs Polyneuropathy Levodopa-responsive parkinsonism Hypoplastic or absent corpus callosum	5% of AR HSP 75% of HSP w/DD & hypoplasia of corpus callosum

Gene [1]	HSP Designation	Type of HSP	Onset	Distinguishing Clinical Features	Other
TECPR2	SPG49	Complicated	Childhood	Central apnea Severe DD Microcephaly Dysmorphic features	Rare
TFG	SPG57	Complicated	Childhood	Optic atrophy Severe polyneuropathy	Rare
USP8	SPG59	Uncomplicated	Childhood	None	Rare
WDR48	SPG60	Complicated	Infancy	Polyneuropathy DD	Rare
ZFYVE26	SPG15	Complicated	Childhood or early adulthood	DD Optic atrophy Ataxia Central retinal degeneration Polyneuropathy	1%-2% of AR HSP

AD = autosomal dominant; AR = autosomal recessive; ALS = amyotrophic lateral sclerosis; DD = developmental delay; HSP = hereditary spastic paraplegia; ID = intellectual disability

Table 3: Hereditary Spastic Paraplegia: Genes and Distinguishing Clinical Features – X-Linked Inheritance (Hedera, 2021)

Gene [1]	HSP Designation	Type of HSP	Onset	Distinguishing Clinical Features	Other
L1CAM [2]	SPG1	Complicated	Infancy	ID Adducted thumbs Corpus callosum hypoplasia Aphasia Obstructive hydrocephalus	Rare

Gene [1]	HSP Designation	Type of HSP	Onset	Distinguishing Clinical Features	Other
PLP1 [3]	SPG2	Complicated	Early-childhood to juvenile onset (in manifesting XX heterozygotes: onset in 4th – 7th decade)	Pure HSP phenotype present in early stages; later, other signs emerge Nystagmus Optic atrophy Dysarthria ID Variable degree of leukodystrophy on MRI	Rare In heterozygous XX individuals: variable phenotype w/relatively late onset & mild clinical manifestations
SLC16A2	SPG22	Complicated	Early childhood	Severe ID Infantile hypotonia Progressive spasticity Ataxia Dystonia ↑ T3 & normal to mildly ↑ TSH ↓ T4 hypomyelination on neuroimaging	Rare SPG22 is a proposed designation. [4] Also referred to as Allan-Herndon-Dudley syndrome [5]

AD = autosomal dominant; AR = autosomal recessive; DD = developmental delay; HSP = hereditary spastic paraplegia; ID = intellectual disability

Table 4: Hereditary Spastic Paraplegia: Gene and Distinguishing Clinical Features – Maternal (Mitochondrial) Inheritance (Hedera, 2021)

Gene	HSP Designation	Type of HSP	Onset	Distinguishing Clinical Features
MT-ATP6	Not assigned	Complicated	Adult	Cardiomyopathy, diabetes mellitus, sensory polyneuropathy

VI. Guidelines and Recommendations

Amyotrophic Lateral Sclerosis (ALS)

Italian Amyotrophic Lateral Sclerosis Genetic (ITALSGEN) Consortium

The following guidelines were created as a result from a workshop on ALS genetic testing.

- "All ALS patients who have a first-degree or second-degree relative with ALS, frontotemporal dementia or both, should be offered genetic testing. At present, however, we do not recommend offering genetic testing to sporadic ALS patients, outside research protocols."
- "Genetic testing at present is not indicated in asymptomatic at-risk subjects and, therefore, should not be proposed."
- The guidelines also note that "two-thirds of mutations are found in four genes, *C9ORF72, SOD1, TARDBP* and *FUS*." Therefore, they state that these genes should be "considered" for routine diagnostic protocol. Furthermore, they note that *C9ORF72* testing is "worthwhile" in sporadic patients. If these four genes are negative, other ALS-related genes may be tested. Finally, *UBQLN2* is a gene that should be tested if there is suspicion of an X-linked dominant inheritance (Chio et al., 2014).

ALS Association

The ALS Association published information on genetic testing on their website. They state that "Knowing whether your ALS is connected to a specific gene mutation could make you eligible for ongoing clinical trials testing treatments targeted at specific genes." The guidelines go on to state that "if genetic testing identifies a disease-causing genetic mutation in a person with ALS, their family members generally have the option to pursue testing themselves" but note that this is a personal decision with significant costs and benefits (ALSA, 2024).

Ataxias (including Friedreich ataxia)

European Federation of Neurological Societies (EFNS) and European Neurological Society (Schuermans et al.)

The EFNS-ENS released joint guidelines on diagnosis and management of chronic ataxias in adulthood. Their genetic testing guidelines are listed below:

"In the case of a family history that is compatible with an autosomal dominant cerebellar ataxia, screening for SCA1, 2, 3, 6, 7 and 17 is recommended (level B). In Asian patients, DRPLA should also be tested for."
"If mutation analysis is negative, we recommend contact with or a referral to a specialized clinic for reviewing the clinical phenotype and further genetic testing (good practice point)."

In the case of a family history compatible with an autosomal recessive cerebellar ataxia, they recommend a three-step diagnostic approach.

Step 1 includes mutation analysis of the FRDA gene for Friedreic''s ataxia (although one can refrain from this in the case of severe cerebellar atrophy).
Step 2 includes mutation analysis of the *SACS*, *POLG*, Aprataxin (*APTX*) and *SPG7* genes (taking into account specific phenotypes).
Step 3 includes "referral to a specialized centre, e.g. for skin or muscle biopsy targeted at diagnoses such as Niemann−Pick type C, recessive ataxia with coenzyme Q deficiency [aarF domain containing kinase 3 (ADCK3)/autosomal recessive spinocerebellar ataxia 9 (SCAR9)] and mitochondrial disorders, or for extended genetic screening using gene panel diagnostics."
"In the case of sporadic ataxia and independent from onset age, we recommend routine testing for SCA1, SCA2, SCA3, SCA6 and DRPLA (in Asian patients) (level B)"(van de Warrenburg et al., 2014).

Friedreich''s Ataxia Research Alliance (FARA)

The FARA notes genetic diagnostic information on their website.

They state that in "more than 95% of abnormal alleles, the mutation is expansion of naturally occurring GAA repeat in first intron (non-coding region) of the frataxin or FRDA gene."
"Genetic testing results in ~98% detection in symptomatic individuals. In rare cases, analysis of frataxin protein levels can be helpful to confirming or ruling out a diagnosis…Carrier testing is recommended for anyone with a positive family history of Friedreich ataxia and for partners of known carriers. Presymptomatic testing for at-risk siblings/relatives is available, however genetic counseling is strongly recommended to assist individuals/families in considering the risks vs benefits for testing an untreatable genetic condition" (FARA, 2022).

An expert working group was convened to review and provide guidelines for FA. This working group reviewed guidelines from a variety of different societies, and drafted their own guidelines based off their review. Their genetic testing items are as follows:

"Any individual in whom the diagnosis of FRDA is considered should undergo genetic testing for FRDA."
"Referral to a clinical geneticist or genetic counselor should be considered on diagnosis of FRDA."
"Requests for pre-symptomatic genetic testing are best managed on a case-by-case basis; there is no evidence to support the routine provision or refusal of pre-symptomatic genetic testing for FRDA."
"The committee did not reach consensus on the issue of whether it is appropriate to conduct presymptomatic testing in a minor. Where a request for presymptomatic testing in a minor occurs, the individual/family should be referred to a team with expertise in this field for discussion about pre-symptomatic genetic testing in which the risks and benefits of pre-symptomatic genetic diagnosis are put forward. The risks and benefits from both the child's and parents' perspectives should be carefully reviewed during the pre-test assessment."
"All patients identified pre-symptomatically and their families would benefit from immediate post-test counseling and psychosocial support and referral for appropriate neurological and cardiac surveillance."
"Carrier testing should be first undertaken on the closest relative" (Corben et al., 2014).

For Friedreich Ataxia due to compound heterozygosity for a *FXN* Intron 1 GAA expansion and point mutation/insertion/deletion:

"If a person compound heterozygous for a *FXN* GAA expansion and a point mutation/insertion/deletion has a similar phenotype to those with FRDA due to homozygosity for GAA expansions, they should be managed as per the guidelines in this document."
"If spastic ataxia is the predominant phenotype, then the main management issue is that of spasticity and the guidelines for management of spasticity should be followed" (Corben et al., 2014).

Ataxia UK

Genetic tests are recommended as part of the secondary care regimen for ataxia. The secondary care is divided into "first line" and "second line" for adults.

The first line genetic tests are for: FRDA, SCA1, 2, 3, 6, 7 (12, 17), and FXTAS. The second line genetic tests are for any remaining genes (de Silva et al., 2019). The guidelines list genes associated with types of ataxia (Bonney et al., 2016).

Spinocerebellar ataxias:

Type (MacLeod et al.)	Gene
1	*ATXN1*
2	*ATXN2*
3	*ATXN3*
5	*SPTBN2*
6	*CACNA1A*
7	*ATXN7*
8	*ATXN8OS*
10	*ATXN10*
11	*TTBK2*
12	*PPP2R2B*
13	*KCNC3*
14	*PRKCG*
15/16	*ITPR1*
17	*TBP*
19/22	*KDND3*
23	*PDYN*
27	*FGF14*
28	*AFG3L2*
31	*BEAN1*
35	*TGM6*
36	*NOP56*
38	*ELOVL5*
40	*CCDC88C*
41	*TRPC3*

The guidelines note that the clinical validity of genetic testing for SCA8 by CAG repeat sizing has not been determined. Therefore, SCA8 should not be offered as a routine test if family history is unknown. However, testing may be appropriate in "large pedigrees where the expansion has been proven to be segregating with the disease" (Bonney et al., 2016).

SCAs are considered autosomal dominant ataxias. Autosomal dominant ataxias also include GSS, DRPLA, POLG1, and EA types 1 and 2.

Autosomal reccessive ataxia genes include *FXN* (Frederich's Ataxia), *APTX, SETX, SACS, SPG7, ATM*, and *TTPA* (Ataxia with Vitamin E deficiency).

Mitochondrial Ataxias include NARP, MELAS, and MERRF.

X-Linked Ataxias include FXTAS (Fragile X associated Tremor and Ataxia Syndrome).

For children, second-line diagnostic tests for chronic ataxias include DNA testing for the *FXN* gene is recommended for suspected Frederich's Ataxia, *ATM* testing is recommended for suspected ataxia-telangiectasia, and general DNA testing is recommended for "other conditions." Finally, "genetic testing of asymptomatic 'at-risk' minors is not generally recommended, but should be considered on a case-by-case basis" (Bonney et al., 2016).

National Ataxia Foundation (NAF)

The National Ataxia Foundation published a "Frequently Asked Questions" document regarding genetic testing for hereditary ataxias. For diagnostic testing, they noted that in sporadic ataxia cases (no prior family history of ataxia), genetic testing should only be done after non-genetic causes of ataxia have been excluded. For predictive testing, a patient "must" know what type of ataxia is present in their family to be eligible (NAF, 2015).

Dystonias

European Federation of Neurological Societies (EFNS)

The EFNS has released guidelines on the genetic testing of dystonias, which are listed below:

"Genetic testing should be performed after establishing the clinical diagnosis. Genetic testing is not sufficient to make a diagnosis of dystonia without clinical features of dystonia (level B). Genetic counselling is recommended."
"*DYT1* testing is recommended for patients with limb-onset, primary dystonia with onset before age 30 (level B), as well as in those with onset after age 30 if they have an affected relative with early-onset dystonia (level B)."
"In dystonia families, *DYT1* testing is not recommended in asymptomatic individuals (good practice point)."
"*DYT6* testing is recommended in early-onset dystonia or familial dystonia with cranio-cervical predominance or after exclusion of *DYT1* (good practice point)."
"Individuals with early-onset myoclonus affecting the arms or neck, particularly if positive for autosomal-dominant inheritance and if triggered by action, should be tested for the *DYT11* gene (good practice point). If direct sequencing of the SGCE gene is negative, gene dosage studies increase the proportion of mutation-positives (level C)."
"Diagnostic testing for the PNKD gene (*DYT8*) is recommended in symptomatic individuals with PNKD (good practice point)."
"Gene testing for mutation in *GLUT1* is recommended in patients with paroxysmal exercise-induced dyskinesias, especially if involvement of *GLUT1* is suggested by low

CSF/serum glucose ratio, epileptic seizures or haemolytic anaemia (good practice point)" (Albanese et al., 2011).

The EFNS also released guidelines on the diagnosis of Huntington's Disease. In it, they recommend that "diagnostic testing for HD is recommended (Level B) when a patient presents with an otherwise unexplained clinical syndrome of a progressive choreatic movement disorder and neuropsychiatric disturbances with or without a positive family history of the disease" (Harbo et al., 2009).

Hereditary Spastic Paraplegia (HSP)

National Organization for Rare Disorders (NORD)

NORD has published a webpage on HSP. This page states that "Individuals seeking genetic counseling for HSP are recommended to consult a genetic counselor or medical geneticist for specific information"; further, "Genetic testing is often helpful in confirming the clinical diagnosis of HSP and in determining the genetic type of HSP. Results of genetic testing can be used, together with clinical information, to provide genetic counseling" (NORD, 2017).

Regarding genetic testing for a HSP diagnosis, NORD states that "Testing for HSP genes is available and performed for individual HSP genes, for panels containing dozens of HSP genes, and by analysis of all genes (whole exome and whole genome analysis). Genetic testing is often helpful to confirm the clinical diagnosis of HSP. Genetic testing is most often able to find causative gene mutations for subjects with HSP who have a family history of a similarly affected first-degree relative. Despite discovery of more than 60 genes in which mutations cause various types of HSP, many individuals with HSP do not have an identified gene mutation… at present, genetic testing results very rarely influence treatment which is largely directed toward reducing symptoms" (NORD, 2017).

Huntington Disease (HD)

Working Group on Genetic Counselling and Testing of the European Huntington's Disease Network (EHDN)

This Working Group was convened to provide guidelines for diagnostic genetic testing for HD. The guidelines list four groups that "should be considered" for genetic testing.

- The first group is "the patient with a positive family history and specific motor symptoms." The authors note that diagnosis of this group is "not difficult" and that the test may be "little more than a formality."

- The second group is "the patient with no family history, but specific symptoms likely to be HD." The authors consider diagnostic testing of this group to be "most clinically useful."
- The third group is "the patient with a positive family history and prodromal symptoms, which suggest the impending onset of HD." The authors state that the motor abnormalities are part of the diagnostic criteria, but other symptoms such as behavioral changes or other mental conditions may present in HD.
- The fourth group"is "the child with a family history of HD and features of juvenile HD." The authors note this group as challenging to diagnose, and alludes to diagnostic criteria set forth by Nance, which are as follows:
    - "a known family history of HD (often, but not exclusively, the father)
    - and two or more of
        - declining school performance
        - Seizures
        - oral motor dysfunction
        - Rigidity
        - gait disturbance" (Craufurd et al., 2015)

Another Working Group was convened to evaluate the predictive testing guidelines for HD in 2013. In those guidelines, they noted that HD testing should not be part of routine blood work and that patients under 18 should not be tested. However, they state that genetic counseling should be offered to those desiring to take the test (MacLeod et al., 2013). MacLeod et al. (2013) was affirmed by the American Association of Neurology on January 14, 2014 (AAN, 2014).

The EDHN performed a literature review of scientific and consensual guidelines in 2019. The only major change had to do with deutetrabenazine (Grade A) as a treatment. Other studies were in agreement wiuth previously noted guidelines and recommendations (Bachoud-Lévi et al., 2019).

Parkinsonism (Parkinson Disease, PD)

European Federation of Neurological Societies (EFNS) and Movement Disorder Society–European Section (MDS-ES)

These guidelines were created by a Task Force comprised of members from both societies. Their genetic testing recommendations for Parkinson disease are listed below:

- "Testing for *SNCA* point mutations and gene multiplications is recommended only in families with multiple affected members in more than one generation suggestive of dominant inheritance, with early- or late-onset PD."
- "*LRRK2* genetic testing for counselling purposes, specifically directed at known pathogenic variants is recommended in patients with a clinical picture of typical PD and a positive family history suggestive of dominant inheritance."
- "In sporadic patients, genetic testing should be limited to the search for known *LRRK2* founder mutations in the appropriate populations (i.e. with known high mutation frequencies)."
- "Genetic testing for *GBA* gene mutations is recommended in patients with typical PD with or without a positive family history, limited to the known founder mutations of established pathogenic role in the appropriate populations."
- "Genetic testing of the *parkin, PINK1* and *DJ-1* genes for counselling purposes is recommended in patients with typical PD and positive family history compatible with recessive inheritance, particularly when the disease onset is before the age of 50 years. For sporadic cases, *parkin, PINK1* and *DJ-1* genetic testing is recommended when onset is very early, particularly before the age of 40."
- "Testing of the *ATP13A2, PLA2G6* and *FBXO7* genes might be considered in cases with very-early-onset PD, if no mutation in *parkin, PINK1* and *DJ-1* gene has been found."

For recommendation III, the guideline lists Ashkenazi Jews, North African Arabs, and Basques as examples of high mutation frequency populations (Berardelli et al., 2013).

Spinal Muscular Atrophies (SMA)

218th European Neuromuscular Centre (ENMC) International Workshop

Researchers, industry representatives, and other representatives from SMA Europe convened to review the current knowledge on the standards of care for SMA. Regarding genetic testing, they noted that "there was consensus that genetic testing is the first line investigation when this condition is suspected in a typical case and that muscle biopsy or electromyography should not be performed in a typical presentation. There was also consensus that, at variance with previous recommendations, the current gold standard is *SMN1* deletion/mutation and *SMN2* copy number testing, with a minimal standard of *SMN1* deletion testing. Other areas concerning the value of *SMN2* copy number were more controversial and a further Delphi round was planned to complete the task" (Finkel et al., 2017).

"Diagnostic testing for HD is recommended (Level B) when a patient presents with an otherwise unexplained clinical syndrome of a progressive choreatic movement disorder

and neuropsychiatric disturbances with or without a positive family history of the disease" (Finkel et al., 2017).

American College of Obstetricians and Gynecologists (ACOG)

ACOG recommended SMA screening for all individuals "considering pregnancy or are currently pregnant." ACOG also noted that if one parent had a family history of SMA, the other parent should be tested for *SMN1* deletion if molecular reports for the first parent were not available. These guidelines were reaffirmed in 2023 (ACOG, 2017).

Wilson Disease (WD)

Hepatology Committee of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)

ESPGHAN has published a position paper regarding Wilson disease in children. The genetic testing-relevant items are listed below:

The paper stated that the scoring system used for diagnosis of Wilson's Disease included identification of a pathogenic mutation, which was considered one point (the scoring system is as follows: 0-1: unlikely, 2-3: probable, 4+, highly likely). The paper also notes that if biochemical and clinical symptoms are present, only one mutation needs to be identified to diagnose Wilson disease. If the patient is asymptomatic, two mutations must be identified to diagnose "with certainty." The diagnostic protocol calls for biochemical (copper metabolism testing), liver (ALT/AST, bilirubin, et al), and clinical evaluation before proceeding to molecular testing, and *ATP7B* is the primary gene mutation mentioned in evaluation of Wilson disease.

"Genetic counseling is essential for families of patients with WD, and screening first-degree relatives is recommended by both European and American guidelines."
"It is essential to screen siblings of any patient newly diagnosed with WD because the chance of being a homozygote and developing clinical disease is 25%. Assessment should include physical examination, serum ceruloplasmin, liver function tests, and molecular testing for *ATP7B* mutations or haplotype studies if not available. Newborn screening is not warranted and screening may be delayed until 1 to 2 years of age" (Socha et al., 2018).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the

determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	CPT Description
81177	ATN1 (atrophin 1) (eg, dentatorubral-pallidoluysian atrophy) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81178	ATXN1 (ataxin 1) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81179	ATXN2 (ataxin 2) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81180	ATXN3 (ataxin 3) (eg, spinocerebellar ataxia, Machado-Joseph disease) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81181	ATXN7 (ataxin 7) (eg, spinocerebellar ataxia) gene analysis, evaluation to det ect abnormal (eg, expanded) alleles
81182	ATXN8OS (ATXN8 opposite strand [non-protein coding]) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81183	ATXN10 (ataxin 10) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81184	CACNA1A (calcium voltage-gated channel subunit alpha1 A) (eg, spinocerebellar ataxia) gene analysis; evaluation to detect abnormal (eg, expanded) alleles
81185	CACNA1A (calcium voltage-gated channel subunit alpha1 A) (eg, spinocerebellar ataxia) gene analysis; full gene sequence
81186	CACNA1A (calcium voltage-gated channel subunit alpha1 A) (eg, spinocerebellar ataxia) gene analysis; known familial variant

CPT	CPT Description
81243	FMR1 (fragile X messenger ribonucleoprotein 1) (eg, fragile X syndrome, X-linked intellectual disability [XLID]) gene analysis; evaluation to detect abnormal (eg, expanded) alleles
81244	FMR1 (fragile X messenger ribonucleoprotein 1) (eg, fragile X syndrome, X-linked intellectual disability [XLID]) gene analysis; characterization of alleles (eg, expanded size and promoter methylation status)
81271	HTT (huntingtin) (eg, Huntington disease) gene analysis; evaluation to detect abnormal (eg, expanded) alleles
81274	HTT (huntingtin) (eg, Huntington disease) gene analysis; characterization of alleles (eg, expanded size)
81284	FXN (frataxin) (eg, Friedreich ataxia) gene analysis; evaluation to detect abnormal (expanded) alleles
81285	FXN (frataxin) (eg, Friedreich ataxia) gene analysis; characterization of alle les (eg, expanded size)
81286	FXN (frataxin) (eg, Friedreich ataxia) gene analysis; full gene sequence
81289	FXN (frataxin) (eg, Friedreich ataxia) gene analysis; known familial variant(s)
81329	SMN1 (survival of motor neuron 1, telomeric) (eg, spinal muscular atrophy) gen e analysis; dosage/deletion analysis (eg, carrier testing), includes SMN2 (survival of motor neuron 2, centromeric) analysis, if performed
81343	PPP2R2B (protein phosphatase 2 regulatory subunit Bbeta) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81344	TBP (TATA box binding protein) (eg, spinocerebellar ataxia) gene analysis, evaluation to detect abnormal (eg, expanded) alleles
81400	Molecular pathology procedure, Level 1 (eg, identification of single germline variant [eg, SNP] by techniques such as restriction enzyme digestion or melt curve analysis)
81401	Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)
81403	Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)
81404	Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)

CPT	CPT Description
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons
81407	Molecular pathology procedure, Level 8 (eg, analysis of 26-50 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of >50 exons, sequence analysis of multiple genes on one platform)
81408	Molecular pathology procedure, Level 9 (eg, analysis of >50 exons in a single gene by DNA sequence analysis)
81479	Unlisted molecular pathology procedure
0136U	ATM (ataxia telangiectasia mutated) (eg, ataxia telangiectasia) mRNA sequence analysis (List separately in addition to code for primary procedure) (Use 0136U in conjunction with 81408) Proprietary test: +RNAinsight™ for ATM Lab/Manufacturer: Ambry Genetics
0231U	CACNA1A (calcium voltage-gated channel subunit alpha 1A) (eg, spinocerebellar ataxia), full gene analysis, including small sequence changes in exonic and intronic regions, deletions, duplications, short tandem repeat (STR) gene expansions, mobile element insertions, and variants in non-uniquely mappable regions Proprietary test: Genomic Unity® CACNA1A Analysis Lab/Manufacturer: Variantyx Inc
0233U	FXN (frataxin) (eg, Friedreich ataxia), gene analysis, including small sequence changes in exonic and intronic regions, deletions, duplications, short tandem repeat (STR) expansions, mobile element insertions, and variants in non-uniquely mappable regions Proprietary test: Genomic Unity® FXN Analysis Lab/Manufacturer: Variantyx Inc
0236U	SMN1 (survival of motor neuron 1, telomeric) and SMN2 (survival of motor neuron 2, centromeric) (eg, spinal muscular atrophy) full gene analysis, including small sequence changes in exonic and intronic regions, duplications and deletions, and mobile element insertions Proprietary test: Genomic Unity® SMN1/2 Analysis Lab/Manufacturer: Variantyx Inc

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

AAN. (2014). Recommendations for the Predictive Genetic Test in Huntington's Disease. https://www.aan.com/Guidelines/home/GuidelineDetail/631

ACOG. (2017). Carrier Screening for Genetic Conditions. https://www.acog.org/clinical/clinical-guidance/committee-opinion/articles/2017/03/carrier-screening-for-genetic-conditions

Albanese, A., Asmus, F., Bhatia, K. P., Elia, A. E., Elibol, B., Filippini, G., Gasser, T., Krauss, J. K., Nardocci, N., Newton, A., & Valls-Solé, J. (2011). EFNS guidelines on diagnosis and treatment of primary dystonias. *European Journal of Neurology*, *18*(1), 5-18. https://doi.org/10.1111/j.1468-1331.2010.03042.x

ALSA. (2024). *Genetic Testing for ALS*. https://www.als.org/understanding-als/who-gets-als/genetic-testing

Bachoud-Lévi, A.-C., Ferreira, J., Massart, R., Youssov, K., Rosser, A., Busse, M., Craufurd, D., Reilmann, R., De Michele, G., Rae, D., Squitieri, F., Seppi, K., Perrine, C., Scherer-Gagou, C., Audrey, O., Verny, C., & Burgunder, J.-M. (2019). International Guidelines for the Treatment of Huntington's Disease [Policy and Practice Reviews]. *Frontiers in Neurology*, *10*(710). https://doi.org/10.3389/fneur.2019.00710

Baig, S. S., Strong, M., Rosser, E., Taverner, N. V., Glew, R., Miedzybrodzka, Z., Clarke, A., Craufurd, D., & Quarrell, O. W. (2016). 22 Years of predictive testing for Huntington's disease: the experience of the UK Huntington's Prediction Consortium. *Eur J Hum Genet*, *24*(10), 1396-1402. https://doi.org/10.1038/ejhg.2016.36

Bandres-Ciga, S., Noyce, A. J., Hemani, G., Nicolas, A., Calvo, A., Mora, G., Tienari, P. J., Stone, D. J., Nalls, M. A., Singleton, A. B., Chiò, A., & Traynor, B. J. (2019). Shared polygenic risk and causal inferences in amyotrophic lateral sclerosis. *Ann Neurol*, *85*(4), 470-481. https://doi.org/10.1002/ana.25431

Berardelli, A., Wenning, G. K., Antonini, A., Berg, D., Bloem, B. R., Bonifati, V., Brooks, D., Burn, D. J., Colosimo, C., Fanciulli, A., Ferreira, J., Gasser, T., Grandas, F., Kanovsky, P., Kostic, V., Kulisevsky, J., Oertel, W., Poewe, W., Reese, J. P., . . . Vidailhet, M. (2013). EFNS/MDS-ES/ENS [corrected] recommendations for the diagnosis of Parkinson's disease. *Eur J Neurol*, *20*(1), 16-34. https://doi.org/10.1111/ene.12022

Bodamer, O. A. (2023, July 28). *Spinal muscular atrophy*. https://www.uptodate.com/contents/spinal-muscular-atrophy

Bonney, H., de Silva, R., Giunti, P., Greenfield, J., & Hunt, B. (2016). Management of the ataxias towards best cllinical practice. https://www.ataxia.org.uk/wp-content/uploads/2020/11/Ataxia_UK_Medical_Guidelines._Third_Edition._v3m_Dec_2016_-_updated_Sep_2019.pdf

Bressman, S. B. (2004). Dystonia genotypes, phenotypes, and classification. *Adv Neurol*, *94*, 101-107.

Chio, A., Battistini, S., Calvo, A., Caponnetto, C., Conforti, F. L., Corbo, M., Giannini, F., Mandrioli, J., Mora, G., Sabatelli, M., Ajmone, C., Mastro, E., Pain, D., Mandich, P., Penco, S., Restagno, G., Zollino, M., & Surbone, A. (2014). Genetic counselling in ALS: facts, uncertainties and clinical suggestions. *J Neurol Neurosurg Psychiatry*, *85*(5), 478-485. https://doi.org/10.1136/jnnp-2013-305546

Chiò, A., Calvo, A., Mazzini, L., Cantello, R., Mora, G., Moglia, C., Corrado, L., D'Alfonso, S., Majounie, E., Renton, A., Pisano, F., Ossola, I., Brunetti, M., Traynor, B. J., & Restagno, G. (2012). Extensive genetics of ALS: a population-based study in Italy. *Neurology*, *79*(19), 1983-1989. https://doi.org/10.1212/WNL.0b013e3182735d36

Chou, K. L. (2024a, March 14). *Clinical manifestations of Parkinson disease*. https://www.uptodate.com/contents/clinical-manifestations-of-parkinson-disease

Chou, K. L. (2024b, April 5). *Diagnosis and differential diagnosis of Parkinson disease*. https://www.uptodate.com/contents/diagnosis-and-differential-diagnosis-of-parkinson-disease

Corben, L. A., Lynch, D., Pandolfo, M., Schulz, J. B., & Delatycki, M. B. (2014). Consensus clinical management guidelines for Friedreich ataxia. *Orphanet J Rare Dis*, *9*, 184. https://doi.org/10.1186/s13023-014-0184-7

Craufurd, D., MacLeod, R., Frontali, M., Quarrell, O., Bijlsma, E. K., Davis, M., Hjermind, L. E., Lahiri, N., Mandich, P., Martinez, A., Tibben, A., & Roos, R. A. (2015). Diagnostic genetic testing for Huntington's disease. *Pract Neurol*, *15*(1), 80-84. https://doi.org/10.1136/practneurol-2013-000790

de Silva, R., Greenfield, J., Cook, A., Bonney, H., Vallortigara, J., Hunt, B., & Giunti, P. (2019). Guidelines on the diagnosis and management of the progressive ataxias. *Orphanet J Rare Dis*, *14*(1), 51. https://doi.org/10.1186/s13023-019-1013-9

Deik, A., & Comelia, C. (2023, May 9). *Etiology, clinical features, and diagnostic evaluation of dystonia*. Wolters Kluwer. https://www.uptodate.com/contents/etiology-clinical-features-and-diagnostic-evaluation-of-dystonia

Dong, E. L., Wang, C., Wu, S., Lu, Y. Q., Lin, X. H., Su, H. Z., Zhao, M., He, J., Ma, L. X., Wang, N., Chen, W. J., & Lin, X. (2018). Clinical spectrum and genetic landscape for hereditary spastic paraplegias in China. *Mol Neurodegener*, *13*(1), 36. https://doi.org/10.1186/s13024-018-0269-1

Dong, Y., Ni, W., Chen, W. J., Wan, B., Zhao, G. X., Shi, Z. Q., Zhang, Y., Wang, N., Yu, L., Xu, J. F., & Wu, Z. Y. (2016). Spectrum and Classification of ATP7B Variants in a Large Cohort of Chinese Patients with Wilson's Disease Guides Genetic Diagnosis. *Theranostics*, *6*(5), 638-649. https://doi.org/10.7150/thno.14596

Elman, L. B., McCluskey, L., & Quinn, C. (2023, February 13). *Clinical features of amyotrophic lateral sclerosis and other forms of motor neuron disease*.

https://www.uptodate.com/contents/clinical-features-of-amyotrophic-lateral-sclerosis-and-other-forms-of-motor-neuron-disease
FARA. (2022). *For Physicians*. http://www.curefa.org/physicians
Fink, J. K. (2014). Hereditary spastic paraplegia: clinical principles and genetic advances. *Semin Neurol*, *34*(3), 293-305. https://doi.org/10.1055/s-0034-1386767
Finkel, R. S., Sejersen, T., & Mercuri, E. (2017). 218th ENMC International Workshop:: Revisiting the consensus on standards of care in SMA Naarden, The Netherlands, 19-21 February 2016. *Neuromuscul Disord*, *27*(6), 596-605. https://doi.org/10.1016/j.nmd.2017.02.014
Harbo, H. F., Finsterer, J., Baets, J., Van Broeckhoven, C., Di Donato, S., Fontaine, B., De Jonghe, P., Lossos, A., Lynch, T., Mariotti, C., Schöls, L., Spinazzola, A., Szolnoki, Z., Tabrizi, S. J., Tallaksen, C., Zeviani, M., Burgunder, J. M., & Gasser, T. (2009). EFNS guidelines on the molecular diagnosis of neurogenetic disorders: general issues, Huntington's disease, Parkinson's disease and dystonias. *European Journal of Neurology*, *16*(7), 777-785. https://doi.org/10.1111/j.1468-1331.2009.02646.x
Hedera, P. (2021). Hereditary Spastic Paraplegia Overview. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews(®)*. University of Washington, Seattle. Copyright © 1993-2020, University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved. https://www.ncbi.nlm.nih.gov/books/NBK1509/
Jacobi, H., du Montcel, S. T., Bauer, P., Giunti, P., Cook, A., Labrum, R., Parkinson, M. H., Durr, A., Brice, A., Charles, P., Marelli, C., Mariotti, C., Nanetti, L., Panzeri, M., Rakowicz, M., Sulek, A., Sobanska, A., Schmitz-Hubsch, T., Schols, L., . . . Klockgether, T. (2015). Long-term disease progression in spinocerebellar ataxia types 1, 2, 3, and 6: a longitudinal cohort study. *Lancet Neurol*, *14*(11), 1101-1108. https://doi.org/10.1016/s1474-4422(15)00202-1
Jankovic, J. (2024, April 15). *Epidemiology, pathogenesis, and genetics of Parkinson disease*. https://www.uptodate.com/contents/epidemiology-pathogenesis-and-genetics-of-parkinson-disease
Kovacs, G. G. (2016). Molecular Pathological Classification of Neurodegenerative Diseases: Turning towards Precision Medicine. *Int J Mol Sci*, *17*(2). https://doi.org/10.3390/ijms17020189
Leotti, V. B., de Vries, J. J., Oliveira, C. M., de Mattos, E. P., Te Meerman, G. J., Brunt, E. R., Kampinga, H. H., Jardim, L. B., & Verbeek, D. S. (2021). CAG Repeat Size Influences the Progression Rate of Spinocerebellar Ataxia Type 3. *Ann Neurol*, *89*(1), 66-73. https://doi.org/10.1002/ana.25919
MacLeod, R., Tibben, A., Frontali, M., Evers-Kiebooms, G., Jones, A., Martinez-Descales, A., Roos, R. A., Editorial, C., & Working Group 'Genetic Testing Counselling' of the European Huntington Disease, N. (2013). Recommendations for the predictive

genetic test in Huntington's disease. *Clinical Genetics*, *83*(3), 221-231. https://doi.org/10.1111/j.1399-0004.2012.01900.x

Maple-Grødem, J., Dalen, I., Tysnes, O. B., Macleod, A. D., Forsgren, L., Counsell, C. E., & Alves, G. (2021). Association of GBA Genotype With Motor and Functional Decline in Patients With Newly Diagnosed Parkinson Disease. *Neurology*, *96*(7), e1036-e1044. https://doi.org/10.1212/wnl.0000000000011411

Maragakis, N., Galvez-Jimenez, Nestor. (2023, November 2). *Epidemiology and pathogenesis of amyotrophic lateral sclerosis*. https://www.uptodate.com/contents/epidemiology-and-pathogenesis-of-amyotrophic-lateral-sclerosis

NAF. (2015). FREQUENTLY ASKED QUESTIONS ABOUT...Gene Testing for Hereditary Ataxia. https://ataxia.org/wp-content/uploads/2017/07/Gene_Testing_for_Hereditary_Ataxia.pdf

Nalls, M. A., Pankratz, N., Lill, C. M., Do, C. B., Hernandez, D. G., Saad, M., DeStefano, A. L., Kara, E., Bras, J., Sharma, M., Schulte, C., Keller, M. F., Arepalli, S., Letson, C., Edsall, C., Stefansson, H., Liu, X., Pliner, H., Lee, J. H., . . . Singleton, A. B. (2014). Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat Genet*, *46*(9), 989-993. https://doi.org/10.1038/ng.3043

NORD. (2017, July 12). *Hereditary Spastic Paraplegia*. https://rarediseases.org/rare-diseases/hereditary-spastic-paraplegia/

Opal, P. (2024, April 22). *Ataxia-telangiectasia*. https://www.uptodate.com/contents/ataxia-telangiectasia

Opal, P., & Ajroud-Driss, S. (2022, August 8). *Hereditary spastic paraplegia*. https://www.uptodate.com/contents/hereditary-spastic-paraplegia

Opal, P., & Zoghbi, H. Y. (2023, December 11). *Friedreich ataxia*. https://www.uptodate.com/contents/friedreich-ataxia

Opal, P., & Zoghbi, H. Y. (2024a, April 26). *Autosomal dominant spinocerebellar ataxias*. https://www.uptodate.com/contents/autosomal-dominant-spinocerebellar-ataxias

Opal, P., & Zoghbi, H. Y. (2024b, February 27). *Overview of the hereditary ataxias*. https://www.uptodate.com/contents/overview-of-the-hereditary-ataxias

Ozelius, L., & Lubarr, N. (2016). DYT1 Early-Onset Isolated Dystonia. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews((R))*. University of Washington, Seattle. University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved. https://www.ncbi.nlm.nih.gov/books/NBK1492/

Reetz, K., Dogan, I., Costa, A. S., Dafotakis, M., Fedosov, K., Giunti, P., Parkinson, M. H., Sweeney, M. G., Mariotti, C., Panzeri, M., Nanetti, L., Arpa, J., Sanz-Gallego, I., Durr, A., Charles, P., Boesch, S., Nachbauer, W., Klopstock, T., Karin, I., . . . Schulz, J. B. (2015). Biological and clinical characteristics of the European Friedreich's Ataxia Consortium

for Translational Studies (EFACTS) cohort: a cross-sectional analysis of baseline data. *Lancet Neurol*, *14*(2), 174-182. https://doi.org/10.1016/s1474-4422(14)70321-7

Schilsky, M. (2024, January 29). *Wilson disease: Epidemiology and pathogenesis*. https://www.uptodate.com/contents/wilson-disease-epidemiology-and-pathogenesis

Schuermans, N., Verdin, H., Ghijsels, J., Hellemans, M., Debackere, E., Bogaert, E., Symoens, S., Naesens, L., Lecomte, E., Crosiers, D., Bergmans, B., Verhoeven, K., Poppe, B., Laureys, G., Herdewyn, S., Van Langenhove, T., Santens, P., De Bleecker, J. L., Hemelsoet, D., . . . for Program for Undiagnosed Rare, D. (2023). Exome Sequencing and Multigene Panel Testing in 1,411 Patients With Adult-Onset Neurologic Disorders. *Neurol Genet*, *9*(3), e200071. https://doi.org/10.1212/NXG.0000000000200071

Shribman, S., Reid, E., Crosby, A. H., Houlden, H., & Warner, T. T. (2019). Hereditary spastic paraplegia: from diagnosis to emerging therapeutic approaches. *Lancet Neurol*, *18*(12), 1136-1146. https://doi.org/10.1016/s1474-4422(19)30235-2

Sidransky, E., Nalls, M. A., Aasly, J. O., Aharon-Peretz, J., Annesi, G., Barbosa, E. R., Bar-Shira, A., Berg, D., Bras, J., Brice, A., Chen, C. M., Clark, L. N., Condroyer, C., De Marco, E. V., Durr, A., Eblan, M. J., Fahn, S., Farrer, M. J., Fung, H. C., . . . Ziegler, S. G. (2009). Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N Engl J Med*, *361*(17), 1651-1661. https://doi.org/10.1056/NEJMoa0901281

Socha, P., Janczyk, W., Dhawan, A., Baumann, U., D'Antiga, L., Tanner, S., Iorio, R., Vajro, P., Houwen, R., Fischler, B., Dezsofi, A., Hadzic, N., Hierro, L., Jahnel, J., McLin, V., Nobili, V., Smets, F., Verkade, H. J., & Debray, D. (2018). Wilson's Disease in Children: A Position Paper by the Hepatology Committee of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr*, *66*(2), 334-344. https://doi.org/10.1097/mpg.0000000000001787

Suchowersky, O. (2023, January 23). *Huntington disease: Clinical features and diagnosis*. https://www.uptodate.com/contents/huntington-disease-clinical-features-and-diagnosis

Toyoshima, Y., Onodera, O., Yamada, M., Tsuji, S., & Takahashi, H. (2012). Spinocerebellar Ataxia Type 17. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews((R))*. University of Washington, Seattle. University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved. https://www.ncbi.nlm.nih.gov/books/NBK1438/

Vajda, A., McLaughlin, R. L., Heverin, M., Thorpe, O., Abrahams, S., Al-Chalabi, A., & Hardiman, O. (2017). Genetic testing in ALS: A survey of current practices. *Neurology*, *88*(10), 991-999. https://doi.org/10.1212/wnl.0000000000003686

van de Warrenburg, B. P. C., van Gaalen, J., Boesch, S., Burgunder, J. M., Dürr, A., Giunti, P., Klockgether, T., Mariotti, C., Pandolfo, M., & Riess, O. (2014). EFNS/ENS Consensus

on the diagnosis and management of chronic ataxias in adulthood. *European Journal of Neurology*, *21*(4), 552-562. https://doi.org/10.1111/ene.12341

Zarkov, M., Stojadinovic, A., Sekulic, S., Barjaktarovic, I., Peric, S., Kekovic, G., Draskovic, B., & Stevic, Z. (2015). Association between the SMN2 gene copy number and clinical characteristics of patients with spinal muscular atrophy with homozygous deletion of exon 7 of the SMN1 gene. *Vojnosanit Pregl*, *72*(10), 859-863.

Zech, M., Boesch, S., Jochim, A., Weber, S., Meindl, T., Schormair, B., Wieland, T., Lunetta, C., Sansone, V., Messner, M., Mueller, J., Ceballos-Baumann, A., Strom, T. M., Colombo, R., Poewe, W., Haslinger, B., & Winkelmann, J. (2017). Clinical exome sequencing in early-onset generalized dystonia and large-scale resequencing follow-up. *Mov Disord*, *32*(4), 549-559. https://doi.org/10.1002/mds.26808

Zoghbi, H. Y., & Orr, H. T. (2023, December 8). *Huntington disease: Genetics and pathogenesis*. https://www.uptodate.com/contents/huntington-disease-genetics-and-pathogenesis

Genetic Testing for Neurofibromatosis and Related Disorders

Policy Number: AHS – M2134 – Genetic Testing for Neurofibromatosis and Related Disorders	Prior Policy Name and Number, as applicable: • M2134-Genetic Testing for Neurofibromatosis
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Neurofibromatoses are a group of three clinically and genetically distinct disorders that cause tumors to form on nerve tissue. Neurofibromatosis type 1 (NF1) is caused by autosomal dominant mutations in the neurofibromin (*NF1*) gene and is characterized by multiple café-au-lait macules and neurofibromas (Korf et al., 2024). Neurofibromatosis type (NF2) is caused by autosomal dominant mutations in the merlin, also known as schwannomin, (*NF2*) gene, and is characterized by multiple tumors of the nervous system, including the more common bilateral vestibular schwannomas as well as intracranial and spinal meningiomas, intrinsic ependymomas, and other spine tumors (Evans, 2023b). Schwannomatosis is caused by inactivating mutations in *SMARCB1* and *LZTR* and is characterized by multiple schwannomas and pain arising in adulthood (Bergner & Yohay, 2024).

Legius syndrome is an NF1-like disorder caused by autosomal dominant mutations in the sprout-related EVH1 [enabled/vasodilator-stimulated phosphoprotein homology 1] domain-containing protein 1 (*SPRED1*) gene, resulting in café-au-lait macules. Constitutional mismatch repair-deficiency syndrome (CMMR-D), caused by mutations in mismatch repair genes, can also result in café-au-lait macules, axillary freckling, and Lisch nodules similar to NF1; however, unlike NF1, CMMR-D can also result in a variety of different malignancies, including glioblastoma and colorectal cancer (Korf et al., 2024).

II. Related Policies

Policy Number	Policy Title

AHS-M2179	Prenatal Screening (Genetic)

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Prior to genetic testing for neurofibromatosis, *NF2-*, *SMARCB1-*, or *LZTR1*-related schwannomatosis, Legius Syndrome, **or** constitutional mismatch repair deficiency (CMMRD), genetic counseling **IS REQUIRED.**

2) For individuals who are clinically suspected of having neurofibromatosis type 1 (NF1), but for whom a definitive diagnosis cannot be made without genetic testing, genetic testing for *NF1* mutations **MEETS COVERAGE CRITERIA** when **one** of the following signs of NF1 is present:
   a) Individual has six or more café-au-lait macules (over 5 mm in greatest diameter in pre-pubertal individuals; over 15 mm in greatest diameter in post-pubertal individuals).
   b) Individual has two or more neurofibromas of any type or one plexiform neurofibroma.
   c) Individual has freckling in the axillary or inguinal regions.
   d) Individual has optic glioma.
   e) Individual has two or more Lisch nodules (iris hamartomas).
   f) Individual has a distinctive osseous lesion, such as sphenoid dysplasia, anterolateral bowing of the tibia, or pseudarthrosis of the long bone.
   g) Individual has a first-degree relative (see Note 1) with NF1 as defined by the above criteria.

3) For asymptomatic individuals who have a close blood relative (see Note 1) with a deleterious *NF1* or *NF2* gene mutation, the following testing **MEETS COVERAGE CRITERIA**:
   a) Testing restricted to the known familial mutation.
   b) Comprehensive genetic testing when the specific familial mutation is unknown (i.e., family member is unavailable for testing or testing results are unavailable).

4) For individuals who have a clinical diagnosis of neurofibromatosis and who are planning to conceive, preconception screening for *NF1* or *NF2* gene mutations, when

the individual has not previously received genetic screening for a pathogenic mutation, **MEETS COVERAGE CRITERIA.**

5) For individuals who are clinically suspected of having *NF2*-related schwannomatosis, but for whom a definitive diagnosis and classification cannot be made without genetic testing, genetic testing for *NF2* gene mutations **MEETS COVERAGE CRITERIA** when **one** of the following signs of *NF2*-related schwannomatosis is present:
    a) Individual has bilateral vestibular schwannomas (VS)
    b) Individual has either two major or one major and two minor criteria:
        i) Major criteria:
            (a) Unilateral VS
            (b) First-degree relative (see Note 1) other than a sibling) with *NF2*-related schwannomatosis
            (c) Two or more meningiomas (note that a single meningioma qualifies as minor criteria)
        ii) Minor criteria:
            (a) Can count >1 of a type (e.g., 2 distinct schwannomas would count as 2 minor criteria): Ependymoma, schwannoma (note that if the major criterion is unilateral VS, at least 1 schwannoma must be dermal in location)
            (b) Can count only once (e.g., bilateral cortical cataracts count as a single minor criterion): Juvenile subcapsular or cortical cataract, retinal hamartoma, epiretinal membrane in a person aged <40 years, meningioma (because multiple meningiomas qualify as a major criteria).

6) For individuals who are negative for *NF2* mutations **and** who have one or more pathologically confirmed schwannoma or hybrid nerve sheath tumor, genetic testing for mutations in *SMARCB1* and *LZTR1* **MEETS COVERAGE CRITERIA**.

7) For individuals who are clinically suspected of having Legius Syndrome, genetic testing of *SPRED1* **MEETS COVERAGE CRITERIA** when **one** of the following conditions is met:
    a) The individual has six or more café-au-lait macules (over 5 mm in greatest diameter in pre-pubertal individuals; over 15 mm in greatest diameter in post-pubertal individuals).
    b) The individual has freckling in the axillary or inguinal regions.

c) The individual has symptoms of NF1, but genetic test results for NF1 were negative.

8) For individuals 25 years and younger who have at least two hyperpigmented skin patches (café-au-lait macules), who have tested negative for *NF1* and *SPRED1* mutations, **and** for whom neither parent has diagnostic signs of NF1 (if known), genetic testing for CMMRD (*MLH1, MSH2, MSH6,* and *PMS2*) **MEETS COVERAGE CRITERIA** when **one** of the following risk factors is present:

   a) Risk factors in the patient include:

      i) Atypical café-au-lait macules (irregular borders and/or pigmentation).
      ii) Hypopigmented skin patches.
      iii) One or more pilomatricoma(s).
      iv) Agenesis of the corpus callosum.
      v) Non-therapy-induced cavernoma.
      vi) Multiple developmental vascular abnormalities (cerebral venous angiomas) in separate regions of the brain.

   b) Familial risk factors include:

      i) Consanguineous parents.
      ii) A genetic diagnosis of Lynch syndrome in one or both of the parental families.
      iii) A sibling with diagnostic NF1 sign(s).
      iv) A sibling, living or deceased, with any type of childhood malignancy.
      v) A first- or second-degree relative (see Note 1) diagnosed before the age of 60 years with one of the following carcinomas from the Lynch syndrome spectrum: colorectal cancer, endometrial cancer, ovarian cancer, gastric cancer, small bowel cancer, cancer of the bile duct or gall bladder, pancreatic cancer, or urothelial cancer.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

9) For all other situations not meeting the criteria outlined above, genetic testing for neurofibromatosis **DOES NOT MEET COVERAGE CRITERIA**

NOTES:

Note 1: Close blood relatives include 1st-degree relatives (e.g., parents, siblings, and children), 2nd-degree relatives (e.g., grandparents, aunts, uncles, nieces, nephews,

grandchildren, and half-siblings), and 3rd-degree relatives (great-grandparents, great-aunts, great-uncles, great-grandchildren, and first cousins), all of whom are on the same side of the family.

Note 2: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
AACR	American Association for Cancer Research
AAP	American Academy of Pediatrics
ACMG	American College of Medical Genetics and Genomics
BVS	Bilateral vestibular schwannoma
C4CMMRD	Care for constitutional mismatch repair deficiency
CALM	Café au lait macule
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMMRD	Constitutional mismatch repair deficiency
CMS	Centers for Medicare and Medicaid Services
EANO	European Association of Neuro-Oncology
EVH1	*Enabled/vasodilator-stimulated phosphoprotein homology 1*
FDA	Food and Drug Administration
LDT	Laboratory-developed test
LZTR1	*Leucin-zipper-like transcriptional regulator 1*
MMR	Measles, mumps, and rubella
MRI	Magnetic resonance imaging
NCCN	National Comprehensive Cancer Network
NF1	Neurofibromatosis type 1
NF2	Neurofibromatosis type 2
NGS	Next-generation sequencing
NSD	Noonan spectrum disorders
SMARCB1	*SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily B, member 1*
SPRED1	*Sprout-Related EVH1 Domain-Containing Protein 1*
sVS	Sporadic vestibular schwannoma
VS	Vestibular schwannoma

V. Scientific Background

Neurofibromatosis type 1

Neurofibromatosis type 1 is relatively common, affecting approximately one in 3,000 individuals (Korf et al., 2024). Almost half of these cases are *de novo* mutations, resulting from the unusually high (~1:10,000) mutation rate in the *NF1* tumor suppressor gene primarily in paternally derived chromosomes (Stephens et al., 1992).

The GTPase protein product of the *NF1* gene, neurofibromin, is expressed in many tissues, including brain, kidney, spleen, and thymus leading to a wide spectrum of clinical manifestations. NF1 typically presents as café-au-lait macules, followed by axillary and/or inguinal freckling, and later Lisch nodules (iris hamartomas), and neurofibromas (Korf et al., 2024). Ocular, neurologic, musculoskeletal, vascular, cardiac, and malignant manifestations have been reported (Hirbe & Gutmann, 2014).

NF1 mutations are highly penetrant and inherited dominantly; however, NF1 is variably expressed resulting in significant clinical variability, not only between unrelated individuals and among affected individuals within a single family but even within a single person with NF1 at different times in life (Friedman, 2023). Despite thousands of *NF1* mutations identified, few genotype/phenotype correlations have been observed (Shofty et al., 2015). Recent reports indicate the growing utility of next generation sequencing to provide solutions for problems like genetic heterogeneity, overlapping clinical manifestations, or the presence of mosaicism, and interactions between *SPRED1* and neurofibromin provide functional insight that will help in the interpretation of pathogenicity of certain missense variants identified in NF1 and Legius syndrome patients (Fisher et al., 2018).

NF1 is diagnosed clinically using the criteria developed by the National Institutes of Health (NIH, 1988), which are both highly specific and sensitive in all but very young children. Approximately 46% of sporadic NF1 cases fail to meet the NIH Diagnostic Criteria by one year of age. Nearly all (97%; 95% confidence interval: 94-98) NF1 patients meet the criteria for diagnosis by eight years old, and all do so by 20 years old (DeBella et al., 2000).

Molecular testing for NF1 includes sequencing of all the coding exons as well as deletions/rearrangements due to the large size of the gene and the heterogeneity of mutations. reported identification of the causative DNA mutation in 64 of 67 patients with a clinical diagnosis of NF1. Korf et al. (2024) states that molecular testing is reported to identify approximately 95 percent of causative mutations. However, a positive *NF1* mutation test does not predict the severity or complications of the disorder (Korf et al., 2024).

Molecular genetic testing is indicated for individuals in whom NF1 is suspected but who do not fulfill the NIH diagnostic criteria (Friedman, 2023). Additionally, there is increasing use of genetic testing in the diagnosis of NF1 for patients who meet only these two NIH criteria; moreover, individuals with only one NIH criterion as a positive genetic test may shorten the period of diagnostic uncertainty, allowing the initiation of appropriate screening evaluations (Korf et al., 2024). Further examples of clinical utility that justify molecular testing include: a young child with a serious tumor (e.g., optic glioma) in whom establishing a diagnosis of NF1 immediately would affect management, an adult with NF1 if prenatal or preimplantation genetic diagnosis in a current or future pregnancy is anticipated (Friedman, 2023).

Prenatal testing is available through direct mutation testing of fetal DNA taken from CVS or from amniocentesis to diagnose *NF1* pathogenic variants in the fetus. An additional option is assessing DNA markers in families with two or more affected individuals; however, many partners do not perform a prenatal assessment because of "the inability to determine disease severity" in the fetus (Ferner et al., 2007). Because the time for prenatal diagnosis is limited, it is common for families to detect pathogenic *NF1* alleles through linkage analysis as a more "rapid and useful" method for diagnosis (Terzi et al., 2009). Additionally, detection of pathogenic *NF1* mutations can be complex and challenging because of wide phenotypic variability and an absence of genotype-phenotype correlation (Terzi et al., 2009).

Preimplantation genetic diagnosis is also available to assist individuals who want to avoid a later termination of a pregnancy. Preimplantation genetic diagnosis occurs using cells removed from an embryo (available at the approximately 3-day mark of embryo development). This helps individuals determine which embryos do not carry the NF1 mutation in order to transfer unaffected embryos for implantation. Additionally, NF1 experts recommend that all NF1 pathogenic mutation affected individuals should receive genetic counseling prior to conception (Ferner et al., 2007).

Lastly, some rare variants of NF1, including spinal NF1, are known to produce a phenotype in which affected individuals may not meet the NIH diagnostic criteria. In this case, molecular testing is indicated for at-risk relatives (Burkitt-Wright et al., 2013).

Neurofibromatosis type 2

Neurofibromatosis type 2 refers to what was originally thought to be a rare subtype of neurofibromatosis type 1, but rather is a distinct entity, both genetically and clinically (Evans, 2023b). It is characterized by bilateral vestibular schwannomas with associated symptoms of tinnitus, hearing loss, and balance dysfunction resulting from mutation in the *NF2* gene. Affected individuals may also develop schwannomas of other cranial and peripheral nerves, meningiomas, ependymomas, and, very rarely, astrocytomas.

Typical age of onset is 18 to 24 years, with almost all affected individuals developing bilateral schwannomas by the age of 30 (Evans, 2023b). The prevalence is about 1:60,000 with a birth incidence of 1:33,000 (Evans et al., 2010). Skin tumors and ocular findings often are the first manifestations and have been underrecognized in children (Ruggieri et al., 2005).

The protein encoded by the *NF2* gene, merlin or schwannomin, is a cell membrane-related tumor suppressor (Evans, 2023b). Inactivation of both alleles is necessary for tumor development. Variable expressivity of *NF2* results in varying size, location, and number of tumors. Despite that these tumors are not malignant, their number and anatomical location contribute significantly to morbidity and mortality with the average age of death being 36 (Baser et al., 2002). However, advances in molecular diagnosis, imaging, and treatment of NF2-associated tumors have resulted in lower mortality (Hexter et al., 2015).

Clinical criteria for NF2 were initially established with those for *NF1* (NIH, 1988), and they were modified as the Manchester criteria to include molecular diagnostics and increase specificity without affecting sensitivity (Evans, 2023b). Most recently, the identification of *LZTR1* as a cause of schwannomatosis reduces the specificity of these more inclusive criteria and even the presence of bilateral VS is now no longer sufficient to be certain that an individual has NF2 (Smith et al., 2017), resulting in further modification of the Manchester criteria.

Detailed molecular testing is reported to identify mutations in NF2 in 93% of families with multiple members affected by NF2 (Evans, 2023b). Early diagnosis of individuals with NF2 facilitates treatment and reduction of mortality (Hexter et al., 2015); however, genetic testing and management is complicated by the well-documented risk of mosaicism (Evans et al., 2012). More so than with *NF1*, the stronger genotype/phenotype correlations in mutations of NF2 (Baser et al., 2004; Baser et al., 2005), high frequency of de novo mutations, and presentation of patients before clinical diagnostic criteria are fulfilled have provided a stronger rationale for the clinical utility of molecular testing than for *NF1*.

Molecular testing approaches can differ for NF2 based on the clinical picture. Patients with the distinctive phenotypic and laboratory findings suggestive of NF2 are likely to be diagnosed using gene-targeted testing (75%), whereas those where the diagnosis of NF2 has not been considered or had met the diagnostic criteria (such as children) are diagnosed after exome sequencing (Evans, 2023a).

Schwannomatosis

Schwannomatosis is an uncommon form of neurofibromatosis characterized by predisposition to develop multiple schwannomas and, less frequently, meningiomas. Its estimated prevalence is 1:70,000 (Dhamija et al., 2018) but is thought to be underestimated (Koontz et al., 2013). Although there is clinical overlap with NF2, schwannomatosis is caused by the concomitant mutational inactivation of two or more tumor suppressor genes. Germline mutations of either the *SMARCB1* or *LZTR1* tumor suppressor genes have been identified in 86% of familial and 40% of sporadic schwannomatosis patients (Kehrer-Sawatzki et al., 2017). *LZTR1* encodes leucin-zipper-like transcriptional regulator 1 and *SMARCB1* (also known as INI1) encodes a subunit of the SWI/SNF chromatin remodeling complex, and both act as tumor suppressors. Biallelic inactivation of these tumor suppressor genes leads to schwannomatosis (Radhika Dhamija, 2023).

The median age of symptom onset is 30 years with pain being the most common presenting symptom in 57 percent of patients. In others (41 percent), a mass was the presenting symptom (Merker et al., 2012). Other symptoms reported at presentation vary based on the location of the tumors, but they can include focal numbness, weakness, and muscle atrophy (Bergner & Yohay, 2024). Peripheral and spinal schwannomas are common in schwannomatosis patients. Severe pain is difficult to treat in these patients and often associated with anxiety and depression (Merker et al., 2012).

Diagnostic criteria for schwannomatosis was first set forth by MacCollin et al. (2005) but has been revised with the addition of molecular diagnostic criteria (Plotkin et al., 2013). More recently combined clinical and molecular criteria from Kehrer-Sawatzki et al., have been proposed (Kehrer-Sawatzki et al., 2017).

"A combined molecular and clinical diagnosis may be made with ≥ 2 tumors with 22q LOH and different somatic *NF2* mutations AND ≥ 2 pathologically confirmed schwannomas or meningiomas"

OR

"Germline *SMARCB1* or *LZTR1* pathogenic mutation AND one pathologically confirmed schwannoma or meningioma"

"A strictly clinical diagnosis may be made with ≥ 2 nonintradermal schwannomas, one pathologically confirmed and no bilateral vestibular schwannoma by high quality MRI (some mosaic NF2 patients will be included in this diagnosis at a young age and some schwannomatosis patients may have unilateral vestibular schwannomas or meningiomas)"

OR

"One pathologically confirmed schwannoma or intracranial meningioma AND an affected first degree relative."

Exclusion criteria for schwannomatosis are as follows:

- Germline pathogenic *NF2* mutation
- First degree relative with NF2
- Fulfillment of diagnostic criteria for NF2
- If schwannomas occur exclusively in a region of previous radiation therapy (Kehrer-Sawatzki et al., 2017)

Kehrer-Sawatzki et al. (2017) also recommended, "Comprehensive mutation analysis of all three genes, *LZTR1*, *SMARCB1,* and *NF2,* in patients with schwannomatosis should be performed to identify the complete mutational spectra and the number of mutational hits that affect these genes. This comprehensive testing may help to classify the tumors according to their mutation-profile. The mutation analysis should also include methods, such as next-generation sequencing, which are well suited to detect somatic mosaicism with mutant cells present in low proportions. This approach should identify tumor heterogeneity and help to distinguish between mosaic NF2 and schwannomatosis, since some NF2 patients with somatic mosaicism for an *NF2* gene mutation fulfil the diagnostic criteria for schwannomatosis" (Kehrer-Sawatzki et al., 2017).

Legius Syndrome

Legius syndrome has similar clinical features to NF1 such as the café-au-lait macules, but does not have the neurofibromas or central nervous system tumors. Furthermore, the primary genetic alteration in Legius syndrome is the sprouty-related EVH1 [enabled/vasodilator-stimulated phosphoprotein homology 1] gene (*SPRED1*) compared to *NF1* for neurofibromatosis 1.

A negative *NF1* mutation test in patients with only café-au-lait macules and axillary freckling should be tested for *SPRED1* mutations followed by the four mismatch repair genes as Legius syndrome, constitutional mismatch repair-deficiency (CMMR-D) syndrome, and Noonan syndrome may present with these indications (Korf et al., 2024).

CMMR-D

Constitutional mismatch repair-deficiency syndrome (CMMR-D) has similar clinical symptoms to neurofibromatosis 1 but leads to different malignancies. Hematologic

malignancies develop in infancy to early childhood, brain tumors (such as glioblastoma) may present in mid-childhood, and colorectal cancer may show up in adolescence or young adulthood (Korf et al., 2024). CMMR-D is a childhood cancer predisposition syndrome that is caused by biallelic pathogenic variants in one of four mismatch repair genes (Hizuka et al., 2021). Individuals with this syndrome may develop hematologic or colorectal malignancies in addition to the neurofibromas seen in NF1 patients (Korf et al., 2024).

One important characteristic of CMMR-D is that it is typically diagnosed in childhood. The Federal Food, Drug, and Cosmetic Act (FD&C Act) defines pediatric patients as persons aged 21 or younger at the time of their diagnosis or treatment and the *Bright Futures* guidelines from the American Academy of Pediatrics identify adolescence as 11 to 21 years of age, dividing the group into early (ages 11–14 years), middle (ages 15–17 years), and late (ages 18–21 years). CMMR-D is most often diagnosed before the age of 18. One collaborative review from the European consortium established the ages of first diagnosis as ranging from 0.4 to 39 years. However, the "vast majority" of patients are diagnosed with a first malignancy before the age of 18 (82% diagnosed before age 18) (Wimmer et al., 2014).

Clinical Utility and Validity

Neurofibromatosis type 1

Giugliano et al. (2019) investigated the clinical and genotypic associations in children with pigmentary features characteristic of a neurocutaneous condition, such as neurofibromatosis type 1. A group of 281 patients were included, with 150 definitively diagnosed with NF1, 95 presenting with only pigmentary features such as café au lait macules (CALMs), and 36 presenting with a clinical suspicion of another "RASopathy" (a condition caused by mutations in the MAPK pathway) or other neurocutaneous disorder. The authors identified the causative pathogenic variant in 239 of 281 cases (leaving 42 undiagnosed). Of the patients diagnosed with NF1, mutations were detected in 98% of cases (147/150) but in patients with only pigmentary features, the detection rate fell to 69.5% (66/95), with *SPRED1* accounting for eight of those cases. In patients presenting with a separate neurocutaneous condition, mutation detection rate was found to be 72.2% (26/36), with pathogenic variants found in 10 genes such as PTPN11. The authors recognized the difficulty of diagnosing these neurocutaneous and concluded that a "combined NGS-based approach can assist clinicians in the diagnosis of NF1 as well as other neurocutaneous disorders and overlapping conditions" (Giugliano et al., 2019).

Castellanos et al. (2020) developed a custom next-generation sequencing (NGS) panel for testing patients with "a clinical suspicion of a RASopathy (n = 48) and children

presenting multiple CALMs [café-au-lait macules] (n = 102)". The authors stated that phenotypic overlaps may exist in children if multiple CALMs are the only clinical symptom present and that genetic testing may differentiate between conditions. Of the 48 patients with clinical suspicion of a RASopathy, 21 were found to harbor a pathogenic mutation (with *NF1* mutations comprising five of 48 cases). Of the patients with multiple CALMs, both *NF1* and *SPRED1* pathogenic mutations were identified. Overall, the authors concluded that "an NGS panel strategy for the genetic testing of these two phenotype-defined groups outperforms previous strategies" (Castellanos et al., 2020).

Witkowski et al. (2020) studied the benefits of adding *NF1* and *SPRED1* sequencing to the Noonan spectrum/ RASopathy NGS panel. Noonan spectrum disorders (NSD) are a group of disorders caused by problems in the MAPK pathway. NSD's are due to gain of function, while NF1 is caused by a loss of function. The study included 28 patients with a negative NSD panel that underwent *NF1* and *SPRED1* sequencing, and a validation panel analyzed 14 RASopathy associated genes in 505 patients. In total, 21% of the 28 patients had disease-causing *NF1/SPRED1* variants. In the validation cohort, only 2% of the patients were found to have disease-causing variants in the *NF1/SPRED1* genes. Adding *NF1* and *SPRED1* to the panel increased the diagnostic yield from 23.5% to 25.7%. The authors concluded that "adding the *NF1* and *SPRED1* genes to Noonan spectrum disorder/RASopathy NGS gene panels modestly increases clinical diagnoses" (Witkowski et al., 2020).

In a retrospective study, Elmas (2022) studied the use of artificial intelligence, Face2Gene, to diagnosis neurofibromatosis type 1. Fourteen patients underwent Face2Gene analysis. As a result, the most detected mutation type was nonsense mutation (42.8%) and suggested NF1 diagnosis for 10 of the 14 patients. The authors concluded that Face2Gene will be used a lot in the routines of medical doctors in the next 10 years (Elmas, 2022).

Neurofibromatosis type 2

Evans et al. (2015) investigated the clinical validity of the primary development of NF2, the bilateral vestibular schwannoma (BVS). The authors observed that out of a database of over 1200 patients, approximately 25% of them over 50 developed a BVS without any other clinical features of NF2. Over 50% of the patients over 70 developed a BVS as well. This lack of other clinical features in addition to the BVS led the authors to suggest that these developments of a BVS were due to chance rather than an NF2 mutation (Evans et al., 2015).

Pathmanaban et al. (2017) analyzed the database of the Manchester Centre for Genomic Medicine to determine the frequency of the known heritable meningioma- or

schwannoma-predisposing mutations in children and young adults presenting with a solitary meningioma or schwannoma. They found that "A significant proportion of young people with an apparently sporadic solitary meningioma or schwannoma had a causative predisposition mutation. This finding has important clinical implications because of the risk of additional tumors and the possibility of familial disease. Young patients presenting with a solitary meningioma or schwannoma should be referred for genetic testing" (Pathmanaban et al., 2017).

Castellanos et al. (2018) recently demonstrated the clinical utility of a careful dermatological inspection and the correct identification of skin plaques in children for an early diagnosis of NF2. Skin plaques from seven patients (four male and three female) were analyzed and histologically characterized as plexiform schwannomas. Genetic analysis of primary Schwann cell cultures derived from them allowed the identification of a constitutional and a somatic NF2 mutation. Genetic testing allowed the early diagnosis of NF2 in a child only exhibiting the presence of skin plaques. Most of the patients with NF2 analyzed had an early presentation of skin plaques and a severe NF2 phenotype. The authors remarked that "Dermatological identification of skin plaque schwannomas in children would facilitate the early diagnosis and treatment of patients with NF2 before development of severe adverse effects" (Castellanos et al., 2018).

A genetic severity score has recently been developed to draw these factors together to enable genotypic data to be routinely factored into clinical and research use. This UK NF2 Genetic Severity Score classifies patients into three categories, which are tissue mosaic (1), classic (2), and severe (3). Within each category are subcategories, which consists of the following in increasing severity: presumed tissue mosaicism (1A), confirmed tissue mosaicism (1B), mild NF2 (2A), moderate NF2 (2B), and severe NF2 (3). These categories are separated by severity of mutation shown below (Halliday et al., 2017).

Genetic Severity	Sub-category	Clinical Characteristics	Definition
1 (Tissue Mosaic)	1A	Presumed tissue mosaicism	Meets clinical criteria for sporadic NF2 but not confirmed molecularly with identical NF2 mutations detected in two separate tissue samples
	1B	Confirmed tissue mosaicism	Mosaic NF2 confirmed molecularly with identical NF2 mutations detected in two or more separate tissue samples

2 (Classic)	2A	Mild NF2	Full or mosaic *NF2* mutation identified in blood excluding those found in group 2B or 3: missense mutations; in-frame deletions and duplications; deletions involving the promoter region or exon 1; splice site mutations in exons 8–15; truncating mutations of exon 1; mosaicism in blood for mutations other than truncating mutations in exons 2–13 Inherited NF2 but no *NF2, SMARCB1* or *LZTR1* mutation identified in blood
	2B	Moderate NF2	Full or mosaic NF2 mutation identified in blood including: splicing mutation involving exons 1–7; large deletion not including the promoter or exon 1; truncating mutations in exons 14–15; mosaic in blood for a truncating mutation in exons 2–13
3 (Severe)	3	Severe NF2	Full *NF2* truncating mutation exons 2–13

Halliday et al. (2017) evaluated the validity of this score in 142 patients (63 in group 1, 35 in group 2, and 19 in group 3 with three with no mutation identified) More severe symptoms such as intracranial meningiomas, BVS, and spinal schwannomas, were more likely to be found in group 3 compared to group 1. For example, BVS and intracranial meningiomas were found in 100% and 94.7% of group 3 patients respectively, compared to 54% and 59% in group 1. Spinal meningiomas were found in 36.8% of group 3 patients compared to 15.3% of group 1, and schwannomas were found in 94.7% of group 3 patients compared to 48.3% of group 1. The authors concluded that "The biggest single factor that determines NF2 severity is the type of mutation, its position within the gene and the proportion of cells carrying it" (Halliday et al., 2017).

Lu et al. (2019) examined the efficacy and safety of bevacizumab for vestibular schwannomas (VS) in neurofibromatosis type 2. The authors included eight articles including 161 patients and 196 VS. The authors identified radiographic response in 41% of cases (termed "partial regression"), no change in 47% of cases, and tumor progression of 7% of cases. Bevacizumab treatment also resulted in hearing improvement in 20% of cases, stability in 69% of cases, and further hearing loss in 6% of cases. Bevacizumab toxicity was observed in 17% of cases, and surgical intervention

was needed in 11% of cases. Overall, the authors concluded that bevacizumab may "arrest" tumor progression and hearing loss in NF2 patients presenting with VS lesions but recommended judicious use of bevacizumab due to serious adverse events (Lu et al., 2019).

Schwannomatosis

Hutter et al. (2014) evaluated the proportion of schwannomatosis cases that come from mutations aside from the germline variants in *SMARCB1* and *LZTR1*. The authors performed whole exome sequencing on 23 patients with sporadic schwannomatosis (without *SMARCB1* mutations) and found only five *LZTR1* or *NF2* mutations. However, since the authors noted the reported frequency of *SMARCB1* mutations to be only 10% in sporadic schwannomatosis patients, they concluded that approximately 65% (or at least the "majority") of sporadic schwannomatosis mutations are caused by an unknown gene (Hutter et al., 2014).

Louvrier et al. (2018) performed targeted next generation sequencing (NGS) to investigate genetic differences between NF2, schwannomatosis, and meningiomatosis. The authors sequenced 196 patients (79 with NF2, 40 with schwannomatosis, 12 with meningiomatosis, and 65 with no clearly established diagnosis) for *NF2, SMARCB1, LZTR1, SMARCE1*, and *SUFU*. The NF2 and schwannomatosis results were as follows: "An NF2 variant was found in 41 of 79 NF2 patients (52%). *SMARCB1* or *LZTR1* variants were identified in 5/40 (12.5%) and 13/40 (~32%) patients in the schwannomatosis cohort. Potentially pathogenic variants were found in 12/65 (18.5%) patients with no clearly established diagnosis. A *LZTR1* variant was identified in 16/47 (34%) NF2/SMARCB1-negative schwannomatosis patients." The authors concluded that targeted NGS was a suitable strategy for identifying NFS mosaicism in blood and for investigation of these tumors (Louvrier et al., 2018).

Sadler et al. (2021) studied which germline pathogenic variants are associated with sporadic vestibular schwannoma (sVS) through genetic analysis of sVS cases of *NF2, LZTR1* and *SMARCB1* genes. NF2 variants were confirmed in 2% of the patients, *LZTR1* was found in 3% of the patients, and there were no pathogenic *SMARCB1* variants identified in this cohort. Therefore, the authors concluded that "loss of *NF2* function is a common event in sVS tumours and may represent a targetable common pathway in VS tumourigenesis. Earlier identification of patients with these syndromes can facilitate more accurate familial risk prediction and prognosis" (Sadler et al., 2021).

Piotrowski et al. (2022) studied the use of targeted massively parallel sequencing to diagnose multiple schwannomas. Thirty-five patients were enrolled in the study and massive parallel sequencing of *LZTR1, SMARCB1*, and *NF2* genomic loci was conducted. The study verified whether any other *LZTR1/SMARCB1/NF2* pathogenic variants could

be found in 16 cases carrying a *SMARCB1* constitutional variant in the 3′-untranslated region. "The 3′-UTR variants c.*17C>T and c.*82C>T showed pathogenicity. Two novel deep intronic *SMARCB1* variants, c.500+883T>G and c.500+887G>A were identified in two individuals. Further resequencing of chromosome 22q in individuals negative for PVs in the *SMARCB1/LZTR1/NF2* demonstrated five potential schwannomatosis-predisposing candidate genes (*MYO18B, NEFH, SGSM1, SGSM3*, and *SBF1*)." The authors conclude that noncoding *SMARCB1/LZTR1* variants are a molecular cause of schwannomatosis, hence it is essential to include them into the molecular diagnostic panel (Piotrowski et al., 2022).

VI. Guidelines and Recommendations

American Academy of Pediatrics (AAP)

In 2008, the AAP committee on genetics published guidelines on health supervision in children with NF1 (Hersh, 2008). The committee stated that genetic consultation and genetic testing should be considered to expedite a diagnosis when there is uncertainty regarding a definitive diagnosis of NF1. The committee also noted that "molecular testing also may represent an option in those instances when a couple in which one person has NF1 is seeking prenatal diagnosis."

This guideline was reaffirmed in 2017.

A Clinical Report from the AAP comments on the role of genetic testing for Neurofibromatosis Type 1. They state that genetic testing:

- "can confirm a suspected diagnosis before a clinical diagnosis is possible;"
- "can differentiate NF1 from Legius syndrome;"
- "may be helpful in children who present with atypical features;"
- "usually does not predict future complications; and"
- "may not detect all cases of NF1; a negative genetic test rules out a diagnosis of NF1 with 95% (but not 100%) sensitivity" (Miller et al., 2019).

American College of Medical Genetics and Genomics (ACMG)

In their guidelines detailing the care of adults with NF1, the ACMG noted that "In most cases, the diagnosis can be easily made based on a history, physical exam, and pedigree review and no additional imaging or NF1 genetic testing is needed". Furthermore, the ACMG stated that genetic testing can quickly establish a diagnosis for children thereby relieving anxiety, but this is not as significant an issue for adults (Stewart et al., 2018).

However, in the ACMG's guidelines for reporting of secondary findings in exome or genome sequencing, mutations in the *NF2* gene were recommended for return as secondary findings related to cancer phenotypes for both pathogenic and likely pathogenic variants (Miller et al., 2021).

European Association of Neuro-Oncology (EANO)

This EANO guideline on "diagnosis and treatment of vestibular schwannoma" comments on neurofibromatosis type 2, stating that NF2 "should be considered when an individual presents with a unilateral vestibular or other sporadic schwannoma at <30 years or meningioma at <25 years. Germline pathogenic variants can be identified in 1-10% of cases. NF2 should also be considered in older patients with two NF2 related tumors (Goldbrunner et al., 2020).

American Association for Cancer Research (AACR) Childhood Cancer Predisposition Workshop

The following recommendations were created based on expert review of the literature and discussion brought to this workshop.

NF1

- "A child who meets one or more clinical criterion should now have NF1 molecular genetic testing (sequencing and deletion/duplication analysis) offered to confirm if NF1 is the correct diagnosis." Genetic testing is especially recommended in children fulfilling only pigmentary features of the criteria.

The clinical diagnostic criteria are as follows:

- Six or more CAL macules, the greatest diameter of which is more than five mm in prepubertal patients and more than 15 mm in post-pubertal patients
- Two or more neurofibromas of any type, or one plexiform neurofibroma
- Axillary or inguinal freckling
- Optic glioma
- Two or more Lisch nodules
- A distinctive osseous lesion such as sphenoid dysplasia or pseudarthrosis
- A first-degree relative with NF1 according to the preceding criteria

The guidelines note that according to the NIH, two or more of these criteria must be present. This contrasts with their own guidelines' statement of only requiring one clinical criterion.

The guidelines summarize their genetic testing recommendations as follows:

- "Children considered at risk of NF1 especially with 6+ CAL macules or diagnosed with NIH criteria should ideally have genetic testing of the *NF1* gene with an RNA-based approach and testing of *SPRED1* if pigmentary features only".
- "Those testing negative should be considered for a panel of genes including *GNAS, MLH1, MSH2, MSH6, NF2, PMS2, PTPN11, SOS1,* and *SPRED1* (if not already tested)" (Evans, Salvador, Chang, Erez, Voss, Schneider, et al., 2017).

NF2

- "All children presenting with either clear diagnostic criteria for NF2, including combined retinal hamartomas, or those with an NF2 tumor (any schwannoma/meningioma) presenting in childhood should undergo genetic testing of *NF2*, ideally in both blood and tumor if available in sporadic cases."

Schwannomatosis

- "Test for mutations in *SMARCB1* and *LZTR1* in children and young adults with one or more non-intradermal schwannoma, including those with VS (vestibular schwannoma) negative for NF2" (Evans, Salvador, Chang, Erez, Voss, Druker, et al., 2017).

European Consortium 'Care for CMMRD'

The C4CMMRD recommends further testing for patients reaching three points on the clinical scoring scale. "Further testing" generally follows the protocols for Lynch syndrome, which involves analysis of microsatellite instability or immunohistochemistry staining of the main mismatch repair proteins (MLH1, MSH2, MSH6 and PMS2). The clinical scoring scale is as follows (K. Wimmer et al., 2014):

Malignancies/premalignancies: one is mandatory; if more than one is present in the patient, add the points.

- Carcinoma from the LS spectrum* at age <25 years 3 points
- Multiple bowel adenomas at age <25 years and absence of APC/MUTYH mutation(s) or a single high-grade dysplasia adenoma at age <25 years 3 points
- WHO grade III or IV glioma at age <25 years 2 points
- NHL (non-Hodgkin's lymphoma) of T-cell lineage or sPNET (supratentorial primitive neuroectodermal tumour) at age <18 years 2 points
- Any malignancy at age <18 years 1 point

Additional features: optional; if more than one of the following is present, add the points

- Clinical sign of NF1 and/or ≥2 hyperpigmented and/or hypopigmented skin alterations Ø>1 cm in the patient 2 points
- Diagnosis of LS in a first-degree or second-degree relative 2 points
- Carcinoma from LS spectrum* before the age of 60 in first-degree, second-degree, and third-degree relative 1 point
- A sibling with carcinoma from the LS spectrum*, high-grade glioma, sPNET or NHL 2 points
- A sibling with any type of childhood malignancy 1 point
- Multiple pilomatricomas in the patient 2 points
- One pilomatricoma in the patient 1 point
- Agenesis of the corpus callosum or non-therapy-induced cavernoma in the patient 1 point
- Consanguineous parents 1 point
- Deficiency/reduced levels of IgG2/4 and/or IgA 1 point

*Colorectal, endometrial, small bowel, ureter, renal pelvis, biliary tract, stomach, bladder carcinoma (K. Wimmer et al., 2014).

The consortium in 2018 issued the selection strategy for CMMR-D testing as follows:

Prerequisites for testing are…

- "Suspicion of NF1 due to the presence of at least one diagnostic NF1 feature, including at least two hyperpigmented skin patches reminiscent of CALMs [café-au-lait macules]
- No *NF1* and *SPRED1* germline mutations detected using comprehensive and highly sensitive mutation analysis protocols.
- Absence of diagnostic NF1 sign(s) in both parents
- Additional features, at least one (either in the family or in the patient) is required
    - In the family
        - Consanguineous parents.
        - Genetic diagnosis of Lynch syndrome in one or both of the parental families.
        - Sibling with diagnostic NF1 sign(s).
        - A (deceased) sibling§ with any type of childhood malignancy.
        - One of the following carcinomas from the Lynch syndrome spectrum: colorectal cancer, endometrial cancer, ovarian cancer, gastric cancer, small bowel cancer, cancer of the bile duct or gall bladder, pancreatic cancer or urothelial cancer before the age of 60 years in first-degree or second-degree relative.
    - In the patient

- Atypical CALMs (irregular borders and/or pigmentation).
- Hypopigmented skin patches.
- One or more pilomatricoma(s) in the patient.
- Agenesis of the corpus callosum.
- Non-therapy-induced cavernoma.
- Multiple developmental vascular abnormalities (also known as cerebral venous angiomas) in separate regions of the brain.

This can be expanded to second-degree and third-degree relatives in populations with a high prevalence of founder mutations" (Suerink et al., 2019).

National Comprehensive Cancer Network (NCCN)

Within the Lynch Syndrome guidelines, the NCCN states, "For patients of reproductive age, advise about the risk of a rare recessive syndrome called CMMRD syndrome… If both partners are a carrier of a pathogenic variant(s) in the same MMR gene, then their future offspring will be at risk of having CMMRD syndrome" (NCCN, 2023).

International consensus group recommendation on neurofibromatosis type 1

An international consensus group revised diagnostic criteria for neurofibromatosis type 1 as well as sought to establish diagnostic criteria for Legius syndrome (Legius et al., 2021). The group involved global experts, advocacy groups, and patient input in a multistep process to establish criteria.

Diagnostic criteria for neurofibromatosis type 1:

> "A: The diagnostic criteria for NF1 are met in an individual who does not have a parent diagnosed with NF1 if two or more of the following are present:
>
> - Six or more café-au-lait macules over 5 mm in greatest diameter in prepubertal individuals and over 15 mm in greatest diameter in postpubertal individuals
> - Freckling in the axillary or inguinal region
> - Two or more neurofibromas of any type or one plexiform neurofibroma
> - Optic pathway glioma
> - Two or more iris Lisch nodules identified by slit lamp examination or two or more choroidal abnormalities (CAs)—defined as bright, patchy nodules imaged by optical coherence tomography (OCT)/near-infrared reflectance (NIR) imaging

- A distinctive osseous lesion such as sphenoid dysplasia, anterolateral bowing of the tibia, or pseudarthrosis of a long bone
- A heterozygous pathogenic *NF1* variant with a variant allele fraction of 50% in apparently normal tissue such as white blood cells.

B: A child of a parent who meets the diagnostic criteria specified in A merits a diagnosis of NF1 if one or more of the criteria in A are present" (Legius et al., 2021).

Diagnostic criteria for Legius syndrome:

"A: The diagnostic criteria for Legius syndrome are met in an individual who does not have a parent diagnosed with Legius syndrome if the following CRITERIA are present:

- Six or more café-au-lait macules bilaterally distributed and no other *NF1*-related diagnostic criteria except for axillary or inguinal freckling
- A heterozygous pathogenic variant in *SPRED1* with a variant allele fraction of 50% in apparently normal tissue such as white blood cells

B: A child of a parent who meets the diagnostic criteria specified in A merits a diagnosis of Legius syndrome if one or more of the criteria in A are present" (Legius et al., 2021).

"The diagnostic criteria for mosaic NF1 are met in an individual if any of the following is present:

1. A pathogenic heterozygous *NF1* variant with a variant allele fraction of significantly less than 50% in apparently normal tissue such as white blood cells AND one other NF1 diagnostic criterion (except a parent fulfilling diagnostic criteria for NF1)
2. An identical pathogenic heterozygous *NF1* variant in two anatomically independent affected tissues (in the absence of a pathogenic *NF1* variant in unaffected tissue)
3. A clearly segmental distribution of café-au-lait macules or cutaneous neurofibromas AND
   a. Another NF1 diagnostic criterion (except a parent fulfilling diagnostic criteria for *NF1*)
   or
   b. Child fulfilling diagnostic criteria for NF1
4. Only one NF1 diagnostic criterion from the following list: freckling in the axillary and inguinal region, optic pathway glioma, two or more Lisch nodules or two or

more choroidal abnormalities, distinctive osseous lesion typical for NF1, two or more neurofibromas or one plexiform neurofibroma AND a child fulfilling the criteria for NF1"(Legius et al., 2021).

"The diagnostic criteria for mosaic Legius syndrome are met in an individual if any of the following is present:

1. A heterozygous pathogenic *SPRED1* variant with a variant allele fraction of significantly less than 50% in apparently normal tissue such as white blood cells AND six or more café-au-lait macules
2. An identical pathogenic heterozygous *SPRED1* variant in two independent affected tissues (in the absence of a pathogenic *SPRED1* variant in unaffected tissue)
3. A clearly segmental distribution of café-au-lait macules AND a child fulfilling the criteria for Legius syndrome" (Legius et al., 2021).

International consensus group recommendation on neurofibromatosis type 2 and schwannomatosis

The international consensus group also provided new recommendations on the nomenclature of NF2 and schwannomatosis. Traditionally, NF2 and SWN were identified based on primarily clinical features; however, the group's consensus is that the "phenotype of these diseases spans a continuum without absolute delineation of subtypes phenotypically" leading to the need for an umbrella (Plotkin et al., 2022). The group chose the term "schwannomatosis" (i.e. as the umbrella term) to showcase the overlapping clinical phenotype of related conditions. Additionally, the group recommended that the type of SWN be further classified based on the gene that harbors a PV (identified through molecular analysis). According to this nomenclature, NF2 would be renamed "NF2-related schwannomatosis" and SWN would fall as either "SMARCB1-related schwannomatosis," "LZTR1-related schwannomatosis," or "22q-related schwannomatosis," depending on the location of the inherited pathogenic. For patients who have clinical features of NF2/SWN but have not had molecular analysis, the group recommends "schwannomatosis-not otherwise specified" as the type categorization or "schwannomatosis-not elsewhere classified" for patients in whom molecular analysis did not successfully detect a PV variant (Plotkin et al., 2022).

Updated diagnostic criteria for NF2-related schwannomatosis:

"A diagnosis of NF2-related schwannomatosis (previously termed neurofibromatosis 2, NF2) can be made when an individual has one of the following:

1. Bilateral vestibular schwannomas (VS)

2. An identical *NF2* pathogenic variant in at least 2 anatomically distinct NF2-related tumors (schwannoma, meningioma, and/or ependymoma). (Note: if the variant allele fraction (VAF) in unaffected tissues such as blood is clearly <50%, the diagnosis is mosaic NF2-related schwannomatosis)
3. Either 2 major or 1 major and 2 minor criteria as described in the following:

Major criteria:

- Unilateral VS
- First-degree relative other than sibling with NF2-related schwannomatosis
- 2 or more meningiomas (Note: single meningioma qualifies as minor criteria).
- *NF2* pathogenic variant in an unaffected tissue such as blood (Note: if the VAF is clearly <50%, the diagnosis is mosaic NF2-related schwannomatosis)"

Minor criteria:

Can count >1 of a type (e.g., 2 distinct schwannomas would count as 2 minor criteria)

- Ependymoma, meningioma (Note: multiple meningiomas qualify as a major criteria), schwannoma (Note: if the major criterion is unilateral VS, at least 1 schwannoma must be dermal in location)

Can count only once (e.g., bilateral cortical cataracts count as a single minor criterion)

- Juvenile subcapsular or cortical cataract, retinal hamartoma, epiretinal membrane in a person aged <40 years, meningioma" (Plotkin et al., 2022).

"Pattern of genetic changes in unaffected and tumor tissue in *NF2*-related schwannomatosis"

Gene locus	Unaffected Tissue	Tumor 1	Tumor 2	Comment
NF2				
Allele 1	PV1	PV1	PV1	Shared *NF2* pathogenic variant
Allele 2	WT	LOH or *NF2* or PV2	LOH or *NF2* or PV3	Tumor-specific partial loss of 22q in trans position or a different *NF2* somatic second PV in every anatomically unrelated tumor"(Plotkin et al., 2022)

Diagnostic criteria for *SMARCB1*- and *LZTR1*-related schwannomatosis

"A diagnosis of *SMARCB1*- or *LZTR1*-related schwannomatosis can be made when an individual meets 1 of the following criteria:

- At least 1 pathologically confirmed schwannoma or hybrid nerve sheath tumor and a *SMARCB1* (or *LZTR1*) pathogenic variant in an unaffected tissue such as blood
- A shared *SMARCB1* or *LZTR1* pathogenic variant in 2 schwannomas or hybrid nerve sheath tumors" (Plotkin et al., 2022).

"Pattern of genetic changes in unaffected and tumor tissue in *SMARCB1*- and *LZTR1*-related schwannomatosis"

Gene locus	Unaffected Tissue	Tumor 1	Tumor 2	Comment
SMARCB1/LZTR1				
Allele 1	PV1	PV1	PV1	Shared *SMARCB1* or *LZTR1* pathogenic variant
Allele 2	WT	LOH	LOH	Tumor-specific partial loss of 22q in trans position, LOH typically entails deletion of 22q region encompassing *LZTR1/SMARCB1/NF2*"(Plotkin et al., 2022).

Gene locus	Unaffected Tissue	Tumor 1	Tumor 2	Comment
NF2				
Allele 1	WT	PV2	PV3	Tumor-specific pathogenic *NF2* variant in cis to pathogenic *SMARCB1* variant
Allele 2	WT	LOH	LOH	Tumor-specific partial loss of 22q in trans position, LOH typically entails deletion of 22q region encompassing *LZTR1/SMARCB1/NF2*"(Plotkin et al., 2022).

Diagnostic criteria for 22q-related schwannomatosis

"A diagnosis of 22q-related schwannomatosis can be made when an individual does not meet criteria for *NF2*-related schwannomatosis, *SMARCB1*-related schwannomatosis, or *LTZR1*-related schwannomatosis, does not have a germline *DGCR8* pathogenic variant, and has both of the following molecular features:

- LOH of the same chromosome 22q markers in 2 anatomically distinct schwannomas or hybrid nerve sheath tumors and
- A different *NF2* pathogenic variant in each tumor, which cannot be detected in unaffected tissue" (Plotkin et al., 2022).

"Pattern of genetic changes in unaffected and tumor tissue in 22q-related schwannomatosis"

Gene locus	Unaffected Tissue	Tumor 1	Tumor 2	Comment
SMARCB1/ LZTR1				
Allele 1	WT	None found	None found	No shared pathogenic *LZTR1* or *SMARCB1* variant
Allele 2	WT	LOH	LOH	Tumor-specific partial loss of the same chromosome 22q, LOH typically entails deletion of 22q region encompassing *LZTR1/SMARCB1/NF2"*(Plotkin et al., 2022)"

Gene locus	Unaffected Tissue	Tumor 1	Tumor 2	Comment
NF2				
Allele 1	WT	PV1	PV2	Tumor-specific pathogenic *NF2* variant trans to the 22q deletion
Allele 2	WT	LOH	LOH	Tumor-specific partial loss of the same chromosome 22q, LOH typically entails deletion of 22q region encompassing *LZTR1/SMARCB1/NF2"*(Plotkin et al., 2022)"

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-

database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81292	MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis
81293	MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; known familial variants
81294	MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; duplication/deletion variants
81295	MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis
81296	MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; known familial variants
81297	MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; duplication/deletion variants
81298	MSH6 (mutS homolog 6 [E. coli]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis
81299	MSH6 (mutS homolog 6 [E. coli]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; known familial variants
81300	MSH6 (mutS homolog 6 [E. coli]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; duplication/deletion variants
81301	Microsatellite instability analysis (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) of markers for mismatch repair deficiency (eg,

	BAT25, BAT26), includes comparison of neoplastic and normal tissue, if performed
81317	PMS2 (postmeiotic segregation increased 2 [S. cerevisiae]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis
81318	PMS2 (postmeiotic segregation increased 2 [S. cerevisiae]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; known familial variants
81319	PMS2 (postmeiotic segregation increased 2 [S. cerevisiae]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; duplication/deletion variants
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons, cytogenomic array analysis for neoplasia)
81408	Molecular pathology procedure, Level 9 (eg, analysis of >50 exons in a single gene by DNA sequence analysis)
81479	Unlisted molecular pathology procedure

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Baser, M. E., Friedman, J. M., Aeschliman, D., Joe, H., Wallace, A. J., Ramsden, R. T., & Evans, D. G. (2002). Predictors of the risk of mortality in neurofibromatosis 2. *Am J Hum Genet*, *71*(4), 715-723. https://doi.org/10.1086/342716

Baser, M. E., Kuramoto, L., Joe, H., Friedman, J. M., Wallace, A. J., Gillespie, J. E., Ramsden, R. T., & Evans, D. G. (2004). Genotype-phenotype correlations for nervous system tumors in neurofibromatosis 2: a population-based study. *Am J Hum Genet*, *75*(2), 231-239. https://doi.org/10.1086/422700

Baser, M. E., Kuramoto, L., Woods, R., Joe, H., Friedman, J. M., Wallace, A. J., Ramsden, R. T., Olschwang, S., Bijlsma, E., Kalamarides, M., Papi, L., Kato, R., Carroll, J., Lazaro, C., Joncourt, F., Parry, D. M., Rouleau, G. A., & Evans, D. G. (2005). The location of constitutional neurofibromatosis 2 (NF2) splice site mutations is associated with the severity of NF2. *J Med Genet*, *42*(7), 540-546. https://doi.org/10.1136/jmg.2004.029504

Bergner, A., & Yohay, K. (2024, January 19). *Schwannomatosis*. UpToDate. https://www.uptodate.com/contents/schwannomatoses-related-to-genetic-variants-other-than-nf2

Burkitt-Wright, E. M. M., Sach, E., Sharif, S., Quarrell, O., Carroll, T., Whitehouse, R. W., Upadhyaya, M., Huson, S. M., & Evans, D. G. (2013). Can the diagnosis of NF1 be excluded clinically? A lack of pigmentary findings in families with spinal neurofibromatosis demonstrates a limitation of clinical diagnosis. *J Med Genet*, *50*(9), 606-613. https://doi.org/10.1136/jmedgenet-2013-101648

Castellanos, E., Plana, A., Carrato, C., Carrio, M., Rosas, I., Amilibia, E., Roca-Ribas, F., Hostalot, C., Castillo, A., Ros, A., Quer, A., Becerra, J. L., Salvador, H., Lazaro, C., Blanco, I., Serra, E., & Bielsa, I. (2018). Early Genetic Diagnosis of Neurofibromatosis Type 2 From Skin Plaque Plexiform Schwannomas in Childhood. *JAMA Dermatol*, *154*(3), 341-346. https://doi.org/10.1001/jamadermatol.2017.5464

Castellanos, E., Rosas, I., Negro, A., Gel, B., Alibes, A., Baena, N., Pineda, M., Pi, G., Pintos, G., Salvador, H., Lazaro, C., Blanco, I., Vilageliu, L., Brems, H., Grinberg, D., Legius, E., & Serra, E. (2020). Mutational spectrum by phenotype: panel-based NGS testing of patients with clinical suspicion of RASopathy and children with multiple cafe-au-lait macules. *Clin Genet*, *97*(2), 264-275. https://doi.org/10.1111/cge.13649

DeBella, K., Szudek, J., & Friedman, J. M. (2000). Use of the national institutes of health criteria for diagnosis of neurofibromatosis 1 in children. *Pediatrics*, *105*(3 Pt 1), 608-614. https://pubmed.ncbi.nlm.nih.gov/10699117/

Dhamija, R., Plotkin, S., Asthagiri, A., Messiaen, L., & Babovic-Vuksanovic, D. (2018). Schwannomatosis [Text]. *Genereviews*. https://doi.org/https://www.ncbi.nlm.nih.gov/books/NBK487394/

Elmas, M. (2022). The road from mutation to next generation phenotyping: contribution of deep learning technology (Face2Gene) to diagnosis neurofibromatosis type 1. https://dergipark.org.tr/en/pub/eurj/issue/68266/894631#article_cite

Evans, D. G. (2023a). *Neurofibromatosis 2*. https://www.ncbi.nlm.nih.gov/books/NBK1201/

Evans, D. G. (2023b, November 2). *NF2-related schwannomatosis (formerly neurofibromatosis type 2)*. UpToDate. https://www.uptodate.com/contents/nf2-related-schwannomatosis-formerly-neurofibromatosis-type-2

Evans, D. G., Freeman, S., Gokhale, C., Wallace, A., Lloyd, S. K., Axon, P., Ward, C. L., Rutherford, S., King, A., Huson, S. M., & Ramsden, R. T. (2015). Bilateral vestibular schwannomas in older patients: NF2 or chance? *J Med Genet*, *52*(6), 422-424. https://doi.org/10.1136/jmedgenet-2014-102973

Evans, D. G., Howard, E., Giblin, C., Clancy, T., Spencer, H., Huson, S. M., & Lalloo, F. (2010). Birth incidence and prevalence of tumor-prone syndromes: estimates from a UK family genetic register service. *Am J Med Genet A*, *152a*(2), 327-332. https://doi.org/10.1002/ajmg.a.33139

Evans, D. G., Raymond, F. L., Barwell, J. G., & Halliday, D. (2012). Genetic testing and screening of individuals at risk of NF2. *Clin Genet*, *82*(5), 416-424. https://doi.org/10.1111/j.1399-0004.2011.01816.x

Evans, D. G. R., Salvador, H., Chang, V. Y., Erez, A., Voss, S. D., Druker, H., Scott, H. S., & Tabori, U. (2017). Cancer and Central Nervous System Tumor Surveillance in Pediatric Neurofibromatosis 2 and Related Disorders. *Clin Cancer Res*, *23*(12), e54-e61. https://doi.org/10.1158/1078-0432.Ccr-17-0590

Evans, D. G. R., Salvador, H., Chang, V. Y., Erez, A., Voss, S. D., Schneider, K. W., Scott, H. S., Plon, S. E., & Tabori, U. (2017). Cancer and Central Nervous System Tumor Surveillance in Pediatric Neurofibromatosis 1. *Clinical Cancer Research*, *23*(12), e46. https://doi.org/10.1158/1078-0432.CCR-17-0589

Ferner, R. E., Huson, S. M., Thomas, N., Moss, C., Willshaw, H., Evans, D. G., Upadhyaya, M., Towers, R., Gleeson, M., Steiger, C., & Kirby, A. (2007). Guidelines for the diagnosis and management of individuals with neurofibromatosis 1. *J Med Genet*, *44*(2), 81-88. https://doi.org/10.1136/jmg.2006.045906

Fisher, M. J., Belzberg, A. J., de Blank, P., De Raedt, T., Elefteriou, F., Ferner, R. E., Giovannini, M., Harris, G. J., Kalamarides, M., Karajannis, M. A., Kim, A., Lazaro, C., Le, L. Q., Li, W., Listernick, R., Martin, S., Morrison, H., Pasmant, E., Ratner, N., . . . Serra, E. (2018). 2016 Children's Tumor Foundation conference on neurofibromatosis type 1, neurofibromatosis type 2, and schwannomatosis. *Am J Med Genet A*, *176*(5), 1258-1269. https://doi.org/10.1002/ajmg.a.38675

Friedman, J. M. (2023). *Neurofibromatosis 1*. https://www.ncbi.nlm.nih.gov/books/NBK1109/

Giugliano, T., Santoro, C., Torella, A., Del Vecchio Blanco, F., Grandone, A., Onore, M. E., Melone, M. A. B., Straccia, G., Melis, D., Piccolo, V., Limongelli, G., Buono, S., Perrotta, S., Nigro, V., & Piluso, G. (2019). Clinical and Genetic Findings in Children with Neurofibromatosis Type 1, Legius Syndrome, and Other Related Neurocutaneous Disorders. *Genes*, *10*(8), 580. https://doi.org/10.3390/genes10080580

Goldbrunner, R., Weller, M., Regis, J., Lund-Johansen, M., Stavrinou, P., Reuss, D., Evans, D. G., Lefranc, F., Sallabanda, K., Falini, A., Axon, P., Sterkers, O., Fariselli, L., Wick, W., & Tonn, J. C. (2020). EANO guideline on the diagnosis and treatment of vestibular schwannoma. *Neuro Oncol*, *22*(1), 31-45. https://doi.org/10.1093/neuonc/noz153

Halliday, D., Emmanouil, B., Pretorius, P., MacKeith, S., Painter, S., Tomkins, H., Evans, D. G., & Parry, A. (2017). Genetic Severity Score predicts clinical phenotype in NF2. *J Med Genet*, *54*(10), 657-664. https://doi.org/10.1136/jmedgenet-2017-104519

Hersh, J. H. (2008). Health supervision for children with neurofibromatosis. *Pediatrics*, *121*(3), 633-642. https://doi.org/10.1542/peds.2007-3364

Hexter, A., Jones, A., Joe, H., Heap, L., Smith, M. J., Wallace, A. J., Halliday, D., Parry, A., Taylor, A., Raymond, L., Shaw, A., Afridi, S., Obholzer, R., Axon, P., King, A. T., Friedman, J. M., & Evans, D. G. (2015). Clinical and molecular predictors of mortality

in neurofibromatosis 2: a UK national analysis of 1192 patients. *J Med Genet*, *52*(10), 699-705. https://doi.org/10.1136/jmedgenet-2015-103290

Hirbe, A. C., & Gutmann, D. H. (2014). Neurofibromatosis type 1: a multidisciplinary approach to care. *Lancet Neurol*, *13*(8), 834-843. https://doi.org/10.1016/s1474-4422(14)70063-8

Hizuka, K., Hagiwara, S. I., Maeyama, T., Honma, H., Kawai, M., Akagi, K., Yasuhara, M., Tomita, N., & Etani, Y. (2021). Constitutional mismatch repair deficiency in childhood colorectal cancer harboring a de novo variant in the MSH6 gene: a case report. *BMC Gastroenterol*, *21*(1), 60. https://doi.org/10.1186/s12876-021-01646-3

Hutter, S., Piro, R. M., Reuss, D. E., Hovestadt, V., Sahm, F., Farschtschi, S., Kehrer-Sawatzki, H., Wolf, S., Lichter, P., von Deimling, A., Schuhmann, M. U., Pfister, S. M., Jones, D. T., & Mautner, V. F. (2014). Whole exome sequencing reveals that the majority of schwannomatosis cases remain unexplained after excluding SMARCB1 and LZTR1 germline variants. *Acta Neuropathol*, *128*(3), 449-452. https://doi.org/10.1007/s00401-014-1311-1

Kehrer-Sawatzki, H., Farschtschi, S., Mautner, V. F., & Cooper, D. N. (2017). The molecular pathogenesis of schwannomatosis, a paradigm for the co-involvement of multiple tumour suppressor genes in tumorigenesis. *Hum Genet*, *136*(2), 129-148. https://doi.org/10.1007/s00439-016-1753-8

Koontz, N. A., Wiens, A. L., Agarwal, A., Hingtgen, C. M., Emerson, R. E., & Mosier, K. M. (2013). Schwannomatosis: the overlooked neurofibromatosis? *AJR Am J Roentgenol*, *200*(6), W646-653. https://doi.org/10.2214/ajr.12.8577

Korf, B. R., Lobbous, M., & Metrock, L. K. (2024, May 13, 2024). *Neurofibromatosis type 1 (NF1): Pathogenesis, clinical features, and diagnosis*. UpToDate. https://www.uptodate.com/contents/neurofibromatosis-type-1-nf1-pathogenesis-clinical-features-and-diagnosis

Legius, E., Messiaen, L., Wolkenstein, P., Pancza, P., Avery, R. A., Berman, Y., Blakeley, J., Babovic-Vuksanovic, D., Cunha, K. S., Ferner, R., Fisher, M. J., Friedman, J. M., Gutmann, D. H., Kehrer-Sawatzki, H., Korf, B. R., Mautner, V.-F., Peltonen, S., Rauen, K. A., Riccardi, V., . . . International Consensus Group on Neurofibromatosis Diagnostic, C. (2021). Revised diagnostic criteria for neurofibromatosis type 1 and Legius syndrome: an international consensus recommendation. *Genetics in Medicine*, *23*(8), 1506-1513. https://doi.org/10.1038/s41436-021-01170-5

Louvrier, C., Pasmant, E., Briand-Suleau, A., Cohen, J., Nitschke, P., Nectoux, J., Orhant, L., Zordan, C., Goizet, C., Goutagny, S., Lallemand, D., Vidaud, M., Vidaud, D., Kalamarides, M., & Parfait, B. (2018). Targeted next-generation sequencing for differential diagnosis of neurofibromatosis type 2, schwannomatosis, and meningiomatosis. *Neuro Oncol*, *20*(7), 917-929. https://doi.org/10.1093/neuonc/noy009

Lu, V. M., Ravindran, K., Graffeo, C. S., Perry, A., Van Gompel, J. J., Daniels, D. J., & Link, M. J. (2019). Efficacy and safety of bevacizumab for vestibular schwannoma in neurofibromatosis type 2: a systematic review and meta-analysis of treatment outcomes. *J Neurooncol*, *144*(2), 239-248. https://doi.org/10.1007/s11060-019-03234-8

MacCollin, M., Chiocca, E. A., Evans, D. G., Friedman, J. M., Horvitz, R., Jaramillo, D., Lev, M., Mautner, V. F., Niimura, M., Plotkin, S. R., Sang, C. N., Stemmer-Rachamimov, A., & Roach, E. S. (2005). Diagnostic criteria for schwannomatosis. *Neurology*, *64*(11), 1838-1845. https://doi.org/10.1212/01.wnl.0000163982.78900.ad

Merker, V. L., Esparza, S., Smith, M. J., Stemmer-Rachamimov, A., & Plotkin, S. R. (2012). Clinical features of schwannomatosis: a retrospective analysis of 87 patients. *Oncologist*, *17*(10), 1317-1322. https://doi.org/10.1634/theoncologist.2012-0162

Miller, D. T., Freedenberg, D., Schorry, E., Ullrich, N. J., Viskochil, D., & Korf, B. R. (2019). Health Supervision for Children With Neurofibromatosis Type 1. *Pediatrics*, *143*(5), e20190660. https://doi.org/10.1542/peds.2019-0660

Miller, D. T., Lee, K., Chung, W. K., Gordon, A. S., Herman, G. E., Klein, T. E., Stewart, D. R., Amendola, L. M., Adelman, K., Bale, S. J., Gollob, M. H., Harrison, S. M., Hershberger, R. E., McKelvey, K., Richards, C. S., Vlangos, C. N., Watson, M. S., & Martin, C. L. (2021). ACMG SF v3.0 list for reporting of secondary findings in clinical exome and genome sequencing: a policy statement of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *23*(8), 1381-1390. https://doi.org/10.1038/s41436-021-01172-3

NCCN. (2023). *Genetic/Familial High-Risk Assessment: Colorectal Version 2.2023 — October 30, 2023*. https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf

NIH. (1988). National Institutes of Health Consensus Development Conference Statement: neurofibromatosis. Bethesda, Md., USA, July 13-15, 1987. *Neurofibromatosis*, *1*(3), 172-178. https://consensus.nih.gov/1987/1987Neurofibramatosis064html.htm

Pathmanaban, O. N., Sadler, K. V., Kamaly-Asl, I. D., King, A. T., Rutherford, S. A., Hammerbeck-Ward, C., McCabe, M. G., Kilday, J. P., Beetz, C., Poplawski, N. K., Evans, D. G., & Smith, M. J. (2017). Association of Genetic Predisposition With Solitary Schwannoma or Meningioma in Children and Young Adults. *JAMA Neurol*, *74*(9), 1123-1129. https://doi.org/10.1001/jamaneurol.2017.1406

Piotrowski, A., Koczkowska, M., Poplawski, A. B., Bartoszewski, R., Króliczewski, J., Mieczkowska, A., Gomes, A., Crowley, M. R., Crossman, D. K., Chen, Y., Lao, P., Serra, E., Llach, M. C., Castellanos, E., & Messiaen, L. M. (2022). Targeted massively parallel sequencing of candidate regions on chromosome 22q predisposing to multiple schwannomas: An analysis of 51 individuals in a single-center experience. *Human Mutation*, *43*(1), 74-84. https://doi.org/10.1002/humu.24294

Plotkin, S. R., Blakeley, J. O., Evans, D. G., Hanemann, C. O., Hulsebos, T. J., Hunter-Schaedle, K., Kalpana, G. V., Korf, B., Messiaen, L., Papi, L., Ratner, N., Sherman, L. S., Smith, M. J., Stemmer-Rachamimov, A. O., Vitte, J., & Giovannini, M. (2013). Update From the 2011 International Schwannomatosis Workshop: From Genetics to Diagnostic Criteria. *Am J Med Genet A*, *0*(3), 405-416. https://doi.org/10.1002/ajmg.a.35760

Plotkin, S. R., Messiaen, L., Legius, E., Pancza, P., Avery, R. A., Blakeley, J. O., Babovic-Vuksanovic, D., Ferner, R., Fisher, M. J., Friedman, J. M., Giovannini, M., Gutmann, D. H., Hanemann, C. O., Kalamarides, M., Kehrer-Sawatzki, H., Korf, B. R., Mautner, V.-F., MacCollin, M., Papi, L., . . . Evans, D. G. (2022). Updated diagnostic criteria and nomenclature for neurofibromatosis type 2 and schwannomatosis: An international consensus recommendation. *Genetics in Medicine*, *24*(9), 1967-1977. https://doi.org/10.1016/j.gim.2022.05.007

Radhika Dhamija, M., Scott Plotkin, MD, PhD, Ashok Asthagiri, MD, Ludwine Messiaen, PhD, and Dusica Babovic-Vuksanovic, MD. (2023). *Schwannomatosis*. https://www.ncbi.nlm.nih.gov/books/NBK487394/

Ruggieri, M., Iannetti, P., Polizzi, A., La Mantia, I., Spalice, A., Giliberto, O., Platania, N., Gabriele, A. L., Albanese, V., & Pavone, L. (2005). Earliest clinical manifestations and natural history of neurofibromatosis type 2 (NF2) in childhood: a study of 24 patients. *Neuropediatrics*, *36*(1), 21-34. https://doi.org/10.1055/s-2005-837581

Sadler, K. V., Bowers, N. L., Hartley, C., Smith, P. T., Tobi, S., Wallace, A. J., King, A., Lloyd, S. K. W., Rutherford, S., Pathmanaban, O. N., Hammerbeck-Ward, C., Freeman, S., Stapleton, E., Taylor, A., Shaw, A., Halliday, D., Smith, M. J., & Evans, D. G. (2021). Sporadic vestibular schwannoma: a molecular testing summary. *Journal of Medical Genetics*, *58*(4), 227-233. https://doi.org/10.1136/jmedgenet-2020-107022

Shofty, B., Constantini, S., & Ben-Shachar, S. (2015). Advances in Molecular Diagnosis of Neurofibromatosis Type 1. *Semin Pediatr Neurol*, *22*(4), 234-239. https://doi.org/10.1016/j.spen.2015.10.007

Smith, M. J., Bowers, N. L., Bulman, M., Gokhale, C., Wallace, A. J., King, A. T., Lloyd, S. K., Rutherford, S. A., Hammerbeck-Ward, C. L., Freeman, S. R., & Evans, D. G. (2017). Revisiting neurofibromatosis type 2 diagnostic criteria to exclude LZTR1-related schwannomatosis. *Neurology*, *88*(1), 87-92. https://doi.org/10.1212/wnl.0000000000003418

Stephens, K., Kayes, L., Riccardi, V. M., Rising, M., Sybert, V. P., & Pagon, R. A. (1992). Preferential mutation of the neurofibromatosis type 1 gene in paternally derived chromosomes. *Hum Genet*, *88*(3), 279-282. https://link.springer.com/article/10.1007/BF00197259

Stewart, D. R., Korf, B. R., Nathanson, K. L., Stevenson, D. A., & Yohay, K. (2018). Care of adults with neurofibromatosis type 1: a clinical practice resource of the American

College of Medical Genetics and Genomics (ACMG). *Genet Med*, *20*(7), 671-682. https://doi.org/10.1038/gim.2018.28

Suerink, M., Ripperger, T., Messiaen, L., Menko, F. H., Bourdeaut, F., Colas, C., Jongmans, M., Goldberg, Y., Nielsen, M., Muleris, M., van Kouwen, M., Slavc, I., Kratz, C., Vasen, H. F., Brugières, L., Legius, E., & Wimmer, K. (2019). Constitutional mismatch repair deficiency as a differential diagnosis of neurofibromatosis type 1: consensus guidelines for testing a child without malignancy. *Journal of Medical Genetics*, *56*(2), 53. https://doi.org/10.1136/jmedgenet-2018-105664

Terzi, Y. K., Oguzkan-Balci, S., Anlar, B., Aysun, S., Guran, S., & Ayter, S. (2009). Reproductive decisions after prenatal diagnosis in neurofibromatosis type 1: importance of genetic counseling. *Genet Couns*, *20*(2), 195-202.

Wimmer, Kratz, C. P., Vasen, H. F., Caron, O., Colas, C., Entz-Werle, N., Gerdes, A. M., Goldberg, Y., Ilencikova, D., Muleris, M., Duval, A., Lavoine, N., Ruiz-Ponte, C., Slavc, I., Burkhardt, B., & Brugieres, L. (2014). Diagnostic criteria for constitutional mismatch repair deficiency syndrome: suggestions of the European consortium 'care for CMMRD' (C4CMMRD). *J Med Genet*, *51*(6), 355-365. https://doi.org/10.1136/jmedgenet-2014-102284

Wimmer, K., Kratz, C. P., Vasen, H. F. A., Caron, O., Colas, C., Entz-Werle, N., Gerdes, A.-M., Goldberg, Y., Ilencikova, D., Muleris, M., Duval, A., Lavoine, N., Ruiz-Ponte, C., Slavc, I., Burkhardt, B., & Brugieres, L. (2014). Diagnostic criteria for constitutional mismatch repair deficiency syndrome: suggestions of the European consortium ‘Care for CMMRD’ (C4CMMRD). *Journal of Medical Genetics*, *51*(6), 355. https://doi.org/10.1136/jmedgenet-2014-102284

Witkowski, L., Dillon, M. W., Murphy, E., S Lebo, M., & Mason-Suares, H. (2020). Expanding the Noonan spectrum/RASopathy NGS panel: Benefits of adding NF1 and SPRED1. *Molecular Genetics & Genomic Medicine*, *8*(4), e1180. https://doi.org/10.1002/mgg3.1180

Genetic Testing for Ophthalmologic Conditions

Policy Number: AHS – M2083 – Genetic Testing for Ophthalmologic Conditions	Prior Policy Name and Number, as applicable: • AHS – M2083 – Genetic Testing for Macular Degeneration
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Genetic eye diseases involve every part of the eye, including the visual system and ocular adnexa (accessory structures attached to the eye, such as the eyelids, extraocular muscles and orbits); conditions within this group of disorders may be rare or common, and they may exhibit a significant impact on vision or may not affect eyesight at all (Lee & Couser, 2016). Many genes involved in ophthalmologic disorders are now mapped and due to this, scientists have developed a greater understanding of how these genes influence vision and eye health (Singh & Tyagi, 2018).

II. Related Policies

Policy Number	Policy Title
AHS-M2144	Genetic Testing for Connective Tissue Disorders

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with clinical signs of an inherited retinal degeneration (see Note 1), single gene or multi-gene panel testing **MEETS COVERAGE CRITERIA.**

2) For individuals with clinical findings suggestive of other ophthalmologic disorders with a known causative gene(s) where identification of a genetic variant will affect clinical management, testing of the known causative gene(s) **MEETS COVERAGE CRITERIA.**

3) For individuals with retinal dystrophy, genetic testing of *RPE65* prior to treatment with Luxturna (voretigene neparvovec-rzyl) **MEETS COVERAGE CRITERIA** and **is required.**

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

4) Genetic testing for age-related macular degeneration **DOES NOT MEET COVERAGE CRITERIA**.

5) For individuals with ophthalmologic conditions, whole exome sequencing (WES) **and/or** whole genome sequencing (WGS) **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: The American Academy of Ophthalmology recommends genetic diagnostic testing for the four major types of inherited retinal degenerations (IRDs):

- Rod-cone degenerations (e.g., retinitis pigmentosa)
- Cone-rod degenerations (e.g., achromatopsia)
- Chorioretinal degenerations (e.g., CHM-associated retinal degeneration [choroideremia])
- Inherited dystrophies that involve the macula (e.g., *ABCA4*-associated macular degeneration [Stargardt disease])

Note 2: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
AAO	American Academy of Ophthalmology
ABC1	*ATP binding cassette 1*
ABCA4	*ATP binding cassette subfamily A member 4*
ABCG1	*ATP binding cassette subfamily G member 1*
AGBL5	*AGBL Carboxypeptidase 5 gene*

AMD	Age-related macular degeneration
Anti-VEGF	Anti-vascular endothelial growth factor
AOA	American Optometric Association
APE1	*Apurinic/apyrimidinic endonuclease 1 gene*
AREDS	Age-Related Eye Disease Study
ARMS2	*Age-related maculopathy susceptibility 2 gene*
HTRA1	*HtrA Serine Peptidase 1 gene*
ASRS	American Society of Retina Specialists
BAP1	*BRCA1 associated protein 1 gene*
C2	*Complement C2 gene*
C3	*Complement C3 gene*
CALM2	*Calmodulin 2*
CAV1/2	Calcium channel, voltage-dependent, L type, alpha 1C subunit
CBS	*Cystathionine beta-synthase*
CDKN2A	*Cyclin dependent kinase inhibitor 2A gene*
CETP	*Cholesteryl ester transfer protein gene*
CF	Complement factor
CFB	Complement factor B
CFH	Complement factor H
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
C-MET	Tyrosine-protein kinase met
CMS	Centers for Medicare and Medicaid
CNGA1	*Cyclic nucleotide gated channel subunit alpha 1 gene*
COL8A1	*Collagen type VIII alpha 1 chain gene*
CRYAA	*Crystallin alpha A gene*
CRYBB2	*Crystallin beta B2 gene*
Cx50/GJA3 & 8	Connexin α8 (GJA8 or Cx50) and connexin α3
CYP1B1	*Cytochrome P450 family 1 subfamily B member 1 gene*
CYP2C19	Cytochrome P450 2C19
CYP51A1	*Cytochrome P450 family 51 subfamily a member 1 gene*
DDEF1	Development and differentiation enhancing factor 1
DNA	Deoxyribose nucleic acid
EIF1AX	*Eukaryotic translation initiation factor 1A X-Linked gene*
EPHA2	*Ephrin type-A receptor 2 gene*
ERN-EYE	European Reference Network for Rare Eye Diseases
EURETINA	European Society of Retina Specialists
FBN1	*Fibrillin 1 gene*
FDA	Food and Drug Administration

FGD6	*FYVE, rhoGEF and ph domain containing 6 gene*
FOXC1	*Forkhead box C1 gene*
GEMIN4	*Gem nuclear organelle associated protein 4 gene*
GNA11	*G protein subunit alpha 11 gene*
GNAQ	*G protein subunit alpha q gene*
HERC2	*HECT and RLD domain containing E3 ubiquitin protein ligase 2 gene*
HGF	*Hepatocyte growth factor gene*
HK1	*Hexokinase 1 gene*
IGF-1	*Insulin-like growth factor 1 gene*
IL-8	*Interleukin 8 gene*
IRDs	Inherited retinal degenerations
LCD	Local Coverage Determination
LDT	Laboratory-developed test
LOX1	*Lectin-type oxidized LDL receptor 1 gene*
LTBP2	*Latent transforming growth factor beta binding protein 2 gene*
MIP	*Major intrinsic protein of lens fiber gene*
MMP-1/2	*Matrix metalloproteinases 1/2 gene*
MMP-9	*Matrix metalloproteinase 9 gene*
MPP-7	*Membrane protein, palmitoylated 7*
MTHFR	*Methylenetetrahydrofolate reductase gene*
MTR	*5-Methyltetrahydrofolate-homocysteine methyltransferase*
MTRR	*5-Methyltetrahydrofolate-homocysteine methyltransferase reductase*
MVL	Molecular vision tests
MYOC	*Myocilin gene*
NAMD	Neovascular age-related macular degeneration
NGS	Next-gene sequencing
NOS2A	*Nitric oxide synthase 2A gene*
OCA2	*Oculocutaneous Albinism type 2 gene*
OPA1	*Optic atrophy 1 gene*
P14arf	ARF tumor suppressor
P4HA2	*Prolyl 4-Hydroxylase Subunit Alpha 2 gene*
PAX6	*Paired box 6 gene*
PCV	Polypoidal choroidal vasculopathy
PDE6A	*Phosphodiesterase 6A gene*
PDE6B	*Phosphodiesterase 6B gene*
PEDF	*Pigment epithelium-derived factor gene*
PITX2	*Paired like homeodomain 2 gene*
POLR3B	*Ribonucleic acid polymerase III subunit b gene*
PPFIA2	*PTPRF interacting protein alpha 2 gene*

PRPF3	*Pre-MRNA processing factor 3 gene*
PRPF31	*Pre-MRNA processing factor 31 gene*
PRPH2	*Peripherin 2*
PRX	*Periaxin gene*
PTEN	*Phosphatase and tensin homolog gene*
PTPRR	*Protein tyrosine phosphatase receptor type r gene*
RAD51B	*RAD51 paralog b gene*
RDH12	*Retinol dehydrogenase 12 gene*
RED	Rare eye diseases
RHO	*Rhodopsin gene*
RIC1	*RIC1 homolog, RAB6A GEF complex partner 1 gene*
RP	Retinitis pigmentosa
RP1	*RP1 axonemal microtubule associated gene*
RP2	*RP2 activator of ARL3 GTPase gene*
RPE65	*Retinal pigment epithelium-specific 65 gene*
RPGR	*Retinitis pigmentosa GTPase regulator gene*
SERPING1	*Serpin family g member 1 gene*
SF3B1	*Splicing factor 3b subunit 1 gene*
SLC16A8	*Solute carrier family 16 member 8 gene*
Snps	Single nucleotide polymorphisms
STGD	Stargardt Disease
TAF1A	*TATA-Box Binding Protein Associated Factor, RNA Polymerase I Subunit A gene*
TAPT1	*Transmembrane Anterior Posterior Transformation 1 gene*
TEK	*Tyrosine, kinase, endothelial gene*
TGFBR2	*Transforming growth factor beta receptor 2 gene*
TIE2	*TEK receptor tyrosine kinase gene*
TIMP3	*Tissue inhibitor of metalloproteinase 3 gene*
UMODL1	*Uromodulin Like 1 gene*
USH2A	*Usherin gene*
VEGF-A	*Vascular endothelial growth factor A gene*
VEGFR-2	*Vascular endothelial growth factor receptor 2 gene*
WDR87	*WD Repeat Domain 87 gene*
WES	Whole exome sequencing
WGS	Whole genome sequencing
XRCC1	*X-ray repair cross complementing 1 gene*
ZNF350	*Zinc finger protein 350 gene*

V. Scientific Background

Approximately 4,000 diseases or syndromes affect humans, and nearly one-third of these diseases are related to the eyes (Singh & Tyagi, 2018). Several ophthalmologic disorders may be inherited, including age-related macular degeneration, cataracts, glaucoma, inherited optic neuropathies, retinitis pigmentosa and Stargardt's disease (Singh & Tyagi, 2018). Early diagnoses, knowledge of family history and genetic testing can positively influence outcomes and treatment regimens. Inherited retinal diseases (IRDs) affect one in 1380 individuals; it is estimated 36% of healthy people could be considered carriers of at least one IRD-related mutation (Hanany et al., 2020).

Genetic testing for eye disorders is growing in popularity. Further, there is considerable overlap between the clinical phenotypes of many eye disorders, highlighting the importance of genetic testing to determine the cause and most effective treatment avenue (Sangermanoa et al., 2020). To date, genetic tests can identify dozens of ophthalmologic conditions (Stone et al., 2014), and panel tests are already used clinically for early-onset glaucoma, retinal dystrophies, inherited optic neuropathies and more (Wiggs, 2017). Further, many genes have been linked to various human eye diseases and disorders. Table 1 below, adapted from Singh and Tyagi (2018), lists genes and gene variants associated with ten different ophthalmologic conditions. However, it's also important to recognize that there is a broad clinical spectrum of disorders and many involved genes in IRD-related disorders. Over 270 genes have currently been associated with IRD and the number of genes and heterogeneity of disease is compounded by variations in familial inheritance patterns (García Bohórquez et al., 2021).

Ocular gene therapy shows promise for both inherited and acquired retinal pathologies. Adeno-associated viruses (AAVs) are the most common and leading platform used in retinal gene therapy. These vectors deliver gene-specific approaches to promote expression of a healthy copy of a disease-causing gene (Michalakis et al., 2021). A combination of factors has led to the adeno-associated virus method as the primary vector option for IRDs. First, AAVs have smaller risks of mutagenesis because they aren't integrated into the host genome. Second, they have low pathogenicity. Lastly, they can transfer genetic material to multiple retinal cell types (Avalyon, 2021).

Recent advancements in AAV ocular gene therapy have been effective in treating certain types of ophthalmologic conditions. For example, Luxturna – the first Food and Drug Administration approved ocular therapy – is a prescription gene therapy product used to treat patients with inherited retinal degenerations (IRDs) due to mutations in the *RPE65* (retinal pigment epithelium-specific 65) gene; however, genetic testing must first be used to determine a potential mutation in this gene (Luxturna, 2022). Therefore,

accurate genetic diagnoses have become imperative for some ophthalmologic treatments.

Other retinal conditions such as choroideremia, achromatopsia, X-linked retinitis pigmentosa, X-linked retinoschisis and AMD are among those being investigated as potential targets for gene therapies using AAVs. In addition, additional viral vectors and non-viral platforms are in the process of consideration because AAVs are limited in the amount of genetic information they can carry, that is, they cannot carry large therapeutic gene sets. For example, larger gene targets (such as the gene associated with Stargardt disease) present a barrier to AAV-specific gene therapy (Avalyon, 2021).

Table 1: Genes/gene variants linked with common human eye diseases/disorders (Singh & Tyagi, 2018)

Disease	Gene/variant	Age of disease or disorder onset
AMD (age-related macular degeneration)	*NOS2A, CFH, CF, C2, C3, CFB, HTRA1/LOC, MMP-9, TIMP-3, SLC16A8*, etc.	Old
Cataract	*GEMIN4, CYP51A1, RIC1, TAPT1, TAF1A, WDR87, APE1, MIP, Cx50/GJA3 & 8, CRYAA, CRYBB2, PRX, POLR3B, XRCC1, ZNF350, EPHA2,* etc.	Old
Glaucoma	*CALM2, MPP-7, Optineurin, LOX1, CYP1B1, CAV1/2, MYOC, PITX2, FOXC1, PAX6, CYP1B1, LTBP2*, etc.	Over 40 except congenital form that can affect an infant
Inherited optic neuropathies	*Complex I* or *ND* genes, *OPA1, RPE65*, etc.	Young males
Marfan syndrome	*FBN1, TGFBR2, MTHFR, MTR, MTRR*, etc.	Born with disorder but may not be diagnosed until later in life

Myopia	*HGF, C-MET, UMODL1, MMP-1/2, PAX6, CBS, MTHFR, IGF-1, UHRF1BP1L, PTPRR, PPFIA2, P4HA2,* etc.	Typically progresses until about age 20
Polypoidal choroidal vasculopathies	*C2, C3, CFH, SERPING1, PEDF, ARMS2-HTRA1, FGD6, ABCG1, LOC387715, CETP,* etc.	Between ages 50 and 65
Retinitis pigmentosa	*RPGR, PRPF3, HK1, AGBL5,* etc.	Between 10 and 30
Stargardt's disease	*ABC1, ABCA4, CRB1,* etc.	Signs may appear in early childhood to middle age
Uveal melanoma	*PTEN, BAP1, GNAQ, GNA11, DDEF1, SF3B1, EIF1AX, CDKN2A, p14ARF, HERC2/OCA2,* etc.	50 to 80

Age-Related Macular Degeneration (AMD)

Age-Related Macular Degeneration is caused by pathologic changes to the deeper retinal layers of the macula and surrounding vasculature, which can result in central vision loss. There are two main types of AMD: neovascular ("dry" AMD) and nonneovascular ("wet" AMD). Nonneovascular AMD accounts for 80-85% of all cases and generally carries a more favorable visual prognosis, whereas Neovascular AMD affects the remaining 15% to 20% and accounts for approximately 80% of severe vision loss (Thomas et al., 2021).

Age-Related Macular Degeneration is caused by a combination of genetic and environmental factors. The strongest genetic association is due to genes involved in complement pathways. For instance, a major polymorphism of complement factor H (CFH) and CFH related genes (*CFHR1*-5) may predispose an individual to AMD (Cipriani et al., 2020). This polymorphism (histidine in place of tyrosine on position 402, CFH Y402H) on chromosome 1 has been associated with higher risk of AMD. One copy of the polymorphism has been associated with a 2.4-4.6 times higher risk of developing AMD whereas both copies of the allele have been associated with a 3.3-7.4 times higher risk. Single nucleotide polymorphisms (SNPs) such as CYP2C19 (G681A) Rs4244285 and CYP1A2 (-163C>A) Rs762551 may also confer added risk for AMD (Stasiukonyte et al., 2017).

Proprietary Testing

Several genetic tests have been developed to identify ophthalmologic conditions. The MVL Vision Panel (v2) by Molecular Vision tests for 581 genes associated with vision-related inherited conditions (MolecularVision, 2023). GeneDx has developed a Glaucoma Panel which tests for 38 glaucoma-related genes (GeneDx, 2024). Invitae has developed the Inherited Retinal Disorders Panel which tests for 248 genes associated with inherited retinal disorders (Invitae, 2024). Blueprint Genetics has developed 25 different ophthalmology panels which test for over 3,900 genes collectively (Blueprint, 2020). Finally, Prevention Genetics has developed the Stargardt Disease and Macular Dystrophies Panel which tests for 28 relevant genes (PreventionGenetics, 2024).

Clinical Utility and Validity

Lenassi et al. (2019) studied the clinical utility of genetic testing in children with inherited eye disorders. A total of 201 children in preschool (aged 0-5) participated in this study; all participants underwent panel testing. This cohort included "74 children with bilateral cataracts, 8 with bilateral ectopia lentis, 28 with bilateral anterior segment dysgenesis, 32 with albinism, and 59 with inherited retinal disorders" (Lenassi et al., 2019). The diagnostic yield for this study was 64% with testing results leading to altered disease management in 33% of probands (Lenassi et al., 2019).

Fauser and Lambrou (2015) analyzed potential biomarker candidates that could be used in a clinical setting to predict response to anti-vascular endothelial growth factor (anti-VEGF) treatment of neovascular AMD (nAMD). SNPs from 39 publications were evaluated and divided into two categories; those associated with AMD pathogenesis and those targeted by anti-VEGF therapies. The authors found that several studies supported an association between anti-VEGF treatment response and two SNPs, CFH rs1061170 and VEGFA rs699947, but results from randomized controlled trials found no such association (Fauser & Lambrou, 2015).

Chew et al. (2014) determined whether genotypes at two major loci associated with late AMD, complement factor H (CFH) and age-related maculopathy susceptibility 2 (*ARMS2*), influenced the relative benefits of Age-Related Eye Disease Study (AREDS) supplements; the original AREDs formulation contained vitamins C and E, zinc, copper and beta-carotene. A total of 1237 AREDS participants, 385 with late AMD, were genotyped. Both *CFH* and *ARMS2* genotypes were noted to individually associate with progression to late AMD. However, the investigators found that the genotypes at the *CFH* and *ARMS2* loci did not significantly alter the benefits of AREDS supplements. The investigators concluded that "genetic testing remains a valuable research tool, but these analyses suggest it provides no benefits in managing nutritional supplementation for patients at risk of late AMD" (Chew et al., 2014).

Hagstrom et al. (2015) evaluated the pharmacogenetic relationship between genotypes of SNPs in the VEGF signaling pathway and response to treatment with ranibizumab or bevacizumab for nAMD. For each of the measures of visual equity evaluated, there was no association with any of the genotypes or with the number of risk alleles. The investigators concluded that there are no pharmacogenetic associations between the studied *VEGF-A* and *VEGFR-2* SNPs and response to anti-VEGF therapy (Hagstrom et al., 2015).

Cascella et al. (2018) aimed to characterize exudative AMD in the Italian population and to identify the susceptibility/protective factors (genetic variants, age, sex, smoking, and dietary habits) that are specific for the onset of disease. The study involved a cohort of 1976 subjects, including 976 patients affected with exudative AMD and 1000 control subjects who underwent genotyping analysis of 20 genetic variants known to be associated with AMD. This analysis revealed that eight genetic variants (*CFH, ARMS2, IL-8, TIMP3, SLC16A8, RAD51B, VEGFA* and *COL8A1*) were significantly associated with AMD susceptibility. Following a multivariate analysis, considering both genetic and non-genetic data available, age, smoking, dietary habits, and sex, together with the genetic variants, were significantly associated with AMD (Cascella et al., 2018).

Chen et al. (2020) completed a study of 2,343 Chinese and Japanese individuals including patients with neovascular age-related macular degeneration (nAMD), polypoidal choroidal vasculopathy (PCV) and healthy controls. PCV is a disease of the choroidal vasculature in the eye. The *TIE2* (tyrosine kinase, endothelial, *TEK*) gene was the main focus in this study. In the analysis of all participants, a SNP of the *TIE2* gene (rs625767) was significantly associated with nAMD and PCV (Chen et al., 2020).

Strunz et al. (2020) completed a transcriptome-wide association study that included data from 6,144 late-stage AMD cases and 17,832 healthy controls. A total of 10 genes were significantly associated with AMD variants in at least one tissue in this study (27 different human tissues were analyzed). The authors conclude by stating that "our study highlights the fact that expression of genes associated with AMD is not restricted to retinal tissue as could be expected for an eye disease of the posterior pole, but instead is rather ubiquitous suggesting processes underlying AMD pathology to be of systemic nature" (Strunz et al., 2020).

Pontikos et al. (2020) conducted a retrospective study of electronic records in families with molecularly characterized IRD, to investigate proportions with disease attributable to gene variants. It was found that depending on the inheritance pattern, different genes were more likely to be implicated; among all the genes encountered, *ABCA4* was most frequent, but when accounting for types of retinitis pigmentosa (RP), the autosomal recessive type was most frequently caused by *USH2A* whereas autosomal

dominant RP was most linked with *RHO, RP1, and PRPF31*. Additionally, many X-linked retinopathies were the result of variants in *RPGR* (about 40%). More families in the study's pediatric cohort were affected by variants in X-linked genes, likely a result of earlier onset and severity of X-linked pathologies and likelihood of earlier diagnoses. The researchers also noted a weak but statistically significant positive correlation with transcription lengths and number of families affected by eye conditions, as longer transcripts are more likely to contain loss of function or premature termination mutations (Pontikos et al., 2020).

Sheck et al. (2021) reported on the performance of a next-gene sequencing (NGS) panel of 176 retinal genes (NGS 176) in patients with IRD. Among 488 patients, a diagnostic yield of 59.4% was recorded, with younger children being more likely to receive a molecular diagnosis than older adults. The clinical diagnoses were also statistically significantly associated with the diagnostic yield after multivariate analyses. Homogeneous IRD phenotypes of achromatopsia and congenital stationary night blindness, which were associated with six and ten genes, respectively, had diagnostic yields of 100% and 94%, respectively. This study demonstrated the effectiveness of using a new sequencing panel in the UK, and other factors, like age and clinical diagnoses that could correlate with a higher diagnostic yield (Sheck et al., 2021).

García Bohórquez et al. (2021) investigated the genetic basis for IRD in 92 patients using two custom NGS panels. At the time of the study, there were 270 genes associated with IRD. Using NGS, the authors found: among 92 patients, 53 had known gene variants, in 12 patients there was just one mutation in a gene found with a known autosomal recessive pattern of inheritance, and 27 patients (29.3%) had zero specified or identified genes, representing "unsolved" cases. A total of 120 pathogenic or likely pathogenic instances were identified. The most common gene variant was *ABCA4*. The *USH2A* gene was the most frequently found gene amongst patients diagnosed with retinitis pigmentosa. Lastly, a total of 10 families had pathogenic variants in more than one IRD-related gene (García Bohórquez et al., 2021).

VI. Guidelines and Recommendations

American Academy of Ophthalmology (AAO)

In 2014, the American Academy of Ophthalmology (AAO) Task Force on Genetic Testing published recommendations for genetic testing of inherited eye diseases. The Task Force stated that standard clinical diagnostic methods like biomicroscopy, ophthalmoscopy, tonography, and perimetry will be more accurate for assessing a patient's risk of vision loss from a complex disease than the assessment of a small number of genetic loci. The authors also state that "skilled counseling should be provided to all individuals who undergo genetic testing to maximize the benefits and

minimize the risks associated with each test" (Stone et al., 2014). The recommendations include:

- "Offer genetic testing to patients with clinical findings suggestive of a Mendelian disorder whose causative gene(s) have been identified. If unfamiliar with such testing, refer the patient to a physician or counselor who is. In all cases, ensure that the patient receives counseling from a physician with expertise in inherited disease or a certified genetic counselor.
- Use Clinical Laboratories Improvement Amendments– approved laboratories for all clinical testing. When possible, use laboratories that include in their reports estimates of the pathogenicity of observed genetic variants that are based on a review of the medical literature and databases of disease-causing and non–disease-causing variants.
- Provide a copy of each genetic test report to the patient so that she or he will be able independently to seek mechanism-specific information, such as the availability of gene-specific clinical trials, should the patient wish to do so.
- Avoid direct-to-consumer genetic testing and discourage patients from obtaining such tests themselves. Encourage the involvement of a trained physician, genetic counselor, or both for all genetic tests so that appropriate interpretation and counseling can be provided.
- Avoid unnecessary parallel testing— order the most specific test(s) available given the patient's clinical findings. Restrict massively parallel strategies like whole-exome sequencing and whole-genome sequencing to research studies conducted at tertiary care facilities.
- Avoid routine genetic testing for genetically complex disorders like age-related macular degeneration and late-onset primary open-angle glaucoma until specific treatment or surveillance strategies have been shown in 1 or more published prospective clinical trials to be of benefit to individuals with specific disease-associated genotypes. In the meantime, confine the genotyping of such patients to research studies.
- Avoid testing asymptomatic minors for untreatable disorders except in extraordinary circumstances. For the few cases in which such testing is believed to be warranted, the following steps should be taken before the test is performed: (1) the parents and child should undergo formal genetic counseling, (2) the certified counselor or physician performing the counseling should state his or her opinion in writing that the test is in the family's best interest, and (3) all parents with custodial responsibility for the child should agree in writing with the decision to perform the test" (Stone et al., 2014).

In 2019, the AAO published the Age-Related Macular Degeneration Preferred Practice Pattern guidelines and state that "The primary risk factors for the development of

advanced AMD include increasing age, northern European ancestry, and genetic factors… The routine use of genetic testing is not recommended at this time" (AAO, 2019). In 2023, they reaffirmed that the AAO "does not currently recommend genetic testing for AMD" (Mukamal, 2021).

In 2022, the AAO published recommendations on clinical assessment of patients with inherited retinal degenerations (IRDs). These clinical guidelines state that "Genetic testing and genetic counseling are essential components of the management of patients with IRDs as genetic testing may confirm the diagnosis, provide information to optimize management of the patient and family members, and potentially confirm eligibility to participate in clinical trials." They also note that "genetic testing for patients with IRDs can take multiple forms, including single gene analyses, panel-based tests that include many IRD disease genes, or more expansive testing such as whole exome and whole genome sequencing. Because of the genetic heterogeneity of the other phenotypes (>80), next generation sequencing testing using a retinal dystrophy panel provides an efficient first step for genetic testing. Whether the patient has syndromic features or not, testing should include genes known to be associated with syndromic forms of retinal disease, since some patients may only show the syndromic features later. Some 'syndromic genes' can be associated with a non-syndromic retinal degeneration." AAO also reiterates the importance of genetic testing for gene therapy: "patients would need to have genetic testing to determine if they are eligible for the FDA-approved voretigene neparvovec or be considered for any of the numerous clinical trials of gene-based therapies" (AAO, 2022).

European Reference Network for Rare Eye Diseases (ERN-EYE)

The ERN-EYE released a position statement on the need for eliminating gaps in genetic testing, as collectively, rare eye diseases (RED) are the "leading cause of visual impairment and blindness for children and young adults in Europe." There are still critical gaps in the administration of genomic testing that need to be addressed, especially in Europe's smaller countries where no formal genomic testing pathways exist. However, the ERN-EYE emphasizes promoting access to genetic testing to RED and the clinical need and relevance of it with increasing evidence for clinical utility (Black et al., 2021).

American Society of Retina Specialists (ASRS)

The ASRS states that there is no clinical evidence that changing treatment based on genetic risk is beneficial to the patient. At present there is "insufficient data to support the use of genetic testing in patients with AMD prior to recommendation of current Age-Related Eye Disease Study (AREDS) nutritional supplement use" (Csaky et al., 2017).

Italian IRD Working Group

An interdisciplinary panel of IRD experts convened to discuss IRD. They established parameters surrounding eligibility for *RPE65*-associated IRD gene therapy. The working group published "a strong consensus" recommendation for the use of "a targeted multi-gene NGS approach, including all the genes known to be responsible for IRDs, both isolated and syndromic forms." The authors also specify that larger panels such as clinical exome or whole-exome sequencing may also be used. They write, "The use of a larger panel (i.e. either a clinical exome or a whole-exome sequencing) is not excluded but, due to the issue of possible incidental findings, requires a more careful pre-test counselling" (Sodi et al., 2021).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81401	Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)
81404	Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)

81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons)
81408	Molecular pathology procedure, Level 9 (eg, analysis of >50 exons in a single gene by DNA sequence analysis)
81434	Hereditary retinal disorders (eg, retinitis pigmentosa, Leber congenital amaurosis, cone-rod dystrophy), genomic sequence analysis panel, must include sequencing of at least 15 genes, including ABCA4, CNGA1, CRB1, EYS, PDE6A, PDE6B, PRPF31, PRPH2, RDH12, RHO, RP1, RP2, RPE65, RPGR, and USH2A
81479	Unlisted molecular pathology procedure
81599	Unlisted multianalyte assay with algorithmic analysis

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

AAO. (2019). *Age-Related Macular Degeneration PPP 2019*. https://www.aao.org/preferred-practice-pattern/age-related-macular-degeneration-ppp

AAO. (2022). *Recommendations on Clinical Assessment of Patients with Inherited Retinal Degenerations - 2022*. https://www.aao.org/education/clinical-statement/guidelines-on-clinical-assessment-of-patients-with#top

Avalyon, J. Y., Glenn. (2021). Ocular gene therapy: The next generation. *Retina Specialist*. https://www.retina-specialist.com/article/ocular-gene-therapy-the-next-generation

Black, G. C., Sergouniotis, P., Sodi, A., Leroy, B. P., Van Cauwenbergh, C., Liskova, P., Grønskov, K., Klett, A., Kohl, S., Taurina, G., Sukys, M., Haer-Wigman, L., Nowomiejska, K., Marques, J. P., Leroux, D., Cremers, F. P. M., De Baere, E., Dollfus, H., & group, E.-E. s. (2021). The need for widely available genomic testing in rare eye diseases: an ERN-EYE position statement. *Orphanet journal of rare diseases*, *16*(1), 142-142. https://doi.org/10.1186/s13023-021-01756-x

Blueprint. (2020). *Ophthalmology*. https://blueprintgenetics.com/tests/panels/ophthalmology/

Cascella, R., Strafella, C., Longo, G., Ragazzo, M., Manzo, L., De Felici, C., Errichiello, V., Caputo, V., Viola, F., Eandi, C. M., Staurenghi, G., Cusumano, A., Mauriello, S.,

Marsella, L. T., Ciccacci, C., Borgiani, P., Sangiuolo, F., Novelli, G., Ricci, F., & Giardina, E. (2018). Uncovering genetic and non-genetic biomarkers specific for exudative age-related macular degeneration: significant association of twelve variants. *Oncotarget*, *9*(8), 7812-7821. https://doi.org/10.18632/oncotarget.23241

Chen, Z. J., Ma, L., Brelen, M. E., Chen, H., Tsujikawa, M., Lai, T. Y., Ho, M., Sayanagi, K., Hara, C., Hashida, N., Tam, P. O., Young, A. L., Nishida, K., Tham, C. C., Pang, C. P., & Chen, L. J. (2020). Identification of TIE2 as a susceptibility gene for neovascular age-related macular degeneration and polypoidal choroidal vasculopathy. *Br J Ophthalmol*. https://doi.org/10.1136/bjophthalmol-2019-315746

Chew, E. Y., Klein, M. L., Clemons, T. E., Agron, E., Ratnapriya, R., Edwards, A. O., Fritsche, L. G., Swaroop, A., & Abecasis, G. R. (2014). No clinically significant association between CFH and ARMS2 genotypes and response to nutritional supplements: AREDS report number 38. *Ophthalmology*, *121*(11), 2173-2180. https://doi.org/10.1016/j.ophtha.2014.05.008

Cipriani, V., Lores-Motta, L., He, F., Fathalla, D., Tilakaratna, V., McHarg, S., Bayatti, N., Acar, I. E., Hoyng, C. B., Fauser, S., Moore, A. T., Yates, J. R. W., de Jong, E. K., Morgan, B. P., den Hollander, A. I., Bishop, P. N., & Clark, S. J. (2020). Increased circulating levels of Factor H-Related Protein 4 are strongly associated with age-related macular degeneration. *Nat Commun*, *11*(1), 778. https://doi.org/10.1038/s41467-020-14499-3

Csaky, K. G., Schachat, A. P., Kaiser, P. K., Small, K. W., & Heier, J. S. (2017). The Use of Genetic Testing in the Management of Patients With Age-Related Macular Degeneration: American Society of Retina Specialists Genetics Task Force Special Report. *Journal of VitreoRetinal Diseases*, *1*(1), 75-78. https://doi.org/10.1177/2474126416680671

Fauser, S., & Lambrou, G. N. (2015). Genetic predictive biomarkers of anti-VEGF treatment response in patients with neovascular age-related macular degeneration. *Surv Ophthalmol*, *60*(2), 138-152. https://doi.org/10.1016/j.survophthal.2014.11.002

García Bohórquez, B., Aller, E., Rodríguez Muñoz, A., Jaijo, T., García García, G., & Millán, J. M. (2021). Updating the Genetic Landscape of Inherited Retinal Dystrophies [Original Research]. *Frontiers in Cell and Developmental Biology*, *9*. https://doi.org/10.3389/fcell.2021.645600

GeneDx. (2024). *Glaucoma Panel*. https://www.genedx.com/tests/detail/glaucoma-panel-940

Hagstrom, S. A., Ying, G. S., Maguire, M. G., Martin, D. F., Gibson, J., Lotery, A., & Chakravarthy, U. (2015). VEGFR2 Gene Polymorphisms and Response to Anti-Vascular Endothelial Growth Factor Therapy in Age-Related Macular Degeneration. *Ophthalmology*, *122*(8), 1563-1568. https://doi.org/10.1016/j.ophtha.2015.04.024

Hanany, M., Rivolta, C., & Sharon, D. (2020). Worldwide carrier frequency and genetic prevalence of autosomal recessive inherited retinal diseases. *Proceedings of the*

National Academy of Sciences, *117*(5), 2710-2716. https://doi.org/doi:10.1073/pnas.1913179117

Invitae. (2024). *Invitae Inherited Retinal Disorders Panel*. https://www.invitae.com/en/inherited-retinal-disorders-panel/

Lee, K., & Couser, N. (2016). Genetic Testing for Eye Diseases: A Comprehensive Guide and Review of Ocular Genetic Manifestations from Anterior Segment Malformation to Retinal Dystrophy. *Genetic Counseling and Clinical Testing 4*, 41-48. https://link.springer.com/article/10.1007/s40142-016-0087-0

Lenassi, E., Clayton-Smith, J., Douzgou, S., Ramsden, S. C., Ingram, S., Hall, G., Hardcastle, C. L., Fletcher, T. A., Taylor, R. L., Ellingford, J. M., Newman, W. D., Fenerty, C., Sharma, V., Lloyd, I. C., Biswas, S., Ashworth, J. L., Black, G. C., & Sergouniotis, P. I. (2019). Clinical utility of genetic testing in 201 preschool children with inherited eye disorders. *Genet Med*. https://doi.org/10.1038/s41436-019-0722-8

Luxturna. (2022). *Could LUXTURNA® be right for you?* https://luxturna.com/about-luxturna/

Michalakis, S., Gerhardt, M., Rudolph, G., Priglinger, S., & Priglinger, C. (2021). Gene Therapy for Inherited Retinal Disorders: Update on Clinical Trials. *Klin Monbl Augenheilkd*, *238*(3), 272-281. https://doi.org/10.1055/a-1384-0818 (Gentherapie für erbliche Netzhauterkrankungen: Übersicht zu aktuellen klinischen Studien.)

MolecularVision. (2023). *Browse Our Test Menu*. https://www.molecularvisionlab.com/mvl-vision-panel/

Mukamal, R. (2021, 01/19/2021). *Genetics and Age-Related Macular Degeneration*. https://www.aao.org/eye-health/diseases/age-related-macular-degeneration-amd-genetics

Pontikos, N., Arno, G., Jurkute, N., Schiff, E., Ba-Abbad, R., Malka, S., Gimenez, A., Georgiou, M., Wright, G., Armengol, M., Knight, H., Katz, M., Moosajee, M., Yu-Wai-Man, P., Moore, A. T., Michaelides, M., Webster, A. R., & Mahroo, O. A. (2020). Genetic Basis of Inherited Retinal Disease in a Molecularly Characterized Cohort of More Than 3000 Families from the United Kingdom. *Ophthalmology*, *127*(10), 1384-1394. https://doi.org/10.1016/j.ophtha.2020.04.008

PreventionGenetics. (2024). *Stargardt Disease (STGD) and Macular Dystrophies Panel*. https://www.preventiongenetics.com/testInfo.php?sel=test&val=Stargardt+Disease+%28STGD%29+and+Macular+Dystrophies+Panel

Sangermanoa, R., Scotta, H., Wagner, N., Place, E., & Bujakowska, K. M. (2020). *Genetics and Genomics of Eye Diseases: Chapter 14 - Genetic testing of various eye disorders*.

Sheck, L. H. N., Esposti, S. D., Mahroo, O. A., Arno, G., Pontikos, N., Wright, G., Webster, A. R., Khan, K. N., & Michaelides, M. (2021). Panel-based genetic testing for inherited retinal disease screening 176 genes. *Mol Genet Genomic Med*, e1663. https://doi.org/10.1002/mgg3.1663

Singh, M., & Tyagi, S. C. (2018). Genes and genetics in eye diseases: a genomic medicine approach for investigating hereditary and inflammatory ocular disorders. *Int J Ophthalmol*, *11*(1), 117-134. https://doi.org/10.18240/ijo.2018.01.20

Sodi, A., Banfi, S., Testa, F., Della Corte, M., Passerini, I., Pelo, E., Rossi, S., Simonelli, F., & Italian, I. R. D. W. G. (2021). RPE65-associated inherited retinal diseases: consensus recommendations for eligibility to gene therapy. *Orphanet journal of rare diseases*, *16*(1), 257. https://doi.org/10.1186/s13023-021-01868-4

Stasiukonyte, N., Liutkeviciene, R., Vilkeviciute, A., Banevicius, M., & Kriauciuniene, L. (2017). Associations between Rs4244285 and Rs762551 gene polymorphisms and age-related macular degeneration. *Ophthalmic Genet*, *38*(4), 357-364. https://doi.org/10.1080/13816810.2016.1242018

Stone, E. M., Aldave, A. J., Drack, A. V., Maccumber, M. W., Sheffield, V. C., Traboulsi, E., & Weleber, R. G. (2014). Recommendations for Genetic Testing of Inherited Eye Diseases - 2014. Retrieved 1/29/2015, from https://www.aao.org/clinical-statement/recommendations-genetic-testing-of-inherited-eye-d

Strunz, T., Lauwen, S., Kiel, C., Hollander, A. D., & Weber, B. H. F. (2020). A transcriptome-wide association study based on 27 tissues identifies 106 genes potentially relevant for disease pathology in age-related macular degeneration. *Sci Rep*, *10*(1), 1584. https://doi.org/10.1038/s41598-020-58510-9

Thomas, C. J., Mirza, R. G., & Gill, M. K. (2021). Age-Related Macular Degeneration. *Medical Clinics of North America*, *105*(3), 473-491. https://doi.org/10.1016/j.mcna.2021.01.003

Wiggs, J. L. (2017). Progress in Diagnostic Genetic Testing for Inherited Eye Disease. *JAMA Ophthalmol*, *135*(12), 1385-1386. https://doi.org/10.1001/jamaophthalmol.2017.4957

Genetic Testing for Polyposis Syndromes

Policy Number: AHS – M2024 – Genetic Testing for Polyposis Syndromes	Prior Policy Name and Number, as applicable: • AHS – M2024 – Familial Adenomatous Polyposis and *MUTYH*-Associated Polyposis
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Familial adenomatous polyposis (FAP) is characterized by development of adenomatous polyps and an increased risk of colorectal cancer (CRC) caused by an autosomal dominant mutation in the *APC* (Adenomatous Polyposis Coli) gene, affecting one in 5,000-10,000 individuals in the United States (Kinzler & Vogelstein, 1996; NORD, 2014). Depending on the location of the mutation in the *APC* gene, FAP can present as the more severe classic FAP with hundreds to thousands of polyps developing at the ages of 10-12 years associated with a significantly increased risk of CRC, or attenuated FAP (AFAP) with fewer polyps, developing later in life with lower risk of CRC (Brosens et al., 2015; Spirio et al., 1993). Two other subtypes of FAP include Gardner syndrome, which causes non-cancer tumors of the skin, soft tissues, and bones, and Turcot syndrome, a rare inherited condition in which individuals have a higher risk of adenomatous polyps and colorectal cancer. In classic FAP, the most common type, patients usually develop cancer in one or more polyps as early as age 20, and almost all classic FAP patients have CRC by the age of 40 if their colon has not been removed (Society, 2023).

MUTYH-associated polyposis (MAP) results from an autosomal recessive mutation of both alleles of the *MUTYH* gene and is characterized by increased risk of CRC with development of adenomatous polyps. This condition, however, may present without these characteristic polyps (Nielsen, 2015).

Two other polyposis syndromes are Juvenile Polyposis Syndrome (JPS) and Peutz-Jeghers Syndrome (PJS). These syndromes are characterized by polyps in the GI tract

and are often associated with *SMAD4* or *BMPR1A* mutations and *STK11* mutations, respectively (Chung, 2023; Chung & Delgado, 2024).

II. Related Policies

Policy Number	Policy Title
AHS-M2004	Lynch Syndrome
AHS-M2026	Testing for Colorectal Cancer Management
AHS-M2179	Prenatal Screening (Genetic)

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Genetic counseling **IS REQUIRED** for individuals being considered for genetic testing for polyposis syndromes.

2) For individuals (see Note 1) in a family with a pathogenic familial polyposis gene variant, the following testing **MEETS COVERAGE CRITERIA:**
   a) Testing restricted to the known pathogenic familial variant.
   b) Comprehensive genetic testing, including muti-gene panel testing (see Note 2), when the specific familial mutation is unknown.

3) For individuals (see Note 1) who have no known familial pathogenic variant(s), multi-gene panel testing (see Note 2, Note 3) for polyposis syndrome risk factors **MEETS COVERAGE CRITERIA** in **any** of the following situations:
   a) For individuals with a personal history of 10 or more cumulative adenomas.
   b) For individuals with a personal history of 2 or more hamartomatous polyps.
   c) For individuals with multifocal/bilateral or unilateral congenital hypertrophy of retinal pigment epithelium (CHRPE).
   d) For individuals with a personal history of **any** of the following:
      i) Primary brain tumor (e.g., medulloblastoma).
      ii) Desmoid tumor.
      iii) Hepatoblastoma.
      iv) Osteomas.

v) Supernumerary teeth.

e) For individuals who meet the criteria for serrated polyposis syndrome (SPS):

i) Individual has 5 or more serrated lesions/polyps proximal to the rectum, all being greater than or equal to 5 mm in size, with 2 or more being greater than or equal to 10 mm in size.

ii) Individual has greater than 20 serrated lesions/polyps of any size, distributed throughout the large bowel, with 5 or more being proximal to the rectum.

4) In an unaffected reproductive partner of an individual with *MUTYH*-associated polyposis (MAP), comprehensive sequencing of *MUTYH* **MEETS COVERAGE CRITERIA.**

5) Genetic testing of *SMAD4* and *BMPR1A* **MEETS COVERAGE CRITERIA** in **any** of the following situations:

a) For individuals with a known family history of juvenile polyposis syndrome (JPS) or known pathogenic familial *SMAD4* or *BMPR1A* mutations (testing restricted to known pathogenic familial mutation).

b) For individuals with at least five juvenile polyps in the colorectum.

c) For individuals with any number of juvenile polyps in other regions of the GI tract.

6) Genetic testing of *STK11* (formerly known as *LKB1*) **MEETS COVERAGE CRITERIA** in **any** of the following situations:

a) For individuals with a known family history of Peutz-Jeghers syndrome or known pathogenic familial *STK11* mutation (testing restricted to known pathogenic familial mutation).

b) For individuals with mucocutaneous hyperpigmentation of the mouth, lips, nose, eyes genitalia, or fingers.

c) For individuals with two or more histologically proven Peutz-Jeghers-type hamartomatous polyps of the GI tract.

7) For individuals less than 18 years of age who have one biological parent with MAP and one unaffected parent, sequencing of the *MUTYH* gene **DOES NOT MEET COVERAGE CRITERIA**.

8) For all other situations not described above, multi-gene panel testing **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: For individuals under 18 years of age, "genetic testing is generally not recommended unless results would impact medical management, such as initiation of early colonoscopy surveillance. Clear exceptions include when familial adenomatous polyposis (FAP), JPS, PHS, or constitutional MMR deficiency (CMMRD) syndrome are suspected or known to be present in a family, in which case testing prior to age 18 is recommended to guide medical management" (NCCN, 2023).

Note 2: Per the NCCN, "multigene panel[s] should include all polyposis and CRC [colorectal cancer] genes" (NCCN, 2023). At minimum, multigene panels should include the following polyposis and CRC risk genes: *APC, ATM, AXIN2, BLM, BMPR1A, CHEK2, EPCAM, GALNT12, GREM1, MBD4, MLH1, MLH3, MSH2, MSH3, MSH6, MUTYH, NTHL1, POLD1, POLE, PMS2, PTEN, RNF43, RPS20, SMAD4, STK11,* and *TP53*.

Note 3: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162-Reimbursement Policy.

IV. Table of Terminology

Term	Definition
ACG	American College of Gastroenterology
ACMG	American College of Medical Genetics and Genomics
AFAP	Attenuated familial adenomatous polyposis
AGA	American Gastrointestinal Association
APC	*Adenomatous polyposis coli gene*
ASCRS	American Society of Colon and Rectal Surgeons
ASGE	American Society for Gastrointestinal Endoscopy
AXIN2	*Axis inhibition protein 2 gene*
BMPR1A	*Bone morphogenetic protein receptor, type 1A gene*
CHRPE	Congenital hypertrophy of retinal pigment epithelium
CLIA	Clinical Laboratory Improvement Amendments
CMMRD	Constitutional MMR deficiency
CMS	Centers for Medicare and Medicaid Services
CRC	Colorectal cancer
EPCAM	*Epithelial cell adhesion molecule gene*
EPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
ESMO	European Society for Medical Oncology
EXO1	*Exonuclease 1 gene*
FAP	Familial adenomatous polyposis

GI	Gastrointestinal
GREM1	*Gremlin 1 gene*
HHT	Hereditary hemorrhagic telangiectasia
JPS	Juvenile polyposis syndrome
KRAS	*Kirsten rat sarcoma virus gene*
LDTs	Laboratory Developed Tests
MAP	*MUTYH* Associated Polyposis
MLH1	*MutL protein homolog 1 gene*
MLPA	Multiplex ligation dependent probe amplification
MMR	Mismatch repair
MSH2	*MutS homolog 2 gene*
MSH3	*MutS homolog 3 gene*
MSH6	*MutS homolog 6 gene*
MUTYH	*mutY DNA glycosylase gene*
OGG1	*8-oxoguanine glycosylase gene*
NCCN	National Comprehensive Cancer Network
NGS	Next-generation sequencing
NIPT	Noninvasive prenatal testing
NSGC	National Society of Genetic Counselors
NTHL1	*Endonuclease III-like protein 1 gene*
PJS	Peutz-Jeghers syndrome
PMS2	*PMS1 homolog 2, mismatch repair system component gene*
POLD1	*Polymerase delta 1 gene*
POLE	*DNA polymerase epsilon, catalytic subunit gene*
POLQ	*DNA polymerase theta gene*
PTEN	*Phosphatase and tensin homolog gene*
SMAD4	*SMAD family member 4/*Mothers against decapentaplegic homolog 4 *gene*
SPS	Serrated polyposis syndrome
STK11	*Serine/threonine kinase 11 gene*
TGFβ	*Transforming growth factor beta gene*
WES	Whole exome sequencing

V. Scientific Background

Familial Adenomatous Polyposis (FAP) and MUTYH-Associated Polyposis (MAP)

Inherited syndromes that express adenomatous polyps and confer a significantly increased risk of CRC include familial adenomatous polyposis (FAP) and *MUTYH*-associated polyposis (MAP) (Jasperson et al., 2010). Both FAP and MAP account for less

than one percent of all colorectal cancer cases (Chung & Rodgers, 2024; Grover & Stoffel, 2022).

Familial Adenomatous Polyposis results from mutations in the adenomatous polyposis coli (*APC*) tumor suppressor gene. Mutant or absent *APC* results in increased transcription of cell proliferation genes regulated through the Wnt/β-catenin pathway and the earliest malignancies (microadenomas and other small polyps) have lost the second *APC* allele. The *APC* gene is thought to prevent accumulation of β-catenin, and mutations in this gene result in failure of these β-catenin regulatory domains. β-catenin is thought to regulate the proliferation and differentiation of intestinal epithelial cells, and failure of this regulatory mechanism results in cell proliferation. Somatic mutations of this gene are present in 80% of sporadic CRCs and a single germline mutation of this gene is responsible for FAP (Frucht & Lucas, 2023). The prevalence of FAP is about 1:13,000 (Brosens et al., 2015). More than 300 different mutations have been reported, and the clinical presentation is dependent on the location of the mutation in the *APC* gene (Brosens et al., 2015; Spirio et al., 1993). Mutations in the central part of the gene (Exons 169 to 1393) result in classic FAP characterized by the presence of 100 or more adenomatous colorectal polyps (Chung & Rodgers, 2024). When fully developed, patients can have up to thousands of colorectal adenomas and nearly 100% risk of CRC. About 50% of patients developed adenomas by age 15 and 95% by age 35. If left untreated, FAP patients will develop CRC at an average age of 39 (Brosens et al., 2015). Patients with FAP are also at risk for extracolonic malignancies, such as desmoid tumors, duodenal adenomas, or even brain tumors (Chung & Rodgers, 2024).

In contrast, mutations in either end of the gene predispose to attenuated FAP (AFAP) (Spirio et al., 1993). AFAP is characterized by fewer colorectal adenomas with a later age of onset and an 80% lifetime risk of CRC compared to FAP. The diagnosis should be considered in patients 40-50 years old with 10-100 adenomas cumulatively. Patients with AFAP are diagnosed on average about 14 years later when compared with classic FAP (44 years of age versus 58 years of age, respectively). Overall, AFAP is a milder, but very similar form, of FAP (Chung & Rodgers, 2024).

MUTYH-associated polyposis is caused by biallelic mutations in the *MUTYH* gene base excision repair gene whose protein repairs oxidative damage on the *APC* gene (Sieber et al., 2003). Failure of base excision repair results in transversions in multiple genes, including the *APC* and *KRAS* genes. The two most common mutations in the *MUTYH* gene are Y179C and G396D, but more than 100 unique *MUTYH* gene mutations have been reported. *MUTYH*-associated polyposis is usually characterized by development of between 10 to 100 colorectal polyps by ages 50-60; however, *MUTYH* mutations have been identified in CRC with few or no colorectal polyps. Adenomas are the

primary polyp type in patients with *MUTYH*-associated polyposis, but hyperplastic and sessile serrated polyps have been reported in some patients (Grover & Stoffel, 2022). The genes that are mutated strongly influence the polyposis phenotype with the *KRAS* gene mutation resulting in different phenotypes compared to *MUTYH* (Boparai et al., 2008). Furthermore, the genotype of the condition may also make a difference in the clinical presentation. Multiple studies have suggested that the mutation G396D is less severe than the mutation Y179C, with the patients of the G396D genotype tending to develop polyps later and experiencing a later age of onset for those polyps (Guarinos et al., 2014; Nielsen et al., 2021).

Although both FAP and *MUTYH*-associated polyposis both cause numerous colorectal adenomas, there are notable differences between the two conditions. Mutations of *MUTYH* typically do not result in FAP. FAP is characterized by mutations in the *APC* gene and may be transmitted from parent to child (although 25% of FAP cases are *de novo*), whereas *MUTYH*-associated polyposis is not inherited in this manner. Diagnosis of *MUTYH*-associated polyposis requires identification of biallelic pathogenic germline variants of *MUTYH* (Grover & Stoffel, 2022).

A study of 8676 patients who had undergone mutation analysis of the *APC* and *MUTYH* genes was performed by Grover et al. Of these 8676, 7225 had colorectal adenomas. Overall, 1457 patients had classical FAP, and 3253 had AFAP. The study found *APC* mutations in 80% of patients with ≥1000 adenomas (95/119), 56% of patients with 100-999 adenomas (756/1338), 10% of patients with 20-99 adenomas (326/3253) and five percent of patients with 10-19 adenomas (50/970). *MUTYH* mutations were found in two percent (2/119), seven percent (94/1338), seven percent (233/3253), and four percent (37/970) of patients, respectively. The authors concluded that *APC* mutation rate increased as number of adenomas increased, but *MUTYH* mutation rate was relatively constant over all categories. There were 2098 patients out of 8676 (24%) who had a pathogenic *APC* or *MUTYH* mutation, and 6578 (76%) had a non-pathogenic mutation or no mutation in either gene (Grover et al., 2012).

Ciavarella et al. (2018) investigated genetic causes of unexplained adenomatous polyposis in eight cases of polyposis with no causative germline variant in *APC* or *MUTYH*. They identified *APC* mosaicism in 50% of patients. In three cases mosaicism was restricted to the colon, while in one it also extended to the duodenum and saliva. One patient without *APC* mosaicism carried an *APC* in-frame deletion of uncertain significance and was found to harbor rare germline variants in *OGG1, POLQ,* and *EXO1* genes. The authors concluded that restrictive selection criteria improved the detection of mosaic *APC* patients and that an oligogenic inheritance of rare variants may have a role in sporadic colorectal polyposis (Ciavarella et al., 2018).

Guidelines have been established by several organizations to reduce morbidity and mortality from hereditary forms of polyposis and resulting CRC by identifying individuals at risk and implementing a highly targeted program of cancer surveillance and management guided by the causative mutations identified (Hampel et al., 2015; Hegde et al., 2014; Provenzale et al., 2016; Syngal et al., 2015).

In a study by M. Yang et al. (2020), next-generation sequencing (NGS) panel, multiplex ligation-dependent probe amplification (MLPA), whole-exome sequencing (WES), and Sanger sequencing were used to determine a diagnostic method for variant-negative FAP patients. Although definite pathogenic variants of the *APC* gene are identified in the majority of FAP patients, there are still numerous variant-negative patients. NGS and MLPA did not identify any variants of the *APC* gene; however, WES recognized three patients with a point variant (c.-190G>A) in the noncoding region of the *APC* gene. Sanger sequencing identified a variant carrier during screening of the family. This study showed that the c.-190G>A variant can cause classic FAP but can be missed by conventional genetic testing. Therefore, "utilizing sequencing technologies covering a larger area can help us to further explore the pathogenesis in variant-negative FAP cases" (M. Yang et al., 2020).

Peutz-Jeghers Syndrome (PJS)

Peutz-Jeghers Syndrome is another uncommon polyposis syndrome that occurs one in 8,300 to one in 20,000 births (Giardiello & Trimbath, 2006). This condition is characterized by two clinical signs: pigmented mucocutaneous macules (melanin spots) and multiple hamartomatous gastrointestinal polyps. Those affected are at higher risk for both gastrointestinal and extra-intestinal cancers. Pathogenic mutations in the *STK11* gene is most strongly associated with PJS; although not every genetic mutation associated with PJS has been identified (Chung & Delgado, 2024).

Over 95% of PJS patients present with mucocutaneous macules, which are typically found on the lips or around the lips, palms, soles of the feet, or on the buccal mucosa. However, these macules tend to be most prevalent in the first two years and typically fade after puberty. Most patients will also present with hamartomatous polyps, typically developing in the first decade of life. These polyps do not have any particularly distinguishing features and may be indicative of several other syndromes, such as Cowden syndrome (Chung, 2023).

Jia et al. (2018) analyzed clinical features of 46 patients with Peutz-Jeghers syndrome (PJS). The authors identified "black spots, abdominal pain, hematochezia, and anemia" as the main clinical features. Histologically, "20 patients were classified as hamartomatous polyps, 18 as adenomatous polyps, 14 as inflammatory polyps, and 10 as zigzag polyps". Eleven patients underwent gene sequencing with a panel of 20

genes, and five were found to have gene mutations. Three of these patients were found to have mutations in the *STK11* gene (Jia et al., 2018).

In a study by Wu et al. (2020), direct sequencing using the QIAamp DNA Blood Mini Kit and multiplex ligation-dependent probe amplification (MLPA) tests were used to detect germline *STK11* mutations in 38 patients clinically diagnosed with Peutz-Jeghers syndrome and their healthy relatives. RNA sequencing was performed in polyps of PJS patient and control groups to evaluate the difference of *STK11* expression. A clinical PJS diagnosis was made when an individual had two of the following: two or more histologically confirmed Peutz-Jeghers-type hamartomatous polyps, mucocutaneous hyperpigmentation of the mouth, lips, nose, eyes, genitalia, or fingers, and family history of PJS. Germline mutation screening of the *STK11* gene detected a pathogenic variant in all probands with a 100% mutation detection rate. "Twenty variants were nucleotide substitutions or indels that were detected by Sanger sequencing (seven were missense variants, and 13 variants were truncating). All mutations fell within the coding region spanning exon one and exon eight, and no mutation in exon nine was identified in any of these PJS individuals" (Wu et al., 2020). While missense mutations did not influence *STK11* expression, truncated mutations resulted in lower *STK11* expression which may cause greater damage to the gene product and a more severe PJS phenotype. In this study, the 13 patients with a truncated *STK11* variant did have earlier onset for PJS symptoms, including intestinal obstruction and first operation events, than those with missense mutations. This indicates that patients with truncated variant need earlier management to prevent complications. In addition, this study identified a fetus with a *STK11* pathogenic variant through non-invasive prenatal testing (NIPT). The parents chose to give birth to this fetus, and melanin spots appeared on the lips at approximately one year old and have gradually increased. This indicates that there are broad application prospects for prenatal testing and preimplantation genetic diagnosis. Due to the significance of genetic testing in this study, the author states that "it is important to detect *STK11* gene mutations to make early diagnoses and treatments to reduce the occurrence of GI complications and malignancies" (Wu et al., 2020).

Juvenile Polyposis Syndrome (JPS)

Juvenile Polyposis Syndrome is another condition thought to confer additional risk for colorectal and gastric cancer. JPS is caused by variants in the *BMPR1A* or *SMAD4* genes, but no genetic variant is found in 20-30% of the cases. These genes code for a protein that play a role in the TGFβ signal transduction system. In patients with *SMAD4* gene variant, severe polyposis in the stomach or duodenum is highly likely (NHS, 2020). Similar to syndromes discussed above, this condition is characterized by numerous polyps in the GI tract. More than half of affected JPS patients will present with rectal

bleeding and will be symptomatic by 20 years old. Differentiating JPS from other hamartomatous syndromes can be difficult, but patients meeting the clinical diagnosis criteria for JPS will often undergo genetic testing for the *BMPR1A* and *SMAD4* genes (Chung & Delgado, 2024).

Gonzalez et al. (2017) evaluated the clinicopathological features of 22 patients with "abundant gastric juvenile-type or hyperplastic-like polyps". There were 14 patients that were diagnosed with JPS an average of 40 years. Out of the 22 cases, 18 cases showed "complete or near-complete carpeting of the gastric mucosa by innumerable polyps", and *SMAD4* immunohistochemical staining revealed "patchy loss" in polyps in 19 of 20 tested cases. Furthermore, five of six patients tested harbored a *SMAD4* mutation (Gonzalez et al., 2017).

VI. Guidelines and Recommendations

National Comprehensive Cancer Network

The NCCN recommends *APC* or *MUTYH* gene testing for individuals with a personal history of ≥20 adenomas, individuals with a known deleterious familial mutation, and individuals with multifocal or bilateral congenital hypertrophy of retinal pigment epithelium (CHRPE). The NCCN recommends that testing be considered in individuals with a personal or family history of CHRPE, osteomas, supernumerary teeth, desmoid tumor, hepatoblastoma, brain cancer (typically medulloblastoma), or cribriform variant of papillary thyroid cancer (Gupta et al., 2017; NCCN, 2023). If an *APC* variant is found, high-quality colonoscopy every 12 months, beginning at 10 to 15 years of age, is recommended. Colonoscopy is preferred over flexible sigmoidoscopy due to the possibility of missing right-sided polyps when limiting to sigmoidoscopy. However, based on patient and family preference or clinical judgment, sigmoidoscopy may also be considered. (NCCN, 2023).

If a patient has a personal or family history of a known pathogenic variant of a colorectal polyposis or cancer gene, further evaluation is warranted. When there is no known familial or personal mutation, the NCCN recommends determining the patient or familial history of the following clinical signs:

- ten or more adenomatous polyps
- two or more hamartomatous polyps or
- five or more serrated polyps proximal to the rectum

If any of these features are identified, the NCCN recommends a detailed risk assessment/genetic evaluation for possible polyposis syndromes. The NCCN also recommends within the algorithm concerning risk assessment/genetic evaluation for

possible polyposis syndromes that for individuals for more than 10 adenomas to test for FAP, AFAP, MAP, and rare genetic causes of multiple adenomatous polyps. Within this latter group, the genes associated "include, but are not limited to monoallelic pathogenic variants in GREM1, POLE, POLD1, and AXIN2, and biallelic pathogenic variants in NTHL1 and MSH3."

The NCCN also notes the following: "When colonic polyposis is present only in the proband and/or in siblings, consider recessive inheritance or *de novo APC* gene mutations. For example MAP follows a recessive pattern of inheritance, so *MUTYH* testing should be considered if a recessive pattern is apparent in the pedigree…*MUTYH* testing is not indicated based solely on a personal history of a desmoid tumor, hepatoblastoma, or cribriform-morular variant of papillary thyroid cancer…" (NCCN, 2023).

The NCCN also makes this note for siblings of a patient with MAP: they are recommended to have site-specific testing for the familial pathogenic/likely pathogenic mutations. "Full sequencing of *MUTYH* may be considered in an unaffected parent when the other parent has MAP. If the unaffected parent is not tested, then comprehensive testing of *MUTYH* should be considered in the children. If the unaffected parent is found to have one *MUTYH* pathogenic variant, then testing the children for the familial *MUTYH* [pathogenic/likely pathogenic] variants is clinically indicated. Testing of children of *MUTYH* heterozygotes should be offered if the other parent is also a heterozygote or could still be offered if the other parent is not a heterozygote and management would change, if they have an first-degree relative affected with CRC, or to inform reproductive risks since their future children could be at risk for MAP" (NCCN, 2023).

The NCCN notes that a classical diagnosis of FAP is suspected when there are "at least 100 cumulative adenomas in the large bowel" present at a young age; however, genetic testing with multi-gene panel is recommended to differentiate between FAP, AFAP, MAP polyposis due to a mutation in a rare gene for which testing is available, and colonic polyposis of unknown etiology (NCCN, 2023).

NCCN lists clinical scenarios for which multigene testing "may be considered", such as adenomatous polyposis, a patient with personal or family history meeting criteria for more than one hereditary cancer syndrome, a colonic polyposis with uncertain histology, second-line testing with inconclusive first-line testing, if family cancer history does not meet established testing guidelines, or if an individual with limited or unknown family history is concerned about cancer predisposition. However, the NCCN also recommends against multi-gene testing in the following scenarios: if the mutation is known and there is no other reason for multi-gene testing or if genetic testing is

performed as first-line testing with a family history that is strongly suggestive of a known hereditary syndrome. In these situations, the NCCN states that a syndrome-specific panel may be considered instead. Overall, the NCCN states that multi-gene panels that include tumor and family-based criteria associated with Lynch Syndrome is recommended. Panel testing may be an option if the personal and family histories are "strongly suggestive" of an inherited condition. The panel states that "at a minimum, a germline multigene panel should include the following genes associated with CRC risk: *APC, MUTYH, MLH1, MSH2, MSH6, PMS2, EPCAM, BMPR1A, SMAD4, PTEN, STK11* and *TP53*." The NCCN also recommends genetic counseling before and after genetic testing is done (NCCN, 2023).

The NCCN recommends genetic testing for juvenile polyposis syndrome patients, noting that 50% of cases occur due to pathogenic *SMAD4* or *BMPR1A* mutations. In families with a known BMPR1A pathogenic variant, "genetic testing should be performed by age 12-15 when surveillance would begin (or sooner if symptoms warrant evaluation)." If there is a known familial mutation of *SMAD4*, genetic testing should be performed within the first 6 months of life. The NCCN also remarks that the majority of Peutz-Jeghers Syndrome cases occur due to pathogenic variants in the *STK11/LKB1* gene (NCCN, 2023).

The NCCN recommendations follow the American Society Clinical Oncology (ASCO), which issued an updated statement regarding genetic testing in 2015. ASCO states that informed consent, as well as the possibility of discovery of unexpected and harmful mutations, should be communicated carefully to the patient. ASCO states that genetic counseling is imperative both before and after genetic testing, as many genes have uncertain clinical utility and a specialist may help provide informed clinical decision-making (NCCN, 2023; Robson et al., 2015).

The NCCN also notes several genes that may decide treatment. For patients with pathogenic variants in *GREM1, POLD1, POLE, AXIN2, NTHL1*, and *MSH3*, they recommend beginning a colonoscopy no later than 25-30 years old and performing one every one to two years if negative. If polyps are found, endoscopic evaluation of the rectum every six to twelve months is recommended, depending on polyp burden. However, the NCCN does note that recommendations for these genes are still "evolving" at this time and that caution is needed when determining surveillance regimes.

Some general considerations and best practices for genetic testing from the NCCN included the following:

"Patients who have received an allogeneic bone marrow transplant should not have molecular genetic testing via blood or saliva samples due to unreliable test results

from contamination by donor DNA in such cases, DNA of the individual being tested should be extracted from a fibroblast culture from a skin punch biopsy. If this is not possible, buccal cells may be considered as an alternative source of DNA."

"In children < 18y, genetic testing is generally not recommended unless results would impact medical management, such as initiation of early colonoscopy surveillance. Clear exceptions include when FAP, JPS, PJS, or constitutional MMR deficiency (CMMRD) syndrome are suspected or known to be present in a family, in which case testing prior to age 18 is recommended to guide medical management" (NCCN, 2023).

American College of Gastroenterology

The ACG recommends that "individuals who have a personal history of >10 cumulative colorectal adenomas, a family history of one of the adenomatous polyposis syndromes, or a history of adenomas and FAP-type extracolonic manifestations (duodenal/ampullary adenomas, desmoid tumors (abdominal>peripheral), papillary thyroid cancer, congenital hypertrophy of the retinal pigment epithelium ((CHRPE), epidermal cysts, osteomas) should undergo assessment for the adenomatous polyposis syndromes. Genetic testing of patients with suspected adenomatous polyposis syndromes should include *APC* and *MUTYH* gene mutation analysis" (Syngal et al., 2015). The ACG recommends screening for CRC in patients with or at risk for "classic AP syndromes" by annual colonoscopy or flexible sigmoidoscopy starting at puberty. The ACG also recommends surveillance by colonoscopy in families with AFAP or MAP (Syngal et al., 2015).

The ACG further states that failure to identify a mutation does not rule out the diagnosis of adenomatous polyposis. Testing for any possible underlying genes should be considered if clinical suspicion is high. Failure to find a mutation means that all close relatives must still be screened, but finding a mutation confirms the diagnosis and allows relatives to be tested accurately. Once an affected patient has been genotyped, all at-risk relatives can be screened properly (Syngal et al., 2015).

The ACG also notes that "Individuals with perioral or buccal pigmentation and/or two or more histologically characteristic GI hamartomatous polyp(s) or a family history of PJS should be evaluated for PJS." Further, they state that genetic evaluation of a patient with "possible" PJS should include testing for *STK11* mutations. Regarding JPS, ACG recommends that "Individuals with five or more juvenile polyps in the colorectum or any juvenile polyps in other parts of the GI tract should undergo evaluation for JPS." A genetic evaluation of a patient with "possible" JPS should include testing for *SMAD4* and *BMPR1A* mutations (Syngal et al., 2015).

American College of Medical Genetics and Genomics

The ACMG recommends testing for FAP in individuals with "100 ≤ polyps with autosomal dominant inheritance, and for at-risk family members of individuals with known familial mutations". The ACMG also recommends testing for FAP in individuals with conditions such as congenital hypertrophy of retinal pigment epithelium or osteomas. It also recommended that "FAP testing be performed using full sequencing of the *APC* gene. If no mutation is detected, then testing for large gene rearrangements should be performed" (Hegde et al., 2014). The ACMG notes that mutations are detected in 80% of patients with FAP with DNA sequencing detecting 87% of smaller mutations, such as deletions or point mutations. The remaining mutations are larger mutations, such as gross duplications, which can be detected by RT-PCR or MLPA. ACMG recommends considering testing for AFAP in individuals with <100 adenomas. They note that individuals with 100 or more polyps at 35-40 years or older may be found to have AFAP. According to ACMG, frequent right-sided distribution of polyps is usually noted in these individuals and adenomas and cancers at an age older than that for classic FAP and other GI manifestations are found (Hegde et al., 2014).

The ACMG recommends *MUTYH* gene testing for individuals with colorectal cancer diagnosed at less than 40, the presence of 10 or more adenomatous polyps without *APC* gene mutation, and a family history of colon cancer with an autosomal recessive inheritance including colon cancers with or without polyps (Hegde et al., 2014). ACMG indicates that *MUTYH* testing should begin with testing for the two common mutations p.Y165C and p.G382D, and if none or one mutation is identified, then full sequencing of the *MUTYH* gene should be considered. The ACMG notes that 80% of mutations in Caucasian and North European populations are of these two variants but sequencing of the entire gene may detect up to 99% of mutations. The ACMG also recommends that testing of the *MUTYH* gene should also be offered to at-risk family members. Sanger sequencing and NGS are both recommended methods for sequencing. Finally, if heterozygosity for only one common mutation is detected, or no mutation is detected at all, then sequencing of the entire *MUTYH* gene may be considered (Hegde et al., 2014).

ACMG and the National Society of Genetic Counselors

The ACMG and NSGC recommend that referral for genetic counseling should be considered for "any individual with a personal history of or first-degree relative with a total of ≥10 adenomatous colon polyps with or without a colorectal or other FAP-associated cancer, a cribriform morular variant of papillary thyroid cancer; a desmoid tumor; or hepatoblastoma diagnosed before age five".

The guidelines also list clinical symptoms that should warrant assessment for cancer predisposition for JPS and PJS. For JPS, they note the following symptoms:

- "three to five cumulative histologically proven juvenile polyps in the same person"
- "Multiple juvenile polyps throughout the GI tract in the same person"
- "Any number of juvenile polyps with a family history positive of JPS"

For PJS:

- "≥ two cumulative histologically proven PJ polyps in the same person"
- "≥ one PJ polyp and mucocutaneous hyperpigmentation in the same person"
- "Any number of PJ polyps and a positive family history of PJS" (Hampel et al., 2015).

European Society for Medical Oncology (ESMO)

The ESMO published a 2019 update for hereditary gastrointestinal cancers, including some polyposis syndromes. These recommendations are as follows:

- For FAP, "Patients with multiple colorectal adenomas (>10) should be considered for panel germline genetic testing that includes *APC, MUTYH, POLE, POLD1* and *NTHL1* genes. *APC* analysis should include large rearrangements"
- "Biallelic *MUTYH* mutations should be suspected in cases of AFAP or FAP with a recessive pattern of inheritance, diagnosis before the age of 50 years, and multiple colonic polyps"
- "A multigene single analysis of *APC, MUTYH* (all exons), *POLE, POLD1* and *NTHL1* is recommended"
- "For *POLE*- and *POLD1*-mutation-positive PPAP and *NTHL1*-mutation-positive adenomatous polyposis, colonoscopic surveillance should follow MAP recommendations" (Stjepanovic et al., 2019).

The ESMO recommends germline testing of *APC* and *MUTYH* for patients with 10 or more colorectal adenomas. Full germline testing should include DNA sequencing and large rearrangement analysis.

Testing for *MUTYH* may start with the two most common mutations (Y179C, G396D), followed by analysis of the entire gene in heterozygotes. Founder mutations present in certain ethnic groups should also be considered. If a mutation is detected, testing may also be offered to at-risk family members (Balmaña et al., 2013).

American Society of Colon and Rectal Surgeons (ASCRS)

The ASCRS has released guidelines on inherited polyposis syndromes. A polyposis diagnosis should be considered "in patients with over 20 adenomas, patients with history of desmoid tumor, extracolonic manifestations, or family members of individuals with known FAP, AFAP, or MAP". Germline testing of the *APC* gene is recommended for these individuals. The ASCRS lists 20 as the cutoff as the risk of finding a genetic mutation rises above 10% at this mark. Genetic counseling is recommended prior to genetic testing. The ASCRS recommends patients with clinical polyposis but without an identified mutation to be treated according to their phenotype. However, this was noted to be a weak recommendation based on low quality evidence (Herzig et al., 2017).

European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Polyposis Working Group

This Working Group released guidelines on both Juvenile Polyposis Syndrome (JPS) and Peutz-Jeghers Syndrome (PJS).

For JPS, the Working Group recommends routine predictive testing for at-risk children at 12-15 years of age. If a child has rectal bleeding before this age, a colonoscopy should be performed, and if polyps are found, that child should undergo genetic testing.

Pediatric patients with a *SMAD4* mutation should be evaluated for Hereditary Hemorrhagic Telangiectasia (HHT), including screening for cerebral and pulmonary arteriovenous malformations.

"Children with *BMPR1A* mutation and early onset polyposis and/or a severe phenotype and/or extraintestinal manifestations should be evaluated for PTEN mutation".

"If a specific gene mutation has been detected in a child, then genetic testing should be offered to all first-degree family members. If no specific gene mutation was detected, then first-degree relatives should be referred for screening colonoscopy at the age of 12 to 15 years" (Cohen et al., 2019).

Regarding PJS, the ESPGHAN recommends offering predictive genetic testing for an asymptomatic at-risk child as early as 3 years of age. Symptomatic at-risk children should have genetic testing performed earlier.

However, the ESPGHAN notes that "No clear genotype-phenotype correlation has been demonstrated in PJS. Furthermore there have been no clear clinical differences found between cases with and without detectable germline *STK11* mutations" (Latchford et al., 2019).

American Society for Gastrointestinal Endoscopy (ASGE)

The ASGE released recommendations for the role of genetic testing in the management of patients with FAP syndromes. As family history may not be present due to germline mutations of the *APC* gene, ASGE recommends genetic testing to make a confirmatory FAP diagnosis before moving forward with morbid surgery or invasive endoscopic screening. Genetic testing is also recommended when a patient presents with ten or more cumulative adenomatous polyps on a single colonoscopy, if a patient presents with ten or more adenomas and a history of CRC, or if a patient has twenty or more adenomatous polyps in a lifetime. In addition, genetic counseling is recommended for all patients with or suspected to have FAP syndromes and first-degree relatives (J. Yang et al., 2020).

The *APC* gene testing is recommended in children at the age of 10-12 years. If AFAP or MAP is suspected, patients should undergo genetic testing at the age of 18-20 years. Younger children, aged six months to five years, can undergo confirmatory *APC* gene testing if parents agree to screen for hepatoblastoma with alpha-fetoprotein test and liver function test every six months. Otherwise, testing is deferred until 10-12 years old. Children without *APC* gene abnormalities should follow average-risk screening guidelines (J. Yang et al., 2020).

Finally, the guideline comments that "Once an individual is found to be affected with MAP, his or her relatives should also be screened for mutations in *MUTYH*…Similar to FAP, genetic testing for mutations in *MUTYH* should be considered in those with (1) 20 or more colorectal adenomas over multiple colonoscopies, (2) a known family history of MAP, (3) 10 or more adenomas found on a single colonoscopy, or (4) criteria for serrated polyposis syndrome with at least some adenomas noted on examination". The guideline further notes "serrated polyposis syndrome" is defined by the WHO as one of the following conditions: "(1) at least 5 serrated polyps proximal to the sigmoid colon with two or more >10 mm in size, (2) any number of serrated polyps proximal to the sigmoid colon in an individual who has a first-degree relative with serrated polyposis syndrome, or (3) >20 serrated polyps of any size distributed throughout the colon." The guideline does remark that genetic testing for *MUTYH* in children should be postponed until adulthood due to the later onset of the condition.

American Gastroenterological Association (AGA)

The AGA released recommendations on genetic testing for young adult-onset colorectal cancer. AGA recommends genetic testing to all young adult CRC patients based on the patient's family history of hereditary CRC, other cancer syndromes, and the presence of polyps. AGA also recommends germline testing for those who do not fit clinical criteria for one hereditary syndrome or have no family history of cancer. AGA

encourages early integration of genetic counselors as increased genetic testing could lead to the chances of finding genetic variants of unknown significance or a pathogenic variant that does not have clear management guidelines (Boardman et al., 2020).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, please visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

On January 18, 2019, the FDA approved the *MUTYH*-Associated Polyposis (MAP) testing by 23andMe, Inc (FDA, 2024).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	CPT Description
81201	APC (adenomatous polyposis coli) (eg, familial adenomatosis polyposis [FAP], attenuated FAP) gene analysis; full gene sequence
81202	APC (adenomatous polyposis coli) (eg, familial adenomatosis polyposis [FAP], attenuated FAP) gene analysis; known familial variants
81203	APC (adenomatous polyposis coli) (eg, familial adenomatosis polyposis [FAP], attenuated FAP) gene analysis; duplication/deletion variants
81404	Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)

81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons, cytogenomic array analysis for neoplasia)
81479	Unlisted molecular pathology procedure

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Balmaña, J., Balaguer, F., Cervantes, A., Arnold, D., & ESMO. (2013). Familial risk-colorectal cancer: ESMO Clinical Practice Guidelines†. *Annals of Oncology*, *24*(suppl_6), vi73-vi80. https://doi.org/10.1093/annonc/mdt209

Boardman, L. A., Vilar, E., You, Y. N., & Samadder, J. (2020). AGA Clinical Practice Update on Young Adult–Onset Colorectal Cancer Diagnosis and Management: Expert Review. *Clinical Gastroenterology and Hepatology*, *18*(11), 2415-2424. https://doi.org/10.1016/j.cgh.2020.05.058

Boparai, K. S., Dekker, E., Van Eeden, S., Polak, M. M., Bartelsman, J. F., Mathus-Vliegen, E. M., Keller, J. J., & van Noesel, C. J. (2008). Hyperplastic polyps and sessile serrated adenomas as a phenotypic expression of MYH-associated polyposis. *Gastroenterology*, *135*(6), 2014-2018. https://doi.org/10.1053/j.gastro.2008.09.020

Brosens, L. A., Offerhaus, G. J. A., & Giardiello, F. M. (2015). Hereditary Colorectal Cancer: Genetics and Screening. *Surg Clin North Am*, *95*(5), 1067-1080. https://doi.org/10.1016/j.suc.2015.05.004

Chung, D. C. (2023, 01/03/2023). *Peutz-Jeghers syndrome: Clinical manifestations, diagnosis, and management*. https://www.uptodate.com/contents/peutz-jeghers-syndrome-clinical-manifestations-diagnosis-and-management

Chung, D. C., & Delgado, K. (2024, March 14, 2024). *Juvenile polyposis syndrome*. https://www.uptodate.com/contents/juvenile-polyposis-syndrome

Chung, D. C., & Rodgers, L. H. (2024, April 8 2024). *Clinical manifestations and diagnosis of familial adenomatous polyposis*. https://www.uptodate.com/contents/clinical-manifestations-and-diagnosis-of-familial-adenomatous-polyposis

Ciavarella, M., Miccoli, S., Prossomariti, A., Pippucci, T., Bonora, E., Buscherini, F., Palombo, F., Zuntini, R., Balbi, T., Ceccarelli, C., Bazzoli, F., Ricciardiello, L., Turchetti, D., & Piazzi, G. (2018). Somatic APC mosaicism and oligogenic inheritance in

genetically unsolved colorectal adenomatous polyposis patients. *Eur J Hum Genet*. https://doi.org/10.1038/s41431-017-0086-y

Cohen, S., Hyer, W., Mas, E., Auth, M., Attard, T. M., Spalinger, J., Latchford, A., & Durno, C. (2019). Management of Juvenile Polyposis Syndrome in Children and Adolescents: A Position Paper From the ESPGHAN Polyposis Working Group. *J Pediatr Gastroenterol Nutr*, *68*(3), 453-462. https://doi.org/10.1097/mpg.0000000000002246

FDA. (2024). https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm

Frucht, H., & Lucas, A. L. (2023, 12/08/2023). *Molecular genetics of colorectal cancer*. https://www.uptodate.com/contents/molecular-genetics-of-colorectal-cancer

Giardiello, F. M., & Trimbath, J. D. (2006). Peutz-Jeghers Syndrome and Management Recommendations. *Clinical Gastroenterology and Hepatology*, *4*(4), 408-415. https://doi.org/10.1016/j.cgh.2005.11.005

Gonzalez, R. S., Adsay, V., Graham, R. P., Shroff, S. G., Feely, M. M., Drage, M. G., Lewin, D. N., Swanson, E. A., Yantiss, R. K., Bagci, P., & Krasinskas, A. M. (2017). Massive gastric juvenile-type polyposis: a clinicopathological analysis of 22 cases. *Histopathology*, *70*(6), 918-928. https://doi.org/10.1111/his.13149

Grover, S., Kastrinos, F., Steyerberg, E. W., Cook, E. F., Dewanwala, A., Burbidge, L. A., Wenstrup, R. J., & Syngal, S. (2012). Prevalence and phenotypes of APC and MUTYH mutations in patients with multiple colorectal adenomas. *Jama*, *308*(5), 485-492. https://doi.org/10.1001/jama.2012.8780

Grover, S., & Stoffel, E. (2022, 06/24/2022). *MUTYH-associated polyposis - UpToDate*. https://www.uptodate.com/contents/mutyh-associated-polyposis

Guarinos, C., Juarez, M., Egoavil, C., Rodriguez-Soler, M., Perez-Carbonell, L., Salas, R., Cubiella, J., Rodriguez-Moranta, F., de-Castro, L., Bujanda, L., Serradesanferm, A., Nicolas-Perez, D., Herraiz, M., Fernandez-Banares, F., Herreros-de-Tejada, A., Aguirre, E., Balmana, J., Rincon, M. L., Pizarro, A., . . . Jover, R. (2014). Prevalence and characteristics of MUTYH-associated polyposis in patients with multiple adenomatous and serrated polyps. *Clin Cancer Res*, *20*(5), 1158-1168. https://doi.org/10.1158/1078-0432.Ccr-13-1490

Gupta, S., Provenzale, D., Regenbogen, S. E., Hampel, H., Slavin, T. P., Jr., Hall, M. J., Llor, X., Chung, D. C., Ahnen, D. J., Bray, T., Cooper, G., Early, D. S., Ford, J. M., Giardiello, F. M., Grady, W., Halverson, A. L., Hamilton, S. R., Klapman, J. B., Larson, D. W., . . . Ogba, N. (2017). NCCN Guidelines Insights: Genetic/Familial High-Risk Assessment: Colorectal, Version 3.2017. *J Natl Compr Canc Netw*, *15*(12), 1465-1475. https://doi.org/10.6004/jnccn.2017.0176

Hampel, H., Bennett, R. L., Buchanan, A., Pearlman, R., & Wiesner, G. L. (2015). A practice guideline from the American College of Medical Genetics and Genomics and the National Society of Genetic Counselors: referral indications for cancer predisposition assessment. *Genet Med*, *17*(1), 70-87. https://doi.org/10.1038/gim.2014.147

Hegde, M., Ferber, M., Mao, R., Samowitz, W., & Ganguly, A. (2014). ACMG technical standards and guidelines for genetic testing for inherited colorectal cancer (Lynch syndrome, familial adenomatous polyposis, and MYH-associated polyposis). *Genet Med*, *16*(1), 101-116. https://doi.org/10.1038/gim.2013.166

Herzig, D., Hardiman, K., Weiser, M., You, N., Paquette, I., Feingold, D. L., & Steele, S. R. (2017). The American Society of Colon and Rectal Surgeons Clinical Practice Guidelines for the Management of Inherited Polyposis Syndromes. *Dis Colon Rectum*, *60*(9), 881-894. https://doi.org/10.1097/dcr.0000000000000912

Jasperson, K. W., Tuohy, T. M., Neklason, D. W., & Burt, R. W. (2010). Hereditary and Familial Colon Cancer. *Gastroenterology*, *138*(6), 2044-2058. https://doi.org/10.1053/j.gastro.2010.01.054

Jia, Y., Fu, H., Li, N., Kang, Q., & Sheng, J. (2018). [Diagnosis and treatment for 46 cases of Peutz-Jeghers syndrome]. *Zhong Nan Da Xue Xue Bao Yi Xue Ban*, *43*(12), 1323-1327. https://doi.org/10.11817/j.issn.1672-7347.2018.12.007

Kinzler, K. W., & Vogelstein, B. (1996). Lessons from hereditary colorectal cancer. *Cell*, *87*(2), 159-170. https://doi.org/10.1016/S0092-8674(00)81333-1

Latchford, A., Cohen, S., Auth, M., Scaillon, M., Viala, J., Daniels, R., Talbotec, C., Attard, T., Durno, C., & Hyer, W. (2019). Management of Peutz-Jeghers Syndrome in Children and Adolescents: A Position Paper From the ESPGHAN Polyposis Working Group. *J Pediatr Gastroenterol Nutr*, *68*(3), 442-452. https://doi.org/10.1097/mpg.0000000000002248

NCCN. (2023, October 30). *NCCN Clinical Practice Guidelines in Oncology: Genetic/Familial High-Risk Assessment: Colorectal Version 2.2023*. https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf

NHS. (2020). Juvenile polyposis syndrome. https://www.genomicseducation.hee.nhs.uk/documents/juvenile-polyposis-syndrome/

Nielsen, M., Joerink-van de Beld, M. C., Jones, N., Vogt, S., Tops, C. M., Vasen, H. F., Sampson, J. R., Aretz, S., & Hes, F. J. (2021). Analysis of MUTYH genotypes and colorectal phenotypes in patients With MUTYH-associated polyposis. *Gastroenterology*, *136*(2), 471-476. https://doi.org/10.1053/j.gastro.2008.10.056

Nielsen, M. L., H., Infante, E., Brand, R. (2015). *MUTYH-Associated Polyposis*. https://www.ncbi.nlm.nih.gov/books/NBK107219

NORD. (2014). Familial Adenomatous Polyposis. https://rarediseases.org/rare-diseases/familial-adenomatous-polyposis/

Provenzale, D., Gupta, S., Ahnen, D. J., Bray, T., Cannon, J. A., Cooper, G., David, D. S., Early, D. S., Erwin, D., Ford, J. M., Giardiello, F. M., Grady, W., Halverson, A. L., Hamilton, S. R., Hampel, H., Ismail, M. K., Klapman, J. B., Larson, D. W., Lazenby, A. J., . . . Darlow, S. (2016). Genetic/Familial High-Risk Assessment: Colorectal Version

1.2016, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw*, *14*(8), 1010-1030. https://doi.org/10.6004/jnccn.2016.0108

Robson, M. E., Bradbury, A. R., Arun, B., Domchek, S. M., Ford, J. M., Hampel, H. L., Lipkin, S. M., Syngal, S., Wollins, D. S., & Lindor, N. M. (2015). American Society of Clinical Oncology Policy Statement Update: Genetic and Genomic Testing for Cancer Susceptibility. *J Clin Oncol*, *33*(31), 3660-3667. https://doi.org/10.1200/jco.2015.63.0996

Sieber, O. M., Lipton, L., Crabtree, M., Heinimann, K., Fidalgo, P., Phillips, R. K., Bisgaard, M. L., Orntoft, T. F., Aaltonen, L. A., Hodgson, S. V., Thomas, H. J., & Tomlinson, I. P. (2003). Multiple colorectal adenomas, classic adenomatous polyposis, and germ-line mutations in MYH. *N Engl J Med*, *348*(9), 791-799. https://doi.org/10.1056/NEJMoa025283

Society, A. C. (2023). Colorectal Cancer Risk Factors. https://www.cancer.org/cancer/colon-rectal-cancer/causes-risks-prevention/risk-factors.html

Spirio, L., Olschwang, S., Groden, J., Robertson, M., Samowitz, W., Joslyn, G., Gelbert, L., Thliveris, A., Carlson, M., Otterud, B., & et al. (1993). Alleles of the APC gene: an attenuated form of familial polyposis. *Cell*, *75*(5), 951-957. https://doi.org/10.1016/0092-8674(93)90538-2

Stjepanovic, N., Moreira, L., Carneiro, F., Balaguer, F., Cervantes, A., Balmana, J., & Martinelli, E. (2019). Hereditary gastrointestinal cancers: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-updagger. *Ann Oncol*, *30*(10), 1558-1571. https://doi.org/10.1093/annonc/mdz233

Syngal, S., Brand, R. E., Church, J. M., Giardiello, F. M., Hampel, H. L., & Burt, R. W. (2015). ACG clinical guideline: Genetic testing and management of hereditary gastrointestinal cancer syndromes. *Am J Gastroenterol*, *110*(2), 223-262; quiz 263. https://doi.org/10.1038/ajg.2014.435

Wu, B.-D., Wang, Y.-J., Fan, L.-L., Huang, H., Zhou, P., Yang, M., & Shi, X.-L. (2020). Clinical and Genetic Analyses of 38 Chinese Patients with Peutz-Jeghers Syndrome. *BioMed Research International*, *2020*, 9159315. https://doi.org/10.1155/2020/9159315

Yang, J., Gurudu, S. R., Koptiuch, C., Agrawal, D., Buxbaum, J. L., Abbas Fehmi, S. M., Fishman, D. S., Khashab, M. A., Jamil, L. H., Jue, T. L., Law, J. K., Lee, J. K., Naveed, M., Qumseya, B. J., Sawhney, M. S., Thosani, N., Wani, S. B., & Samadder, N. J. (2020). American Society for Gastrointestinal Endoscopy guideline on the role of endoscopy in familial adenomatous polyposis syndromes. *Gastrointest Endosc*, *91*(5), 963-982.e962. https://doi.org/10.1016/j.gie.2020.01.028

Yang, M., Zhu, L., Zhu, L., Xu, D., & Yuan, Y. (2020). Role of a Rare Variant in APC Gene Promoter 1B Region in Classic Familial Adenomatous Polyposis. *Digestion*. https://doi.org/10.1159/000509234

Genetic Testing for PTEN Hamartoma Tumor Syndrome

Policy Number: AHS – M2087 – Genetic Testing for PTEN Hamartoma Tumor Syndrome	Prior Policy Name and Number, as applicable:
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Phosphatase and tensin homolog (PTEN) hamartoma tumor syndromes (PHTS) are primarily characterized by hamartomatous tumors (disorganized growths of native cells in native tissues) caused by *PTEN* germline mutations. PHTS includes Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), *PTEN*-related Proteus syndrome (PS), and Proteus-like syndrome (PLS)(C. Eng, 2016; Stanich & Roberts, 2023).

Genetic counseling is strongly recommended for individuals pursuing genetic testing for PHTS.

II. Related Policies

Policy Number	Policy Title
AHS-M2003	Genetic Testing for Breast, Ovarian, Pancreatic, and Prostate Cancers
AHS-M2108	Molecular Markers in Fine Needle Aspirates Of The Thyroid

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

All major and minor criteria are defined in Note 1

1) For asymptomatic individuals who are in a family with a known pathogenic *PTEN* mutation, the following testing **MEETS COVERAGE CRITERIA**:
   a) Testing restricted to the known familial mutation.
   b) Comprehensive *PTEN* gene sequencing when the specific *PTEN* mutation is unknown.

2) For individuals suspected of having Cowden Syndrome (CS)/PTEN hamartoma tumor syndrome (PHTS), genetic testing for a *PTEN* mutation **MEETS COVERAGE CRITERIA** when at least one of the following clinical signs is present:
   a) Three or more major criteria (one must be macrocephaly, Lhermitte-Duclos disease, or gastrointestinal hamartomas).
   b) Two major and three minor criteria.

3) For individuals not meeting the clinical diagnostic criteria above, genetic testing for a *PTEN* mutation **MEETS COVERAGE CRITERIA** when the individual has a personal history of any one of the following:
   a) Adult Lhermitte-Duclos disease (cerebellar tumors).
   b) Autism spectrum disorder and macrocephaly.
   c) Two or more biopsy-proven trichilemmomas.
   d) Two or more major criteria (one must be macrocephaly).
   e) Three major criteria without macrocephaly.
   f) Two major and two or more minor criteria (for individuals without macrocephaly).
   g) One major and three or more minor criteria.
   h) Four or more minor criteria.

4) For individuals with a personal history of Bannayan-Riley-Ruvalcaba syndrome (BRRS), genetic testing for a *PTEN* mutation **MEETS COVERAGE CRITERIA**.

5) For at-risk individuals with a relative who has been clinically but not genetically diagnosed with CS/PHTS or BRRS, genetic testing for a *PTEN* mutation **MEETS COVERAGE CRITERIA** when at least **one** of the following conditions are met:
   a) The at-risk individual has any one major criterion.
   b) The at-risk individuals has two minor criteria.

6) For individuals with a *PTEN* pathogenic/likely pathogenic variant detected by tumor profiling on any tumor type in the absence of germline analysis, genetic testing for a *PTEN* mutation **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

7) For all other situations not described above, genetic testing for a PTEN mutation **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: The NCCN provides the following major and minor testing criteria for Cowden Syndrome/PTEN Hamartoma Tumor Syndrome (Category 2A recommendation: based on lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate):

Major Testing Criteria

- Breast cancer
- Endometrial cancer
- Follicular thyroid cancer
- Multiple GI hamartomas or ganglioneuromas
- Macrocephaly (megalocephaly) (ie, ≥97%, 58 cm in adult female, 60 cm in adult male)
- Macular pigmentation of glans penis
- Mucocutaneous lesions (any of the following):
  - One biopsy-proven trichilemmoma
  - Multiple palmoplantar keratoses
  - Multifocal or extensive oral mucosal papillomatosis
  - Multiple cutaneous facial papules (often verrucuous)

Minor Testing Criteria

- Autism spectrum disorder
- Colon cancer
- ≥3 Esophageal glycogenic acanthoses
- Lipomas
- Intellectual disability (i.e., IQ ≤75)
- Papillary or follicular variant of papillary thyroid cancer
- Thyroid structural lesions (e.g., adenoma, nodule[s], goiter)

- Renal cell carcinoma
- Single GI hamartoma or ganglioneuroma
- Testicular lipomatosis
- Vascular anomalies (including multiple intracranial developmental venous anomalies)

IV. Table of Terminology

Term	Definition
ACG	American College of Gastroenterology
aCGH	Array comparative genomic hybridization
ACMG	American College of Medical Genetics and Genomics
AKT	Protein Kinase B
ALK	*Anaplastic lymphoma kinase gene*
ASDs	Autism spectrum disorders
BEN	Benign
BP	Base pair
BRCA1	*Breast cancer type 1 gene*
BRCA2	*Breast cancer type 2 gene*
BRRS	Bannayan-Riley-Ruvalcaba Syndrome
CC	Cleveland Clinic
CC15	Cleveland Clinic Score of 15
CGH	Comparative genomic hybridization
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CONF	Conflicting
CS	Cowden Syndrome
DNA	Deoxyribonucleic acid
DTC	Differentiated thyroid carcinoma
ERCC2	*ERCC excision repair 2 gene*
FDA	Food and Drug Administration
FTC	Follicular thyroid carcinoma
GI	Gastrointestinal
HRAS	*HRas proto-oncogene*
IQ	Intelligence quotient
LBEN	Likely benign
LDD	Lhermitte-Duclos Disease
LDTs	Laboratory-developed tests
LPATH	Likely pathogenic
MLPA	Multiplex ligation-dependent probe amplification

mTOR	Mechanistic Target of Rapamycin
MUTYH	*mutY DNA glycosylase*
NCCN	National Comprehensive Cancer Network
NGS	Next-generation sequencing
NORD	National Organization for Rare Disorders
PATH	Pathogenic
PCR	Polymerase chain reaction
PHTS	PTEN Hamartoma Tumor Syndromes
PI3K	Phosphatidylinositol 3-Kinase
PLS	Proteus-Like Syndrome
PS	Proteus Syndrome
PTEN	*Phosphatase and tensin homolog*
qPCR	Quantitative polymerase chain Reaction
RET	*Ret proto-oncogene*
RNA	Ribonucleic acid
SOLAMEN	Segmental Overgrowth, Lipomatosis, Arteriovenous Malformation, and
TSC2	*Tuberous sclerosis complex 2 gene*
VUS	Variant of uncertain significance

V. Scientific Background

Tumor suppressor genes serve several purposes within the body; these genes are able to regulate cell division, restore DNA errors, and tell cells when to die (a process known as apoptosis). Communication of these processes occurs via various cell signaling pathways. In particular, the tumor suppressor gene known as phosphatase and tensin homolog (*PTEN*) is an important phosphatase regulator of the phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway and the mechanistic target of rapamycin (mTOR) signaling pathway (Sansal & Sellers, 2004; Stanich & Roberts, 2023). The PI3K/AKT and mTOR signaling pathways are critical for cell proliferation, cell cycle progression, and apoptosis.

Loss of function of the *PTEN* gene contributes to an increased risk of both benign and malignant tumors and is implicated in increased lifetime risks of breast, thyroid, uterine, renal, and other cancers; patients may also display clinical features such as cognitive changes, skin changes, macrocephaly (enlarged head) and intestinal polyposis (Ngeow & Eng, 2020). Further, loss of *PTEN* gene functionality results in a spectrum of autosomal dominant disorders known *PTEN* hamartoma tumor syndromes (PHTS); this includes Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), *PTEN*-related Proteus syndrome (PS), and Proteus-like syndrome (PLS) (C. Eng, 2016; Charis Eng, 2016). Autism spectrum disorders (ASDs) with macrocephaly and

adult Lhermitte-Duclos disease (LDD) have also been associated with PHTS (Pilarski, 2019; Stanich & Roberts, 2023).

A diagnosis of PHTS is established by identification of a germline pathogenic variant in *PTEN* via molecular genetic testing. A single-gene sequence analysis will detect up to 80% of CS cases, 60% of BRRS cases, 50% PLS cases, and 20% of PS cases. Deletion and duplication analysis or promoter region analysis may also detect additional cases, but further research is required. In addition to single gene analysis, gene panels or further comprehensive testing may assist with clinical management (C. Eng, 2016). All types of mutations have been reported including missense, nonsense, splice site, insertions, and deletions; therefore, *PTEN* mutation testing usually requires sequence analysis of the entire coding region and deletion/duplication analysis(C. Eng, 2016; Marsh et al., 1999). Mutations have also been reported in the *PTEN* promoter, and a test for mutations in this region of the gene has become clinically available (Stanich & Roberts, 2023). Mingo et al. (2018) recently found that the pathogenicity of frequent *PTEN* mutations targeting the N-terminus "may be related, at least in part, with the retention of *PTEN* in the nucleus. This could be important for the implementation of precision therapies for patients with alterations in the *PTEN* pathway." Germline *PTEN* mutations are found in approximately 20 to 34% of individuals who meet clinical criteria for CS or who meet criteria for genetic testing (Pilarski, 2019; Pilarski et al., 2011; Tan et al., 2011). This number seems to be highly variable as Giorgianni et al. (2013) state that approximately 80% of CS patients have a *PTEN* mutation at locus 10q23.2.

Cowden Syndrome (CS)

Cowden Syndrome is defined by multiple hamartomas with a high risk for benign and malignant tumors in the thyroid, breast, and endometrium. Besides multiple hamartomas in a variety of tissues, patients typically exhibit macrocephaly as well as characteristic dermatologic manifestations, such as trichilemmomas (benign tumors originating from hair follicles) and papillomatous papules (wart-like growths) (Garofola & Gross, 2024). For individuals with CS, the lifetime risk of developing breast cancer is as high as 85%; for thyroid cancer, which is usually follicular carcinoma, the lifetime risk is as high as 35%; finally, for endometrial cancer, the risk may reach as high as 28% (C. Eng, 2016; Charis Eng, 2016). The estimated prevalence of CS is 1 in 200,000 to 250,000, though it is likely underreported (Stanich & Roberts, 2023). Of all PHTS, only CS has been documented to confer a predisposition to cancer; however, it has been suggested that any patients with *PTEN* mutations should be assumed to have cancer risks similar to CS (Stanich & Roberts, 2023).

Bannayan-Riley-Ruvalcaba Syndrome (BRRS)

Bannayan-Riley-Ruvalcaba Syndrome is characterized by hamartomas along with macrocephaly, lipomas, and pigmented penile macules. It is also associated with high birth weight, developmental delay or deficiency, proximal muscle myopathy, joint hypermobility, high palate, and scoliosis (Stanich & Roberts, 2023). Similar mutations in the *PTEN* gene are found in both CS and BRRS, which are now considered phenotypically distinct presentations of a similar genetic abnormality (Lachlan et al., 2007; Marsh et al., 1999).

PTEN-related *Proteus Syndrome (PS)*

PTEN-related Proteus Syndrome is characterized by hamartomatous overgrowth of multiple tissues with hyperostosis (excessive bone growth), vascular malformations, dysregulation of fatty tissues, connective tissue nevi, and epidermal nevi. Phenotypic features of PS are usually present at birth and progress over an individual's lifetime (Hobert & Eng, 2009).

Proteus-like Syndrome (PLS)

Proteus-like Syndrome is undefined but typically refers to individuals with similar clinical features to PS who do not meet the diagnostic criteria (C. Eng, 2016; Charis Eng, 2016; Hobert & Eng, 2009). Overall, limited information is available on PLS. Stanich and Roberts (2023) report that PLS cases typically exhibit "a distinct type of epidermal nevus (also referred to as segmental overgrowth, lipomatosis, arteriovenous malformation, and epidermal nevus (SOLAMEN) syndrome or type 2 segmental Cowden syndrome)."

Autism Spectrum Disorders (ASDs)

Autism Spectrum Disorders are a collection of disorders presenting with common abnormalities associated with social and communication behaviors. A few studies have been published which research the relationship between *PTEN* mutations and ASDs. Schaefer and Mendelsohn (2013) completed a literature review of at least seven different studies and determined that 15 of 318 (5%) individuals with an ASD diagnosis had a pathogenic *PTEN* mutation; further, macrocephaly was present in all 15 cases.

Lhermitte-Duclos Disease (LDD)

Lhermitte-Duclos Disease is a rare disease characteristic of benign tumor development in the granule cells of the cerebellum. It has been reported that "Most LDD patients appear to have a germline loss of the *PTEN* allele and go on to lose the remaining *PTEN* allele at some point, thereby allowing abnormal growth of the granule cells" (Giorgianni et al., 2013). LDD often develops in conjunction with CS, but may also develop singularly (Stanich & Roberts, 2023).

Proprietary Testing

Ambry Genetics, GeneDx, Invitae, and Blueprint Genetics all have genetic tests for *PTEN* mutations. Invitae and Blueprint Genetics both use a next-generation sequencing (NGS) assay that performs full *PTEN* gene sequencing and deletion/duplication analysis (Ambry, 2024; Blueprint, 2024; GeneDx, 2024; Invitae, 2022). The Invitae assay "achieves >99% analytical sensitivity and specificity for single nucleotide variants, insertions, and deletions <15bp [base pair] in length, and exon-level deletions and duplications." The Blueprint Genetics NGS sequencing assay for single genes performs similarly with a >99% specificity and sensitivity for single nucleotide variants, including insertions, deletions, and indels up to 50 bp. The Invitae assay has a clinical sensitivity of 85% for CS, 70% for BRRS, 20-50% for Proteus-Like Syndrome, and 10% for PTEN-related macrocephalic autism spectrum disorder. The clinical sensitivity is not provided by Blueprint Genetics; however, clinical sensitivity is said to vary based on patient clinical presentation (Blueprint, 2024; Invitae, 2022).

GeneDx utilizes a PCR-amplified assay with capillary sequencing to confirm variants of clinical or uncertain significance. Their assay also performs deletion/duplication testing using "either exon-level CGH [comparative genomic hybridization] or MLPA [multiplex ligation-dependent probe amplification]. Confirmation of copy number changes is performed by MLPA, qPCR [quantitative polymerase chain reaction], or repeat aCGH [array CGH] analysis." GeneDx has found that "for those probands with Cowden syndrome, sequence analysis of the coding and promoter regions is expected to detect 47-80% of causative variants, while large deletions and duplications have been reported. For individuals with Bannayan-Riley-Ruvalcaba syndrome, ~60% of causative pathogenic variants will be detected by sequencing while 11% will be detected by deletion/duplication analysis" (GeneDx, 2024). Ambry Genetics also utilizes a PCR-amplified assay with NGS, as well as Sanger Sequencing for any regions "missing or with insufficient read depth coverage for reliable heterozygous variant detection." Their assay also performs gross deletion and duplication analysis, with any copy number changes detected from NGS confirmed by targeted chromosomal microarray or MLPA. Their analytical sensitivity is >99.9% for described mutations in the *PTEN* gene when present and has an identical clinical sensitivity to that described by Invitae (Ambry, 2024).

Clinical Utility and Validity

Yehia et al. (2018) conducted a four-year multicenter study; all participants had suspected BRRS or Cowden/Cowden-like (CS/CS-like) syndromes without *PTEN* mutations. Exome sequencing and targeted analysis was completed on 59 genes supported by the American College of Medical Genetics and Genomics

(ACMG), as well as on 24 additional genes known to be associated with inherited cancer syndromes. "Pathogenic or likely pathogenic cancer susceptibility gene alterations were found in seven of the 87 (8%) CS/CS-like and BRRS patients and included *MUTYH*, *RET, TSC2*, *BRCA1*, *BRCA2*, *ERCC2* and *HRAS*" (Yehia et al., 2018).

Ngeow et al. (2015) estimated the cost effectiveness of each *PTEN* mutation detected in CS-like patients using the PTEN Cleveland Clinic (CC) score. This is a risk assessment tool which helps to estimate an individual's risk of having a *PTEN* mutation. Several questions are answered through an online survey, and a total risk score between 0 and 100 is provided (CC, 2020). The authors found that the cost to detect one *PTEN* mutation was between $3720 to $4573 at a CC score of 15 (CC15). The authors also concluded that "In sensitivity analyses, CC15 is robustly the most cost-effective strategy for probands who are younger than 60 years" (Ngeow et al., 2015).

Isik et al. (2020) researched the genotype to phenotype correlation of patients with *PTEN* mutations. A total of ten molecularly confirmed PHTS patients participated in this study; these participants originated from seven different families. The authors note that "Macrocephaly was the most common clinical finding, involving all patients. This was followed by skin lesions, neurodevelopmental delay, and pathologic cranial magnetic resonance imaging findings" (Isik et al., 2020). Further, sequencing of the *PTEN* gene identified seven different *PTEN* variants; four variants were located in exon five. The authors conclude by highlighting the importance of screening for *PTEN* mutations in patients with macrocephaly, particularly due to an increased risk of cancer.

Jonker et al. (2020) conducted an extensive literature search to identify evidence to support a thyroid carcinoma surveillance program among children with PHTS. They found that children with PHTS were at an increased risk of developing differentiated thyroid carcinoma (DTC), "with 4 years being the youngest age reported at presentation and FTC [follicular thyroid carcinoma] being overrepresented." This finding supports genetic testing for *PTEN* mutations, as that could provide guidance and awareness for potential thyroid cancer development at an early age and reduce morbidity with early diagnosis. "Consensus within the study team was reached to recommend surveillance from the age of 10 years onwards, since at that age the incidence of DTC seems to reach 5%" (Jonker et al., 2020). This recommendation was also supported by Baran et al. (2021), who further retrospectively investigated patients at the Children's Hospital of Philadelphia with a PHTS diagnosis between January 2003 and June 2019. They found "the most common clinical feature at presentation was macrocephaly (85.1%), followed by impaired development (42.0%), skin/oral lesions (30.9%), and autism spectrum disorder (27.2%)," which also appears to corroborate the physical findings of Isik et al. (2020).

Cummings et al. (2022) conducted a systematic review and meta-analysis of the behavioral and psychological features associated with *PTEN* mutations and the prevalence of autism spectrum disorder characteristics. Using a random effects model, the researchers estimated a pooled prevalence of ASD at 25%, with frequent reporting of intellectual disability and developmental delay, including issues that pertained globally, to motor, and to speech and language. Less frequently were mentions of emotional difficulties ad impaired cognitive functioning. This indicates that further research using appropriate comparison groups may provide greater depth of the association with aberrations affecting *PTEN*.

VI. Guidelines and Recommendations

The International Cowden Consortium Operational Criteria for the Diagnosis of Cowden Syndrome *Ver 2000*

The following recommendations were provided:

Pathognomonic criteria

Mucocutaneous lesions

- Trichilemmomas, facial
- Acral keratoses
- Papillomatous papules
- Mucosal lesions

Major criteria

- Breast carcinoma
- Thyroid carcinoma (non-medullary), especially follicular thyroid carcinoma
- Macrocephaly (megalencephaly) (say, ≥97th centile)
- Lhermitte-Duclos disease (LDD)
- Endometrial carcinoma

Minor criteria

- Other thyroid lesions (e.g., adenoma or multinodular goitre)
- Mental retardation (say, IQ
- GI hamartomas
- Fibrocystic disease of the breast
- Lipomas
- Fibromas
- Genito-urinary tumours (e.g., renal cell carcinoma, uterine fibroids) or malformation

According to the International Cowden Consortium, an operational diagnosis of Cowden Syndrome is made if the individual meets any of the following:

(1) Mucocutaneous lesions alone if:

(a) there are six or more facial papules, of which three or more must be trichilemmoma, or
(b) cutaneous facial papules and oral mucosal papillomatosis, or
(c) oral mucosal papillomatosis and acral keratoses, or
(d) palmoplantar keratoses, six or more

(2) Two major criteria but one must include macrocephaly or LDD

(3) One major and three minor criteria

(4) Four minor criteria

According to the International Cowden Consortium, an operational diagnosis of Cowden Syndrome in a family where one person is diagnostic for Cowden syndrome is made if any of the following criteria are met:

(1) The pathognomonic mucocutaneous lesion

(2) Any one major criterion with or without minor criteria

(3) Two minor criteria

Pilarski et al. (2013) published revised evidence-based criteria covering the spectrum of PTEN-related clinical disorders. The revised guidelines define the operational diagnosis of PTEN Hamartoma Tumor syndrome in an individual who meets either one of the following:

- Three or more major criteria, but one must include macrocephaly, Lhermitte-Duclos disease, or gastrointestinal hamartomas; or
- Two major and three minor criteria

The operational diagnosis in a family where one individual meets the revised PTEN Hamartoma Tumor syndrome clinical diagnostic criteria or has a PTEN mutation is as follows (Pilarski et al., 2013):

- Any two major criteria with or without minor criteria; or
- One major and two minor criteria; or
- Three minor criteria

Pilarski et al. (2013) defined the major and minor criteria as follows:

Major Criteria:

- Breast Cancer
- Endometrial cancer (epithelial)
- Thyroid Cancer (follicular)
- Gastrointestinal hamartomas (including ganglioneuromas, adenomas, hyperplastic polyps; ≥3)
- Lhermitte-Duclos disease (adult)
- Macrocephaly (megalocephaly) (i.e., ≥97th percentile, 58 cm in adult women, 60 cm in adult men)
- Macular pigmentation of glans penis
- Multiple mucocutaneous lesions (any of the following):
  - Multiple trichilemmomas (≥3, at least one biopsy proven)
  - Acral keratoses (≥3 palmoplantar keratotic pits and/or acral hyperkeratotic papules) 4 – GT63
  - Mucocutaneous neuromas (≥3)
  - Oral papillomas (particularly on tongue and gingiva), multiple (≥3) OR biopsy proven OR dermatologist diagnosed

Minor Criteria:

- Autism spectrum disorder
- Colon cancer
- ≥3 esophageal glycogenic acanthoses
- Lipomas (≥3)
- Intellectual disability (i.e., IQ ≤75)
- Renal cell carcinoma
- Testicular lipomatosis
- Thyroid cancer (papillary or follicular variant of papillary)
- Thyroid structural lesions (e.g., adenoma, multinodular goiter)
- Vascular anomalies (including multiple intracranial developmental venous anomalies)

National Comprehensive Cancer Network (NCCN)

The NCCN guidelines for Cowden Syndrome/PTEN Hamartoma Tumor syndrome recommend testing in individuals who meet any of the following criteria:

- "Individual from a family with a known *PTE*^{cc} P/LP variant
- Individual with a personal history of Bannayan-Riley-Ruvalcaba syndrome (BRRS)

- Individual meeting clinical diagnostic criteria[mm] for CS/PHTS
- Individual not meeting the clinical diagnostic criteria[mm] for CS/PHTS with a personal history of:
  - Adult Lhermitte-Duclos disease (cerebellar tumors); or
  - Autism spectrum disorder and macrocephaly; or
  - Two or more biopsy-proven trichilemmomas; or
  - Two or more major criteria (one must be macrocephaly); or
  - Three major criteria, without macrocephaly; or
  - One major and ≥3 minor criteria;[nn] or
  - ≥4 minor criteria

- individual with a relative with a clinical diagnosis of CS/PHTS or BRRS for whom testing has not been performed
  - individual must have the following:
    - Any one major criterion, OR
    - Two minor criteria
- *PTEN* P/LP variant detected by tumor genomic testing on any tumor type in the absence of germline analysis[oo]"

The relevant superscripts are captured below.

"[cc]When this gene is included as part of a multi-gene panel, an individual does not need to meet these testing criteria if testing criteria on other testing criteria pages are met.

[nn]If an individual has two or more major criteria, such as breast cancer and nonmedullary thyroid cancer, but does not have macrocephaly, one of the major criteria may be included as one of the three minor criteria to meet testing criteria.

[oo]This should prompt a careful evaluation of personal and family history of the individual to determine the yield of germline sequencing. Somatic PTEN pathogenic/likely pathogenic variants are common in many tumor types in absence of germline pathogenic/likely pathogenic variant" (NCCN, 2024).

Aforementioned major and minor testing criteria are reported below.

"Major Testing Criteria:

- Breast cancer
- Endometrial cancer
- Follicular thyroid cancer

- Multiple GI hamartomas or ganglioneuromas[pp]
- Macrocephaly (megalocephaly) (ie, ≥97%, 58 cm in adult female, 60 cm in adult male)[qq]
- Macular pigmentation of glans penis
- Mucocutaneous lesions[rr] (any of the following):
  - One biopsy-proven trichilemmoma
  - Multiple palmoplantar keratoses
  - Multifocal or extensive oral mucosal papillomatosis
  - Multiple cutaneous facial papules (often verrucuous)

Minor Testing Criteria:[ss]

- Autism spectrum disorder
- Colon cancer
- ≥3 Esophageal glycogenic acanthoses
- Lipomas
- Intellectual disability (ie, IQ ≤75)
- Papillary or follicular variant of papillary thyroid cancer
- Thyroid structural lesions (e.g., adenoma nodule[s], goiter)
- Renal cell carcinoma
- Single GI hamartoma or ganglioneuroma
- Testicular lipomatosis
- Vascular anomalies (including multiple intracranial developmental venous anomalies)" (NCCN, 2024)

The relevant superscripts are captured below.

[pp]Multiple polyp types are often seen in patients with PHTS, and less commonly may include adenomas, hyperplastic polyps, and other histologies.

[rr]The literature available on mucocutaneous lesions is not adequate to accurately specify the number or extent of mucocutaneous lesions required to be a major criterion for CS/PHTS. Clinical judgment should be used.

[ss]evidence exists in the literature to include fibrocystic disease of the breast, fibromas, and uterine fibroids as diagnostic criteria" (NCCN, 2024)

The NCCN also notes that "Other cancers associated with PTEN but not in the testing criteria include: colorectal, kidney cancer, and melanoma."

In their section outlining the "REVISED CLINICAL DIAGNOSTIC CRITERIA FOR PTEN HAMARTOMA TUMOR SYNDROME," the NCCN recommends the following:

"Operational diagnosis in an individual (either of the following):

1. Three or more major criteria, but one must include macrocephaly, Lhermitte-Duclos disease, or GI hamartomas; or
2. Two major and three minor criteria.

Operational diagnosis in a family where one individual meets revised PTEN hamartoma tumor syndrome clinical diagnostic criteria or has a *PTEN* P/LP variant:

1. Any two major criteria with or without minor criteria; or
2. One major and two minor criteria; or
3. Three minor criteria" (NCCN, 2024).

ClinGen PTEN Expert Panel

This expert panel was convened by the ClinGen Hereditary Cancer Clinical Domain Working Group to develop PTEN-specific genetic variant interpretations. A total of 42 variants were included in the following categories: benign/likely benign (BEN/LBEN), pathogenic/likely pathogenic (PATH/LPATH), uncertain significance (VUS), and conflicting (CONF) ClinVar assertions.

The following variants were considered BEN/LBEN: 9C>G, 903G>A, 1026C>A, 1311T>C, 18A>G, 75G>A, 132C>T, 360A>G, 1104T>G, 79+35C>T, 165-13_165-10delGTTT, 254-39G>T, 801+23G>A, and 1026+32T>G.

The following variants were considered PATH/LPATH: 50_51delAA, 511C>T, 892C>T, 964A>T, 987_990delTAAA, 80-1G>C, 165-1G>A, 493-2A>G, 801+1delG, 802-2A>T, 1026+1G, 103A>G, 389G>A, 407G>A, 517C>T, and 737C>T.

The following variants were considered VUS or CONF: 1170C>T, 209+3A>T, 235G>A, 304_306dupAAA, 1052_1054delTAG, 1171C>T, 764G>A, 44G>A, 78C>T, 209+4_209+7delAGTA, 521A>G (Mester et al., 2018).

American College of Medical Genetics (ACMG)

In 2013, the ACMG published guidelines for identifying the etiology of autism spectrum disorders (ASDs). These guidelines state that "*PTEN* testing [should] be reserved for patients with ASDs with a head circumference above the 98th percentile" (Schaefer & Mendelsohn, 2013). The authors then suggest that of all ASD-related genetic evaluations, about 5% of testing will be due to *PTEN* mutations.

National Organization for Rare Disorders (NORD)

The NORD asserts that "The primary findings in PHTS include increased risk for certain types of cancer, benign tumors and tumor-like malformations (hamartomas) and neurodevelopmental disorders. The symptoms of PHTS vary greatly from person to person and can develop at any age." However, some noteworthy sign and symptoms that indicate PTEN genetic testing are captured below.

- "A small percentage of adults develop a rare tumor known as a cerebellar dysplastic gangliocytoma (Lhermitte-Duclos disease). Symptoms of Lhermitte-Duclos disease include increased intracranial pressure, impaired ability to coordinate voluntary movements (ataxia) and seizures. It is rare when a person with adult-onset Lhermitte-Duclos does not have an underlying PTEN variant, and observing this tumor type is an automatic indicator for PTEN genetic testing."
- "Macrocephaly (large head size) is found in 94% of measured patients with PHTS and can be a helpful screening tool to identify patients for PTEN genetic testing. In most patients, large head size is caused by overgrowth of brain tissue. The head shape may also be longer than average relative to width in some individuals (dolicocephaly)."

The authors go further to state that "Sotos syndrome is a rare genetic disorder characterized by excessive growth that occurs prior to and after birth (prenatally and postnatally)….The overlap of neurodevelopmental disabilities and macrocephaly in both PHTS and Sotos syndrome indicates the need for genetic testing to avoid misdiagnosis" (NORD, 2023). Genetic counseling is also suggested.

The authors report that "A diagnosis of PHTS may be suspected based upon a thorough clinical evaluation, a detailed patient history and the presence of characteristic findings. Recently, a variant risk calculator has been developed which can estimate the risk for adults to have a PTEN variant based on their personal history characteristics; this tool is available online at https://www.lerner.ccf.org/gmi/ccscore/. The diagnosis can only be confirmed when a variant of the PTEN gene is identified (NORD, 2023).

The current suggested screening regimens by age include:

"**Children (under age 18)**

- Yearly thyroid ultrasound starting at 7 years of age
- Yearly skin check with physical examination
- Consider neurodevelopmental evaluation

Adults

- Monthly breast self-examination starting at age 18 years
- Yearly thyroid ultrasound and dermatologic evaluation
- Females: breast screening (at minimum mammogram) yearly beginning at age 35; MRI may also be incorporated
- Females: consider endometrial cancer screening starting at age 35 years. Personalized management including endometrial biopsy every 1-2 years and transvaginal ultrasound as needed (postmenopausal)
- Colonoscopy beginning at age 35 (unless symptomatic or close relative with colon cancer before age 40 years); colonoscopy frequency every 5 years; more frequently if symptomatic or polyps are found
- Biannual (every other year) renal imaging (CT or MRI preferred) beginning at age 40

For patients with a family history of a particular cancer type, screening may be considered 5-10 years prior to the youngest diagnosis in the family. For example, a patient whose mother developed breast cancer at 30 may begin breast surveillance at age 25-30" (NORD, 2023).

American College of Gastroenterology (ACG)

In 2015, the ACG published guidelines on genetic testing and management of hereditary gastrointestinal cancer syndromes. These guidelines clearly state that the "Genetic evaluation of a patient with possible CS should include testing for *PTEN* mutations… CS is caused by mutations in the *PTEN* gene. Once a disease-causing mutation is identified in a patient with CS or related conditions, other family members should undergo mutation-specific testing to determine whether the disease is present or absent so that appropriate surveillance can be undertaken" (Syngal et al., 2015). Further, the ACG also notes that "Surveillance in affected or at-risk CS patients should include screening for colon, stomach, small bowel, thyroid, breast, uterine, kidney, and skin (melanoma) cancers (conditional recommendation, low quality of evidence)" (Syngal et al., 2015).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-

database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81321	PTEN (phosphatase and tensin homolog) (eg, Cowden syndrome, PTEN hamartoma tumor syndrome) gene analysis; full sequence analysis
81322	PTEN (phosphatase and tensin homolog) (eg, Cowden syndrome, PTEN hamartoma tumor syndrome) gene analysis; known familial variant
81323	PTEN (phosphatase and tensin homolog) (eg, Cowden syndrome, PTEN hamartoma tumor syndrome) gene analysis; duplication/deletion variant
0235U	PTEN (phosphatase and tensin homolog) (eg, Cowden syndrome, PTEN hamartoma tumor syndrome), full gene analysis, including small sequence changes in exonic and intronic regions, deletions, duplications, mobile element insertions, and variants in non-uniquely mappable regions Proprietary test: Genomic Unity® PTEN Analysis Lab/Manufacturer: Variantyx Inc

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Ambry. (2024). *PTEN-related Disorders*. https://www.ambrygen.com/providers/genetic-testing/130/oncology/pten-related-disorders

Baran, J. A., Tsai, S. D., Isaza, A., Brodeur, G. M., MacFarland, S. P., Zelley, K., Adams, D. M., Franco, A. T., & Bauer, A. J. (2021). The Clinical Spectrum of PTEN Hamartoma Tumor Syndrome: Exploring the Value of Thyroid Surveillance. *Horm Res Paediatr*, 1-9. https://doi.org/10.1159/000515731

Blueprint. (2024). *PTEN Single Gene Test*. https://blueprintgenetics.com/tests/single-gene-tests/pten-single-gene-test-2/

CC. (2020). *Cleveland Clinic PTEN Risk Calculator*. https://www.lerner.ccf.org/gmi/ccscore/

Cummings, K., Watkins, A., Jones, C., Dias, R., & Welham, A. (2022). Behavioural and psychological features of PTEN mutations: a systematic review of the literature and meta-analysis of the prevalence of autism spectrum disorder characteristics. *J Neurodev Disord*, *14*(1), 1. https://doi.org/10.1186/s11689-021-09406-w

Eng, C. (2016). *PTEN Hamartoma Tumor Syndrome*. University of Washington, Seattle University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved. https://www.ncbi.nlm.nih.gov/books/NBK1488/

Eng, C. (2016). PTEN Hamartoma Tumor Syndrome [Text]. *GeneReviews*. https://www.ncbi.nlm.nih.gov/pubmed/

Garofola, C., & Gross, G. P. (2024). Cowden Disease (Multiple Hamartoma Syndrome). In *StatPearls*. StatPearls Publishing LLC. https://www.ncbi.nlm.nih.gov/pubmed/30252240

GeneDx. (2024). *PTEN Gene Sequencing and Del/Dup*. https://providers.genedx.com/tests/detail/pten-gene-sequencing-and-del-dup-604

Giorgianni, A., Pellegrino, C., De Benedictis, A., Mercuri, A., Baruzzi, F., Minotto, R., Tabano, A., & Balbi, S. (2013). Lhermitte-Duclos disease. A case report. *Neuroradiol J*, *26*(6), 655-660. https://doi.org/10.1177/197140091302600608

Hobert, J. A., & Eng, C. (2009). PTEN hamartoma tumor syndrome: an overview. *Genet Med*, *11*(10), 687-694. https://doi.org/10.1097/GIM.0b013e3181ac9aea

Invitae. (2022). *Invitae PTEN-Related Disorders Test*. https://www.ncbi.nlm.nih.gov/gtr/tests/528497/performance-characteristics/

Isik, E., Simsir, O. S., Solmaz, A. E., Onay, H., Atik, T., Aykut, A., Durmaz, A., Cogulu, O., & Ozkinay, F. (2020). Clinical and molecular aspects of PTEN mutations in 10 pediatric patients. *Ann Hum Genet*. https://doi.org/10.1111/ahg.12380

Jonker, L. A., Lebbink, C. A., Jongmans, M. C. J., Nievelstein, R. A. J., Merks, J. H. M., Nieveen van Dijkum, E. J. M., Links, T. P., Hoogerbrugge, N., van Trotsenburg, A. S. P., & van Santen, H. M. (2020). Recommendations on Surveillance for Differentiated Thyroid Carcinoma in Children with PTEN Hamartoma Tumor Syndrome. *Eur Thyroid J*, *9*(5), 234-242. https://doi.org/10.1159/000508872

Lachlan, K. L., Lucassen, A. M., Bunyan, D., & Temple, I. K. (2007). Cowden syndrome and Bannayan Riley Ruvalcaba syndrome represent one condition with variable expression and age-related penetrance: results of a clinical study of PTEN mutation carriers. *J Med Genet*, *44*(9), 579-585. https://doi.org/10.1136/jmg.2007.049981

Marsh, D. J., Kum, J. B., Lunetta, K. L., Bennett, M. J., Gorlin, R. J., Ahmed, S. F., Bodurtha, J., Crowe, C., Curtis, M. A., Dasouki, M., Dunn, T., Feit, H., Geraghty, M. T., Graham, J.

M., Jr., Hodgson, S. V., Hunter, A., Korf, B. R., Manchester, D., Miesfeldt, S., . . . et al. (1999). PTEN mutation spectrum and genotype-phenotype correlations in Bannayan-Riley-Ruvalcaba syndrome suggest a single entity with Cowden syndrome. *Hum Mol Genet*, *8*(8), 1461-1472. https://pubmed.ncbi.nlm.nih.gov/10400993/

Mester, J. L., Ghosh, R., Pesaran, T., Huether, R., Karam, R., Hruska, K. S., Costa, H. A., Lachlan, K., Ngeow, J., Barnholtz-Sloan, J., Sesock, K., Hernandez, F., Zhang, L., Milko, L., Plon, S. E., Hegde, M., & Eng, C. (2018). Gene-specific criteria for PTEN variant curation: Recommendations from the ClinGen PTEN Expert Panel. *Hum Mutat*, *39*(11), 1581-1592. https://doi.org/10.1002/humu.23636

Mingo, J., Rodriguez-Escudero, I., Luna, S., Fernandez-Acero, T., Amo, L., Jonasson, A. R., Zori, R. T., Lopez, J. I., Molina, M., Cid, V. J., & Pulido, R. (2018). A pathogenic role for germline PTEN variants which accumulate into the nucleus. *Eur J Hum Genet*. https://doi.org/10.1038/s41431-018-0155-x

NCCN. (2024, February 12, 2024). *Genetic/Familial High-Risk Assessment:Breast, Ovarian, and Pancreatic Version*. https://www.nccn.org/professionals/physician_gls/pdf/genetics_bop.pdf

Ngeow, J., & Eng, C. (2020). PTEN in Hereditary and Sporadic Cancer. *Cold Spring Harb Perspect Med*, *10*(4). https://doi.org/10.1101/cshperspect.a036087

Ngeow, J., Liu, C., Zhou, K., Frick, K. D., Matchar, D. B., & Eng, C. (2015). Detecting Germline PTEN Mutations Among At-Risk Patients With Cancer: An Age- and Sex-Specific Cost-Effectiveness Analysis. *J Clin Oncol*, *33*(23), 2537-2544. https://doi.org/10.1200/jco.2014.60.3456

NORD. (2023, 11/7/2023). *PTEN Hamartoma Tumor Syndrome*. https://rarediseases.org/rare-diseases/pten-hamartoma-tumor-syndrome/

Pilarski, R. (2019). PTEN Hamartoma Tumor Syndrome: A Clinical Overview. *Cancers (Basel)*, *11*(6). https://doi.org/10.3390/cancers11060844

Pilarski, R., Burt, R., Kohlman, W., Pho, L., Shannon, K. M., & Swisher, E. (2013). Cowden syndrome and the PTEN hamartoma tumor syndrome: systematic review and revised diagnostic criteria. *J Natl Cancer Inst*, *105*(21), 1607-1616. https://doi.org/10.1093/jnci/djt277

Pilarski, R., Stephens, J. A., Noss, R., Fisher, J. L., & Prior, T. W. (2011). Predicting PTEN mutations: an evaluation of Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome clinical features. *J Med Genet*, *48*(8), 505-512. https://doi.org/10.1136/jmg.2011.088807

Sansal, I., & Sellers, W. R. (2004). The biology and clinical relevance of the PTEN tumor suppressor pathway. *J Clin Oncol*, *22*(14), 2954-2963. https://doi.org/10.1200/jco.2004.02.141

Schaefer, G. B., & Mendelsohn, N. J. (2013). Clinical genetics evaluation in identifying the etiology of autism spectrum disorders: 2013 guideline revisions. *Genet Med*, *15*(5), 399-407. https://doi.org/10.1038/gim.2013.32

Stanich, P. P., & Roberts, M. E. (2023, 08/24/2023). *PTEN hamartoma tumor syndromes, including Cowden syndrome*. https://www.uptodate.com/contents/pten-hamartoma-tumor-syndromes-including-cowden-syndrome

Syngal, S., Brand, R. E., Church, J. M., Giardiello, F. M., Hampel, H. L., & Burt, R. W. (2015). ACG clinical guideline: Genetic testing and management of hereditary gastrointestinal cancer syndromes. *Am J Gastroenterol*, *110*(2), 223-262; quiz 263. https://doi.org/10.1038/ajg.2014.435

Tan, M. H., Mester, J., Peterson, C., Yang, Y., Chen, J. L., Rybicki, L. A., Milas, K., Pederson, H., Remzi, B., Orloff, M. S., & Eng, C. (2011). A clinical scoring system for selection of patients for PTEN mutation testing is proposed on the basis of a prospective study of 3042 probands. *Am J Hum Genet*, *88*(1), 42-56. https://doi.org/10.1016/j.ajhg.2010.11.013

Yehia, L., Ni, Y., Sesock, K., Niazi, F., Fletcher, B., Chen, H. J. L., LaFramboise, T., & Eng, C. (2018). Unexpected cancer-predisposition gene variants in Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome patients without underlying germline PTEN mutations. *PLoS Genet*, *14*(4), e1007352. https://doi.org/10.1371/journal.pgen.1007352

Genetic Testing for Rett Syndrome

Policy Number: AHS – M2088 – Genetic Testing for Rett Syndrome	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Rett syndrome (RTT) is a rare X-linked neurodevelopmental disorder that occurs almost exclusively in females and is usually caused by mutations in the methyl CpG binding protein 2 (*MECP2*) gene (Amir et al., 1999). It is characterized by normal early growth and development, followed by regressions in development, walking, language, and purposeful use of the hands, along with slowed brain and head growth, distinctive hand movements, seizures, and intellectual disability (Colvin et al., 2004; Hagberg et al., 1983; Leonard et al., 2017; Naidu et al., 1986; Neul et al., 2010; Rett, 1966).

Terms such as male and female are used when necessary to refer to sex assigned at birth.

II. Related Policies

Policy Number	Policy Title
AHS – M2176	Testing for Developmental Delay

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For a child with developmental delay/intellectual disability **and** signs/symptoms of Rett syndrome (RTT), but for whom there is uncertainty in the clinical diagnosis, genetic testing of *MECP2*, *CDKL5*, and/or *FOXG1* to confirm a diagnosis of RTT **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

2) For all other situations not described above (e.g., prenatal screening, testing of family members), genetic testing for RTT **DOES NOT MEET COVERAGE CRITERIA**.

IV. Table of Terminology

Term	Definition
AAN	American Academy of Neurology
AAP	American Academy of Pediatrics
ACMG	American College of Medical Genetics
ASD	Autism spectrum disorder
BDNF	Brain-derived neurotrophic factor
CDKL5	Cyclin-dependent kinase-like 5
CMS	Centers for Medicare and Medicaid
CNS	Child Neurology Society
CPS	Canadian Pediatric Society
CTNNB1	*Catenin beta 1*
DNA	Deoxyribonucleic acid
FDA	Food and Drug Administration
FOXG1	*Forkhead box g1*
GABA	Gamma-aminobutyric acid
GDD	Global developmental delay
LDT	Laboratory developed test
MDS	*Methyl CPG binding protein* duplication syndrome
MECP	*Methyl CPG binding protein*
miRNA	Micro ribonucleic acid
MLPA	Multiplex ligation-dependent probe amplification
NGS	Next generation sequencing
qPCR	Quantitative polymerase chain reaction
REST	Rett evaluation of symptoms and treatments
RNA	Ribonucleic acid
RTT	Rett Syndrome
SCN1A	*Sodium voltage-gated channel alpha subunit 1*

WDR45	*WD repeat domain 45*
XLID	X-linked intellectual disability

V. Scientific Background

Rett syndrome (RTT) is a severe neurodevelopmental disorder which affects approximately 1:10,000 live female births in the United States annually (Hagberg, 1985; NORD, 2023). It is a prominent cause of severe intellectual disability in women, accounting for up to 10% of cases inherited genetically (Armstrong, 1997). Originally thought to be lethal in males (Amir et al., 1999; Chahil et al., 2018; Franco & Ballabio, 2006), RTT has been identified in up to 1.3% of male patients with mental retardation (Villard, 2007) and can be associated with a more severe phenotype (Zhang et al., 2017). These males have either an extra X-chromosome (Klinefelter syndrome) or somatic mosaicism of the *MECP2* variant. Reichow et al. (2015) claim to have published the first review of male RTT data in 2015, and they only identified a total of 57 published cases.

Rett syndrome can be inherited as an X-linked dominant disorder; however, more than 99% of cases result from a de novo pathogenic mutation in the *methyl CpG binding protein 2* (*MECP2*) gene (Amir et al., 1999; Christodoulou & Ho, 1993), a transcriptional regulator located on the X chromosome. More than 200 mutations in *MECP2* have been associated with RTT (Suter et al., 2014). Analysis of parental origin of the mutated *MECP2* gene in sporadic cases of RTT showed that 94.4% of mutations were from paternal origin, 90.6% of which were point mutations; further, 5.6% of mutations were from maternal origin (Zhang et al., 2012). This may explain the high occurrence of RTT in females. MECP2 is a multifunctional protein which interprets DNA methylation and regulates chromatin architecture, gene transcription, and RNA splicing (Sun et al., 2018). The complex upstream and downstream pathways of *MECP2* involve microRNAs and neurotrophic factors, such as GABA and BDNF (Kang et al., 2014). Transcriptome level analysis in tissues derived from RTT patients report dysregulations in dendritic connectivity and synapse maturation, mitochondrial dysfunction, and glial cell activity (Shovlin & Tropea, 2018). Researchers have identified two individuals with an RTT diagnosis who lacked a mutation in the *MECP2* gene but had a mutation in other genes previously unassociated with RTT: *CTNNB1* and *WDR45* (Percy et al., 2018).

The *MECP2* gene is critical for neuronal maturation (Fukuda et al., 2005; Smrt et al., 2007), and its deficiency results in impaired dendritic morphogenesis and reduced dendritic spine numbers (Chapleau et al., 2009; Kishi & Macklis, 2010). This results in dysfunctional synaptic transmission and neural network activity (Sun et al., 2018), affecting successive stages of brain development, including prenatal neurogenesis, postnatal development of synaptic connections and function, experience-dependent

synaptic plasticity, and maintenance of adult neural function, including sensory integration (Feldman et al., 2016).

The clinical picture of RTT is characterized by a broad clinical spectrum of signs and symptoms (Pini et al., 2016) and a distinctive course of apparent normal development for the first six to 18 months of life, followed by characteristic developmental stagnation and loss of acquired skills, including loss of intellectual functioning, loss of acquired fine and gross motor skills and communication (Colvin et al., 2004; Dolce et al., 2013; Hagberg et al., 1983; Leonard et al., 2017; Naidu et al., 1986; Neul et al., 2010; Rett, 1966). Purposeful use of the hands is often replaced by repetitive stereotypical hand movements (Dy et al., 2017; Elian & de, 1996; Goldman & Temudo, 2012). Other clinical observations include deceleration of head growth, seizures, disturbed breathing patterns, scoliosis, growth retardation, and gait apraxia (Cianfaglione et al., 2015).

Despite a period of apparently normal early development, the profound neurological regressions characteristic of RTT have been found to result from *MECP2*-related defects in the establishment and refinement of early neural circuits and, later, cortical plasticity (Feldman et al., 2016). Subtle signs, such as hypotonia, jerkiness in limb movement, and limited social interaction can be present during early infancy (Ip et al., 2018).

The severity and rate of progression of this disease can vary with several recognized atypical variants. The milder forms (Zappella) present with less severe regression and milder expression of the clinical characteristics of RTT. In the most severe forms, there is no normal development period (Neul et al., 2010). Both genetic and clinical variants of RTT are associated with distinct electrophysiological profiles reflecting how genetic dysregulation of synapse formation results in differences in neuronal network architecture (Sun et al., 2018) and varying clinical phenotypes (Keogh et al., 2018). The pattern of X-chromosome inactivation can also influence the severity of the clinical disease (Archer et al., 2007; Weaving et al., 2003).

Mutations in the upstream cyclin-dependent kinase-like five (*CDKL5*) gene cause an early seizure (Hanefield) variant of the RTT phenotype (Bahi-Buisson et al., 2008), and mutations in the forkhead box G1 (*FOXG1*) gene have been found in the congenital variant (Rolando) (Ariani et al., 2008). Two cases of females with pathogenic de novo mutations in *SCN1A*, which usually leads to Dravet syndrome, but fulfill the diagnostic criteria for classic RTT have also been reported (Henriksen et al., 2018). In males, *MECP2* duplication phenotypically presents with infantile hypotonia, recurrent respiratory infections, and severe mental retardation (Villard, 2007).

Fu et al. (2020) published a set of "consensus guidelines" with input from several clinical sites, Rett Syndrome-focused centers, two patient advocacy groups, and Rett

Syndrome clinical specialists. Although this guideline focuses on "management" of Rett Syndrome, the guideline does comment on the genetics of Rett Syndrome. The guideline remarks that "nearly" all individuals with Rett Syndrome (RTT) have a loss-of-function mutation on the *MECP2* and that these mutations are "almost always" *de novo* (and thereby not expected to recur in families). Two other genes (*CDKL5* and *FOXG1*) are named as possible causes of RTT. The guideline does not note any specific treatments based on type of mutation, though two other genes (*CDKL5* and *FOXG1*) are named as possible causes of RTT. However, the guideline states that "Alterations in *MECP2*, *CDKL5* and *FOXG1* should be considered in all individuals, male and female, with developmental delays and intellectual disability" (Fu et al., 2020). This consensus guideline also notes there is hope for disease-modifying therapy as reversing symptoms in mice has occurred in a clinical research context (Fu et al., 2020).

Banerjee et al. (2019) published a paper titled "Towards a Better Diagnosis and Treatment of Rett Syndrome: A Model" summarizing the developments in the diagnosis and treatment of Rett syndrome over the past 50 years. They note that the first gene therapy trial for *MECP2* "was modelled after the successful (i.e., improved survival and motor functions) single dose intravenous adeno-associated virus serotype 9 delivery of complementary DNA." Even with promising gene therapy techniques, the authors note that the field has challenges. "The Rett syndrome field is experiencing the same challenges as other neurodevelopmental disorders pursuing neurobiologically based treatments: inadequate outcomes and measures of response." The authors also comment on the complexity of the *MECP2* mutation's role in the syndrome, "*MECP2* mutations is supportive, but not confirmatory because of the limited genotype-phenotype correlations in Rett syndrome" (Banerjee et al., 2019).

Confirmation of the genetic diagnosis can improve the medical management of the patient. It can also end the diagnostic odyssey, provide a general idea of prognosis for the patient, and/or provide closure to the family (Mroch et al., 2012). Complex neurodevelopmental disorders need multi-disciplinary treatment approaches for optimal care. The clinical effectiveness of treatments is limited in patients with rare genetic syndromes and multisystem morbidity such as RTT; single drug strategies may not be sufficient, due to the multiple overlapping physiological systems affected (Singh & Santosh, 2018).

Functional performance for self-care, upper extremity function, and mobility in RTT patients may relate to the type of mutation. Knowledge of these relationships is useful for developing appropriate rehabilitation strategies and prognosis (Pidcock et al., 2016).

Of the clinical criteria for RTT, loss of hand skills was the most significant clinical predictor of a positive genetic test for mutations of a *MECP* gene in females. Gait abnormalities and stereotypic hand movements were also strong predictors of a positive genetic test for mutations of *MECP*. Language delay is the least specific of the major criteria (Knight et al., 2016). A reliable and single multidimensional questionnaire, the Rett Evaluation of Symptoms and Treatments (REST) Questionnaire, is being developed to combine physiological aspects of the disease obtained using wearable sensor technology, along with genetic and psychosocial data to stratify patients and streamline the care pathway (Santosh et al., 2017).

Clinical Utility and Validity

Lallar et al. (2018) used Sanger sequencing to diagnose suspected RTT cases. Participants were divided into two groups: Group one was comprised of females with symptoms of classical and atypical RTT, and Group two was comprised of females with other "Rett like features" that did not fit into the first category. *MECP2* mutations were identified in 74% of females in Group one and in zero percent of females in Group two; females in Group one with classical RTT had a mutation detection rate of 93% (Lallar et al., 2018). This shows that Sanger sequencing is efficient in detecting RTT in patients with the classical form of the disease.

Sheinerman et al. (2019) used brain-enriched microRNAs (miRNAs) to identify miRNA biomarkers of RTT; for this study, 30 patients with RTT were matched with 30 healthy controls of similar age (Sheinerman et al., 2019). Results showed that miRNAs identified RTT patients with 85-100% sensitivity when compared to controls; further, the researchers determined that "the dynamics in levels of miRNAs appear to be associated with disease development (involvement of liver, muscle and lipid metabolism in the pathology)" (Sheinerman et al., 2019). These results suggest that circulating miRNAs could be used to measure RTT disease progression or individual response to treatment.

In at least 95% of Rett syndrome cases, the cause is a de novo mutation in the child; *MECP2* variants are rarely inherited from a carrier mother with a germline mutation in *MECP2*, in whom favorable skewing of X-chromosome inactivation results in minimal to no clinical findings. When the mother is a known carrier, inheritance follows an X-linked dominant pattern with a 50% risk to her offspring of inheriting the *MECP2* variant (Christodoulou & Ho, 1993).

A mutation in *MECP2* does not necessarily equate to a clinical diagnosis of RTT. *MECP2* mutations have also been reported in other clinical phenotypes, including individuals with an Angelman-like picture, nonsyndromic X-linked intellectual disability, autism, in males as PPM-X syndrome (an X-linked genetic disorder characterized by

psychotic disorders, parkinsonism, and intellectual disability), and most commonly as neonatal encephalopathy (Liyanage & Rastegar, 2014; Suter et al., 2014; Williamson & Christodoulou, 2006).

Recent expert opinion in the United Kingdom concluded that genetic testing for all children with unexplained global developmental delay (GDD) should be first-line if an exogenous cause is not already established. All patients, irrespective of severity of GDD, should have investigations for treatable conditions. The yield for treatable conditions is higher than previously thought and that investigations for these conditions should be considered as first-line. Additional second-line investigations can be led by history, examination, and developmental trajectories (Mithyantha et al., 2017).

Vidal et al. (2017) have utilized next generation sequencing (NGS) in a total of 1577 patients with RTT-like clinical diagnoses or patients with potential RTT genetic mutations as determined previously by Sanger Sequencing. Of the 1577 patients with RTT-like clinical diagnoses, the NGS method was able to confirm the RTT diagnosis in 477 patients (about 30%). Further, "Positive results were found in 30% by Sanger sequencing, 23% with a custom panel, 24% with a commercial panel and 32% with whole exome sequencing," suggesting that NGS is a competitive diagnostic RTT tool compared to the aforementioned methods (Vidal et al., 2017).

Vidal et al. (2019) used multiplex ligation-dependent probe amplification (MLPA) in the *MECP2* gene of 21 RTT patients to identify deletions of varying sizes; these researchers identified both total and partial deletions of the *MECP2* gene in each patient, with identified partial deletions ranging from 1,235 bp to 85 kb. Breakpoints were delineated by DNA-qPCR; the results have allowed the researchers to "propose a genotype–phenotype correlation" which will assist in appropriate genetic counseling (Vidal et al., 2019).

Seventy-two classical Rett syndrome (RTT) female patients were included in a cohort study by Khajuria et al. (2020) to analyze exons two-four of *MECP2* gene by Sanger sequencing for sequence variations followed by deletion/duplication analysis using Multiplex Ligation-dependent Probe Amplification (MLPA). Patients were defined as classical when they showed signs of partial or complete loss of acquired purposeful hand skills, partial or complete loss of acquired spoken language, gait abnormalities, impaired or absence of ability to walk, and stereotypic hand movements. Through Sanger Sequencing, *MECP2* sequence variations were identified in 90.3% of patients. With further evaluation using MLPA, large deletions of *MECP2* were identified in 9.7% of the patients, which were negative on DNA sequencing. MLPA analysis increased the detection rate of *MECP2* sequence variants identified in patients from 90.3% to 98.6%.

The authors emphasize that "MLPA analysis of *MECP2* is crucial and needs to be performed in classical RTT patients. Large deletions can be missed using DNA sequencing and reaffirms the view that large *MECP2* deletions are an important cause of classical RTT" (Khajuria et al., 2020). Xiol et al. (2021) performed a clinical review of technological advances in RTT genetics. The authors review summarizes that our understanding of Rett syndrome has evolved "towards a spectrum of overlapping phenotypes with great genetic heterogeneity." The authors note that advances in genetic diagnosis have been impacted by the rise in next generation sequencing (NGS) and whole genome sequencing. Of note are the "90 causative genes" and "significantly overlapping phenotypes" involved in RTT spectrum disorders. To achieve an accurate and quick diagnosis of Rett syndrome, the authors strongly recommend simultaneous multiple gene testing and thorough phenotypic characterization.

Bassuk (2021) published a paper concerning methyl-CpG-binding protein 2 (*MECP2*) and its encoding of an epigenetic reader, MeCP2. The author notes that loss of function of the epigenetic reader may be a factor in RTT, but also that "locus duplications also cause a severe neurodevelopmental disorder, *MECP2* duplication syndrome (MDS)." This suggests that MeCP2 (the protein) could be what is called a "Goldilocks protein," that is, one that requires an activity level that is precise. Using the re-expression of the MeCP2 protein in mouse models, the author presents a case for the development of therapeutic interventions in people and the restoration of the desirable phenotypes. However, gene therapy must be approached with caution, as restoring function to the protein still carries the risk of "MDS overexpression phenotypes" (Bassuk, 2021).

Zade et al. (2024) studied the characteristics of RTT in Ireland. Twenty-five families completed questionnaires about the demographics, genetics, family history, clinical features, and regression. "The results show that Irish individuals with RTT have comparable presentation with respect to individuals in other countries." Importantly, "one of the main findings of this study is the limited genetic information available to individuals to support the clinical diagnosis of RTT." The authors emphasize that the study "highlights the importance of having a swift access to genetic testing to sharpen the characterization of the phenotype and increase the visibility of Irish individuals in the international RTT community" (Zade et al., 2024).

VI. Guidelines and Recommendations

American Academy of Pediatrics (AAP)

A 2014 policy statement from the AAP recommends *MECP2* mutation analysis for females with microcephaly or deceleration of head growth and other features of Rett

syndrome, or who present with stereotypical hand-wringing movements and developmental regression. *MECP2* gene mutations are extremely rare in males but may be considered in boys who present with clinical features of Rett syndrome or severe developmental regression (Moeschler & Shevell, 2014).

Complete *MECP2* deletion, duplication, and sequencing study is also recommended for females with intellectual disability or global developmental delay for whom the chromosomal microarray, specific metabolic testing, and fragile X genetic testing did not produce a diagnosis (Moeschler & Shevell, 2014).

The above guideline was reaffirmed in 2019 (AAP, 2019).

The AAP also published a guideline focusing on children with autism spectrum disorder (ASD). In it, they note that other disorders may meet certain criteria for ASD. However, the AAP notes that these disorders should prompt the "appropriate targeted testing" (or referral to a specialist). The AAP lists an example of Rett Syndrome, stating that "for example, a girl with significant developmental delays, deceleration in head growth velocity, and characteristic midline hand movements should prompt genetic testing for a mutation or deletion or duplication of *MECP2*, the gene implicated in Rett syndrome." In Supplemental Table 13, they list the following findings as representative of Rett Syndrome: "Deceleration of head growth velocity, acquired microcephaly, loss of purposeful hand use, prominent hand stereotypies (especially hand wringing or clasping), apraxia, hyperventilation or breath-holding, seizures" (Hyman et al., 2020).

Canadian Pediatric Society (CPS)

The CPS supports the guidelines mentioned above by the AAP. The CPS stated that "According to the AAP. . . *MECP2* molecular analysis should be ordered when characteristic symptomatology is present (i.e., initially normal development followed by loss of speech and purposeful hand use, stereotypical hand movement, gait abnormalities) or for moderately-to-severely affected girls" (Belanger & Caron, 2018).

RettSearch

The AAP has not provided recommendations on when to use *CDKL5* or *FOXG1* testing. RettSearch members, representing the majority of the international clinical RTT specialists, "participated in an iterative process to come to a consensus on a revised and simplified clinical diagnostic criteria for [RTT]" (Neul et al., 2010). This group provided clarifications for diagnosis of classic or typical RTT and atypical RTT and

provided guidelines for molecular evaluation of specific variant forms of RTT. The authors define RTT as a clinical diagnosis based on distinct clinical criteria, independent of molecular findings. Presence of a *MECP2* mutation is not sufficient for the diagnosis of RTT. Neul et al. (2010) proposed three distinct criteria for diagnosis of variant forms of RTT: preserved speech variant (Zapella variant), early seizure variant (Hanefeld variant) and congenital variant (Rolando variant); identifying the molecular genetics of each variant was also recommended. In the Zapella variant, the molecular analysis for *MECP2* was recommended. In Hanefeld and Rolando variants, recommended mutations for analysis were in the *CDKL5* and *FOXG1* genes, respectively. Further, it was stated that patients found negative for *MECP2* mutations and who have a strong clinical diagnosis of RTT should be considered for further screening for the *CDKL5* gene if early onset seizures or *FOXG1* gene congenital features (e.g., severe postnatal microcephaly) are present (Neul et al., 2010).

American College of Medical Genetics (ACMG)

In 2013, the ACMG revised its evidence-based guidelines for clinical genetics evaluation of autism spectrum disorders. Testing for *MECP2* mutations is recommended as part of the diagnostic workup of females who present with an autistic phenotype. Routine *MECP2* testing in males with autistic spectrum disorders is not recommended. However, when features of *MECP2* duplications (e.g., drooling, recurrent respiratory infections, hypotonic facies) are present, *MECP2* duplication testing in boys with autism and such features may be considered (Schaefer & Mendelsohn, 2013).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for

Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81302	MECP2 (methyl CpG binding protein 2) (eg, Rett syndrome) gene analysis; full sequence analysis
81303	MECP2 (methyl CpG binding protein 2) (eg, Rett syndrome) gene analysis; known familial variant
81304	MECP2 (methyl CpG binding protein 2) (eg, Rett syndrome) gene analysis; duplication/deletion variants
81404	Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons, cytogenomic array analysis for neoplasia)
0234U	MECP2 (methyl CpG binding protein 2) (eg, Rett syndrome), full gene analysis, including small sequence changes in exonic and intronic regions, deletions, duplications, mobile element insertions, and variants in non-uniquely mappable regions Proprietary test: Genomic Unity® MECP2 Analysis Lab/Manufacturer: Variantyx Inc

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

AAP. (2019). AAP Publications Reaffirmed or Retired. *Pediatrics*, *145*(3), e20193991. https://doi.org/10.1542/peds.2019-3991

Amir, R. E., Van den Veyver, I. B., Wan, M., Tran, C. Q., Francke, U., & Zoghbi, H. Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat Genet*, *23*(2), 185-188. https://doi.org/10.1038/13810

Archer, H., Evans, J., Leonard, H., Colvin, L., Ravine, D., Christodoulou, J., Williamson, S., Charman, T., Bailey, M. E., Sampson, J., de Klerk, N., & Clarke, A. (2007). Correlation between clinical severity in patients with Rett syndrome with a p.R168X or p.T158M MECP2 mutation, and the direction and degree of skewing of X-chromosome inactivation. *J Med Genet*, *44*(2), 148-152. https://doi.org/10.1136/jmg.2006.045260

Ariani, F., Hayek, G., Rondinella, D., Artuso, R., Mencarelli, M. A., Spanhol-Rosseto, A., Pollazzon, M., Buoni, S., Spiga, O., Ricciardi, S., Meloni, I., Longo, I., Mari, F., Broccoli, V., Zappella, M., & Renieri, A. (2008). FOXG1 is responsible for the congenital variant of Rett syndrome. *Am J Hum Genet*, *83*(1), 89-93. https://doi.org/10.1016/j.ajhg.2008.05.015

Armstrong, D. D. (1997). Review of Rett syndrome. *J Neuropathol Exp Neurol*, *56*(8), 843-849. https://www.ncbi.nlm.nih.gov/pubmed/9258253

Bahi-Buisson, N., Nectoux, J., Rosas-Vargas, H., Milh, M., Boddaert, N., Girard, B., Cances, C., Ville, D., Afenjar, A., Rio, M., Heron, D., N'Guyen Morel, M. A., Arzimanoglou, A., Philippe, C., Jonveaux, P., Chelly, J., & Bienvenu, T. (2008). Key clinical features to identify girls with CDKL5 mutations. *Brain*, *131*(Pt 10), 2647-2661. https://doi.org/10.1093/brain/awn197

Banerjee, A., Miller, M. T., Li, K., Sur, M., & Kaufmann, W. E. (2019). Towards a better diagnosis and treatment of Rett syndrome: a model synaptic disorder. *Brain*, *142*(2), 239-248.

Bassuk, A. G. (2021). Gene therapy for Rett syndrome. *Genes, Brain and Behavior*, *Early View*.

Belanger, S. A., & Caron, J. (2018). Evaluation of the child with global developmental delay and intellectual disability. *Paediatr Child Health*, *23*(6), 403-419. https://doi.org/10.1093/pch/pxy093

Chahil, G., Yelam, A., & Bollu, P. C. (2018). Rett Syndrome in Males: A Case Report and Review of Literature. *Cureus*, *10*(10), e3414. https://doi.org/10.7759/cureus.3414

Chapleau, C. A., Calfa, G. D., Lane, M. C., Albertson, A. J., Larimore, J. L., Kudo, S., Armstrong, D. L., Percy, A. K., & Pozzo-Miller, L. (2009). Dendritic spine pathologies in hippocampal pyramidal neurons from Rett syndrome brain and after expression of Rett-associated MECP2 mutations. *Neurobiol Dis*, *35*(2), 219-233. https://doi.org/10.1016/j.nbd.2009.05.001

Christodoulou, J., & Ho, G. (1993). MECP2-Related Disorders. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews((R))*. https://www.ncbi.nlm.nih.gov/pubmed/20301670

Cianfaglione, R., Clarke, A., Kerr, M., Hastings, R. P., Oliver, C., & Felce, D. (2015). A national survey of Rett syndrome: age, clinical characteristics, current abilities, and health. *Am J Med Genet A*, *167*(7), 1493-1500. https://doi.org/10.1002/ajmg.a.37027

Colvin, L., Leonard, H., de Klerk, N., Davis, M., Weaving, L., Williamson, S., & Christodoulou, J. (2004). Refining the phenotype of common mutations in Rett syndrome. *J Med Genet*, *41*(1), 25-30. https://www.ncbi.nlm.nih.gov/pubmed/14729826

Dolce, A., Ben-Zeev, B., Naidu, S., & Kossoff, E. H. (2013). Rett syndrome and epilepsy: an update for child neurologists. *Pediatr Neurol*, *48*(5), 337-345. https://doi.org/10.1016/j.pediatrneurol.2012.11.001

Dy, M. E., Waugh, J. L., Sharma, N., O'Leary, H., Kapur, K., D'Gama, A. M., Sahin, M., Urion, D. K., & Kaufmann, W. E. (2017). Defining Hand Stereotypies in Rett Syndrome: A Movement Disorders Perspective. *Pediatr Neurol*, *75*, 91-95. https://doi.org/10.1016/j.pediatrneurol.2017.05.025

Elian, M., & de, M. R. N. (1996). Observations on hand movements in Rett syndrome: a pilot study. *Acta Neurol Scand*, *94*(3), 212-214. https://www.ncbi.nlm.nih.gov/pubmed/8899055

Feldman, D., Banerjee, A., & Sur, M. (2016). Developmental Dynamics of Rett Syndrome. *Neural Plast*, *2016*, 6154080. https://doi.org/10.1155/2016/6154080

Franco, B., & Ballabio, A. (2006). X-inactivation and human disease: X-linked dominant male-lethal disorders. *Curr Opin Genet Dev*, *16*(3), 254-259. https://doi.org/10.1016/j.gde.2006.04.012

Fu, C., Armstrong, D., Marsh, E., Lieberman, D., Motil, K., Witt, R., Standridge, S., Nues, P., Lane, J., Dinkel, T., Coenraads, M., von Hehn, J., Jones, M., Hale, K., Suter, B., Glaze, D., Neul, J., Percy, A., & Benke, T. (2020). Consensus guidelines on managing Rett syndrome across the lifespan. *BMJ Paediatr Open*, *4*(1), e000717. https://doi.org/10.1136/bmjpo-2020-000717

Fukuda, T., Itoh, M., Ichikawa, T., Washiyama, K., & Goto, Y. (2005). Delayed maturation of neuronal architecture and synaptogenesis in cerebral cortex of Mecp2-deficient mice. *J Neuropathol Exp Neurol*, *64*(6), 537-544. https://www.ncbi.nlm.nih.gov/pubmed/15977646

Goldman, S., & Temudo, T. (2012). Hand stereotypies distinguish Rett syndrome from autism disorder. *Mov Disord*, *27*(8), 1060-1062. https://doi.org/10.1002/mds.25057

Hagberg, B. (1985). Rett's syndrome: prevalence and impact on progressive severe mental retardation in girls. *Acta Paediatr Scand*, *74*(3), 405-408. https://www.ncbi.nlm.nih.gov/pubmed/4003065

Hagberg, B., Aicardi, J., Dias, K., & Ramos, O. (1983). A progressive syndrome of autism, dementia, ataxia, and loss of purposeful hand use in girls: Rett's syndrome: report of 35 cases. *Ann Neurol*, *14*(4), 471-479. https://doi.org/10.1002/ana.410140412

Henriksen, M. W., Ravn, K., Paus, B., von Tetzchner, S., & Skjeldal, O. H. (2018). De novo mutations in SCN1A are associated with classic Rett syndrome: a case report. *BMC Med Genet*, *19*(1), 184. https://doi.org/10.1186/s12881-018-0700-z

Hyman, S. L., Levy, S. E., & Myers, S. M. (2020). Identification, Evaluation, and Management of Children With Autism Spectrum Disorder. *Pediatrics*, *145*(1), e20193447. https://doi.org/10.1542/peds.2019-3447

Ip, J. P. K., Mellios, N., & Sur, M. (2018). Rett syndrome: insights into genetic, molecular and circuit mechanisms. *Nat Rev Neurosci*, *19*(6), 368-382. https://doi.org/10.1038/s41583-018-0006-3

Kang, S. K., Kim, S. T., Johnston, M. V., & Kadam, S. D. (2014). Temporal- and Location-Specific Alterations of the GABA Recycling System in Mecp2 KO Mouse Brains. *J Cent Nerv Syst Dis*, *6*, 21-28. https://doi.org/10.4137/JCNSD.S14012

Keogh, C., Pini, G., Dyer, A. H., Bigoni, S., DiMarco, P., Gemo, I., Reilly, R., & Tropea, D. (2018). Clinical and genetic Rett syndrome variants are defined by stable electrophysiological profiles. *BMC Pediatr*, *18*(1), 333. https://doi.org/10.1186/s12887-018-1304-7

Khajuria, R., Gupta, N., Roozendaal, K. V., Sapra, S., Ghosh, M., Gulati, S., Smeets, E., Curfs, L., & Kabra, M. (2020). Spectrum of MECP2 mutations in Indian females with Rett Syndrome - a large cohort study.

Kishi, N., & Macklis, J. D. (2010). MeCP2 functions largely cell-autonomously, but also non-cell-autonomously, in neuronal maturation and dendritic arborization of cortical pyramidal neurons. *Exp Neurol*, *222*(1), 51-58. https://doi.org/10.1016/j.expneurol.2009.12.007

Knight, V. M., Horn, P. S., Gilbert, D. L., & Standridge, S. M. (2016). The Clinical Predictors That Facilitate a Clinician's Decision to Order Genetic Testing for Rett Syndrome. *Pediatr Neurol*, *63*, 66-70. https://doi.org/10.1016/j.pediatrneurol.2016.06.016

Lallar, M., Rai, A., Srivastava, P., Mandal, K., Gupta, N., Kabra, M., & Phadke, S. R. (2018). Molecular Testing of MECP2 Gene in Rett Syndrome Phenotypes in Indian Girls. *Indian Pediatr*, *55*(6), 474-477. https://www.ncbi.nlm.nih.gov/pubmed/29428920

Leonard, H., Cobb, S., & Downs, J. (2017). Clinical and biological progress over 50 years in Rett syndrome. *Nat Rev Neurol*, *13*(1), 37-51. https://doi.org/10.1038/nrneurol.2016.186

Liyanage, V. R., & Rastegar, M. (2014). Rett syndrome and MeCP2. *Neuromolecular Med*, *16*(2), 231-264. https://doi.org/10.1007/s12017-014-8295-9

Mithyantha, R., Kneen, R., McCann, E., & Gladstone, M. (2017). Current evidence-based recommendations on investigating children with global developmental delay. *Arch Dis Child*, *102*(11), 1071-1076. https://doi.org/10.1136/archdischild-2016-311271

Moeschler, J. B., & Shevell, M. (2014). Comprehensive evaluation of the child with intellectual disability or global developmental delays. *Pediatrics*, *134*(3), e903-918. https://doi.org/10.1542/peds.2014-1839

Mroch, A. R., Flanagan, J. D., & Stein, Q. P. (2012). Solving the puzzle: case examples of array comparative genomic hybridization as a tool to end the diagnostic odyssey. *Curr Probl Pediatr Adolesc Health Care*, *42*(3), 74-78. https://doi.org/10.1016/j.cppeds.2011.10.003

Naidu, S., Murphy, M., Moser, H. W., & Rett, A. (1986). Rett syndrome--natural history in 70 cases. *Am J Med Genet Suppl*, *1*, 61-72. https://www.ncbi.nlm.nih.gov/pubmed/3087204

Neul, J. L., Kaufmann, W. E., Glaze, D. G., Christodoulou, J., Clarke, A. J., Bahi-Buisson, N., Leonard, H., Bailey, M. E., Schanen, N. C., Zappella, M., Renieri, A., Huppke, P., Percy, A. K., & RettSearch, C. (2010). Rett syndrome: revised diagnostic criteria and nomenclature. *Ann Neurol*, *68*(6), 944-950. https://doi.org/10.1002/ana.22124

NORD. (2023). *Rett Syndrome*. https://rarediseases.org/rare-diseases/rett-syndrome/

Percy, A. K., Lane, J., Annese, F., Warren, H., Skinner, S. A., & Neul, J. L. (2018). When Rett syndrome is due to genes other than MECP2. *Transl Sci Rare Dis*, *3*(1), 49-53. https://doi.org/10.3233/trd-180021

Pidcock, F. S., Salorio, C., Bibat, G., Swain, J., Scheller, J., Shore, W., & Naidu, S. (2016). Functional outcomes in Rett syndrome. *Brain Dev*, *38*(1), 76-81. https://doi.org/10.1016/j.braindev.2015.06.005

Pini, G., Bigoni, S., Congiu, L., Romanelli, A. M., Scusa, M. F., Di Marco, P., Benincasa, A., Morescalchi, P., Ferlini, A., Bianchi, F., Tropea, D., & Zappella, M. (2016). Rett syndrome: a wide clinical and autonomic picture. *Orphanet J Rare Dis*, *11*(1), 132. https://doi.org/10.1186/s13023-016-0499-7

Reichow, B., George-Puskar, A., Lutz, T., Smith, I. C., & Volkmar, F. R. (2015). Brief report: systematic review of Rett syndrome in males. *J Autism Dev Disord*, *45*(10), 3377-3383. https://doi.org/10.1007/s10803-015-2519-1

Rett, A. (1966). [On a unusual brain atrophy syndrome in hyperammonemia in childhood]. *Wien Med Wochenschr*, *116*(37), 723-726. https://www.ncbi.nlm.nih.gov/pubmed/5300597 (Uber ein eigenartiges hirnatrophisches Syndrom bei Hyperammonamie im Kindersalter.)

Santosh, P., Lievesley, K., Fiori, F., & Singh, J. (2017). Development of the Tailored Rett Intervention and Assessment Longitudinal (TRIAL) database and the Rett Evaluation of Symptoms and Treatments (REST) Questionnaire. *BMJ Open*, *7*(6), e015342. https://doi.org/10.1136/bmjopen-2016-015342

Schaefer, G. B., & Mendelsohn, N. J. (2013). Clinical genetics evaluation in identifying the etiology of autism spectrum disorders: 2013 guideline revisions. *Genet Med*, *15*(5), 399-407. https://doi.org/10.1038/gim.2013.32

Sheinerman, K., Djukic, A., Tsivinsky, V. G., & Umansky, S. R. (2019). Brain-enriched microRNAs circulating in plasma as novel biomarkers for Rett syndrome. *PLoS One*, *14*(7), e0218623. https://doi.org/10.1371/journal.pone.0218623

Shovlin, S., & Tropea, D. (2018). Transcriptome level analysis in Rett syndrome using human samples from different tissues. *Orphanet J Rare Dis*, *13*(1), 113. https://doi.org/10.1186/s13023-018-0857-8

Singh, J., & Santosh, P. (2018). Key issues in Rett syndrome: emotional, behavioural and autonomic dysregulation (EBAD) - a target for clinical trials. *Orphanet J Rare Dis*, *13*(1), 128. https://doi.org/10.1186/s13023-018-0873-8

Smrt, R. D., Eaves-Egenes, J., Barkho, B. Z., Santistevan, N. J., Zhao, C., Aimone, J. B., Gage, F. H., & Zhao, X. (2007). Mecp2 deficiency leads to delayed maturation and altered gene expression in hippocampal neurons. *Neurobiol Dis*, *27*(1), 77-89. https://doi.org/10.1016/j.nbd.2007.04.005

Sun, Y., Gao, Y., Tidei, J. J., Shen, M., Hoang, J. T., Wagner, D., & Zhao, X. (2018). Loss of MeCP2 in immature neurons leads to impaired network integration. *Hum Mol Genet*. https://doi.org/10.1093/hmg/ddy338

Suter, B., Treadwell-Deering, D., Zoghbi, H. Y., Glaze, D. G., & Neul, J. L. (2014). Brief report: MECP2 mutations in people without Rett syndrome. *J Autism Dev Disord*, *44*(3), 703-711. https://doi.org/10.1007/s10803-013-1902-z

Vidal, S., Brandi, N., Pacheco, P., Gerotina, E., Blasco, L., Trotta, J. R., Derdak, S., Del Mar O'Callaghan, M., Garcia-Cazorla, A., Pineda, M., & Armstrong, J. (2017). The utility of Next Generation Sequencing for molecular diagnostics in Rett syndrome. *Sci Rep*, *7*(1), 12288. https://doi.org/10.1038/s41598-017-11620-3

Vidal, S., Pascual-Alonso, A., Rabaza-Gairi, M., Gerotina, E., Brandi, N., Pacheco, P., Xiol, C., Pineda, M., & Armstrong, J. (2019). Characterization of large deletions of the MECP2 gene in Rett syndrome patients by gene dosage analysis. *Mol Genet Genomic Med*, *7*(8), e793. https://doi.org/10.1002/mgg3.793

Villard, L. (2007). MECP2 mutations in males. *J Med Genet*, *44*(7), 417-423. https://doi.org/10.1136/jmg.2007.049452

Weaving, L. S., Williamson, S. L., Bennetts, B., Davis, M., Ellaway, C. J., Leonard, H., Thong, M. K., Delatycki, M., Thompson, E. M., Laing, N., & Christodoulou, J. (2003). Effects of MECP2 mutation type, location and X-inactivation in modulating Rett syndrome phenotype. *Am J Med Genet A*, *118A*(2), 103-114. https://doi.org/10.1002/ajmg.a.10053

Williamson, S. L., & Christodoulou, J. (2006). Rett syndrome: new clinical and molecular insights. *Eur J Hum Genet*, *14*(8), 896-903. https://doi.org/10.1038/sj.ejhg.5201580

Xiol, C., Heredia, M., Pascual-Alonso, A., Oyarzabal, A., & Armstrong, J. (2021). Technological Improvements in the Genetic Diagnosis of Rett Syndrome Spectrum Disorders. *Int J Mol Sci*, *22*(19). https://doi.org/10.3390/ijms221910375

Zade, K., Campbell, C., Bach, S., Fernandes, H., & Tropea, D. (2024). Rett syndrome in Ireland: a demographic study. *Orphanet J Rare Dis*, *19*(1), 34. https://doi.org/10.1186/s13023-024-03046-8

Zhang, Q., Zhao, Y., Bao, X., Luo, J., Zhang, X., Li, J., Wei, L., & Wu, X. (2017). Familial cases and male cases with MECP2 mutations. *Am J Med Genet B Neuropsychiatr Genet*, *174*(4), 451-457. https://doi.org/10.1002/ajmg.b.32534

Zhang, X., Bao, X., Zhang, J., Zhao, Y., Cao, G., Pan, H., Zhang, J., Wei, L., & Wu, X. (2012). Molecular characteristics of Chinese patients with Rett syndrome. *Eur J Med Genet*, *55*(12), 677-681. https://doi.org/10.1016/j.ejmg.2012.08.009

Genetic Testing of CADASIL Syndrome

Policy Number: AHS – M2069 – Genetic Testing of CADASIL Syndrome	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a genetic small vessel disease in which mutations in *notch receptor 3* (*NOTCH3*), located on chromosome 19 (Joutel et al., 1996), result in a clinical syndrome of adult-onset migraines (frequently with aura), progressive strokes, and cognitive decline in adults leading to severe functional impairment by the seventh decade of life (Opherk et al., 2004; Zhu & Nahas, 2016).

II. Related Policies

Policy Number	Policy Title
AHS-M2145	General Genetic Testing, Germline Disorders

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals who have received genetic counseling and who have received a clinical diagnosis of cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) or for whom a definitive diagnosis cannot be made without genetic testing, genetic testing of *NOTCH3* to confirm the diagnosis of CADASIL **MEETS COVERAGE CRITERIA**.

2) For asymptomatic individuals who have a first- or second-degree relative (see Note 1) diagnosed with CADASIL syndrome, the following genetic testing **MEETS COVERAGE CRITERIA**:

Genetic Testing of Mitochondrial Disorders

Policy Number: AHS – M2085 – Genetic Testing of Mitochondrial Disorders	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Mitochondrial disease refers to a heterogeneous group of disorders caused by dysfunctional mitochondria; the organelles responsible for oxidative phosphorylation within the cell. Mitochondrial diseases are classified according to the primary genetic defect, including those affecting respiratory chain proteins or ancillary proteins, mitochondrial RNA translation defects, inner membrane lipid defects, mutations causing depletion of mitochondrial DNA, or mutations to mitochondrial dynamics. Tissues with high energy demands, such as brain, heart, and skeletal muscle, are most affected by mitochondrial diseases (O'Ferrall, 2024). As a result, mitochondrial encephalopathy and cardiomyopathy are the most prominent manifestations (Meyers et al., 2013). Some examples of mitochondrial disorders include mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), myoclonic epilepsy and ragged-red-fibers (MERRF), chronic progressive external ophthalmoplegia (CPEO), Kearns-Sayre syndrome (KSS), and maternally inherited Leigh syndrome (MILS) (Hatakeyama & Goto, 2016).

When pursuing genetic testing for mitochondrial disorders, genetic counseling is strongly recommended.

II. Related Policies

Policy Number	Policy Title

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) As an alternative to muscle biopsy for individuals with clinical signs and symptoms consistent with a specific mitochondrial disorder, but for whom a definitive diagnosis cannot be made without genetic testing, genetic testing to confirm the diagnosis of a mitochondrial disorder **MEETS COVERAGE CRITERIA**.

2) For individuals who are strongly suspected of having a mitochondrial disorder but who do not have symptomology associated with a specific mitochondrial condition, genomic sequencing and large deletion detection of the entire mitochondrial genome, with heteroplasmy detection, **MEETS COVERAGE CRITERIA**.

3) For individuals who are strongly suspected of having a mitochondrial disorder but who do not have symptomology associated with a specific mitochondrial condition, genetic testing for nuclear encoded mitochondrial genes (in conjunction with or after testing for variants in the mitochondrial genome) **MEETS COVERAGE CRITERIA.**

4) For the diagnosis of mitochondrial DNA (mtDNA) depletion syndromes, quantification of mtDNA in tissue **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

5) The combination of mitochondrial genome testing with nuclear genome testing with whole genome sequencing (e.g., Genomic Unity ® Whole Genome Analysis), **DOES NOT MEET COVERAGE CRITERIA**.

6) For all other situations not discussed above, genetic testing for mitochondrial disorders **DOES NOT MEET COVERAGE CRITERIA**.

IV. Table of Terminology

Term	Definition
ACGS	Association for Clinical Genomic Science
BGS	Bigenomic sequencing
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CM	Cardiomyopathy
CMS	Centers for Medicare and Medicaid

COX10	*Cytochrome c oxidase*
CPEO	Chronic progressive external ophthalmoplegia
DNA	Deoxyribonucleic acid
dThd	Crystalline nucleoside of thymine
EAN	European Academy of Neurology
ES	Exome sequencing
FDA	Food and Drug Administration
GLUL	*Glutamine synthetase*
KSS	Kearns-Sayre syndrome
LDTs	Laboratory-developed tests
LHON	Leber hereditary optic neuropathy
LR-PCR	Long range polymerase chain reaction
MD	Mitochondrial disease
MELAS	Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes
MERRF	Myoclonic epilepsy and ragged-red-fibers
MILS	Maternally inherited Leigh syndrome
MNGIE	Mitochondrial neurogastrointestinal encephalomyopathy
MPS	Massively parallel sequencing
MRPS23	*Mitochondrial ribosomal protein S23*
mtDNA	Mitochondrial deoxyribose nucleic acid
MT-RNR1	*Mitochondrially encoded 12S ribosomal RNA*
NADH	Nicotinamide adenine dinucleotide
NAMDC	North American Mitochondrial Disease Consortium
NARP	Neuropathy, ataxia, and retinitis pigmentosa
nDNA	Nuclear deoxyribose nucleic acid
NGS	Next-generation sequencing
nWES	Nuclear whole-exome sequencing
ONT	Oxford Nanopore Technologies
PEO	Progressive external ophthalmoplegia
PGT	Preimplantation genetic testing
PMD	Primary mitochondrial disease
PNPLA4	*Patatin like phospholipase domain containing 4*
POLG	*Polymerase gamma*
POLG1	*Deoxyribose nucleic acid polymerase subunit gamma*
Q10	Coenzyme Q10 (also known as ubiquinone)
QRSL1	*Glutaminyl-trna synthase (glutamine-hydrolyzing)-like 1*
RMND1	*Required for meiotic nuclear division protein 1*
RNA	Ribonucleic acid

TP	Thymidine phosphorylase
VUS	Variant of uncertain significance
WES	Whole-exome sequencing
WGS	Whole-genome sequencing

V. Scientific Background

Mitochondrial diseases are generally inherited and present with an elaborate genetic makeup (Lightowlers et al., 2015). Several different types of mitochondrial diseases and resulting ailments exist which include "mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), myoclonic epilepsy and ragged-red-fibers (MERRF), chronic progressive external ophthalmoplegia (CPEO), Kearns-Sayre syndrome (KSS), neuropathy, ataxia, and retinitis pigmentosa (NARP), maternally inherited Leigh syndrome (MILS), and Leber hereditary optic neuropathy (LHON)" (Hatakeyama & Goto, 2016). These diseases can be caused by mutations in either nuclear DNA (nDNA) or mitochondrial DNA (mtDNA); many different mitochondrial disease mutations have been identified, but clinical symptoms are extremely variable and may even differ between patients carrying the same mtDNA mutation (Hatakeyama & Goto, 2016). Symptoms present in several areas of the body, including the central nervous system, cardiovascular system, gastrointestinal system, endocrine system, and neuromuscular system (Hatakeyama & Goto, 2016). Clinical differences depend on age of onset, affected organ or tissue, and disease progression. Phenotypic variability and severity can create challenges when diagnosing affected patients (O'Ferrall, 2024).

Pathogenic variants in more than 300 genes have been associated with mitochondria-related disorders (Thompson et al., 2019). For example, infantile onset of mitochondrial diseases with multiple mitochondrial respiratory chain defects results from mutation(s) in the Required for Meiotic Nuclear Division protein 1 (*RMND1*) gene (Ng et al., 2016). Further, a pathogenic variant in the ubiquinone biosynthesis protein (*COQ4)* gene has been affiliated with mitochondrial disease development (Lightowlers et al., 2015; Ling et al., 2019). Patients with mutations in the NADH dehydrogenase mitochondrial (*MT-ND1* and *NDUF)* genes share phenotypic and genotypic correlations in Leigh syndrome (Sofou et al., 2018). The individual symptoms are nonspecific, and symptom patterns can overlap considerably. As a result, a patient often cannot be easily classified into one particular syndrome (Chinnery, 2014).

Previously, the prevalence of mitochondrial diseases was considered low. With the advent of advanced genetic analysis tools, numerous studies now report a higher incidence of mitochondrial-associated mutations. A meta-analysis of the prevalence of

the three primary mtDNA mutations that cause LHON in Europe shows a prevalence of approximately 1:45,000 (Mascialino et al., 2012). A longitudinal study in Sweden reports an incidence of mitochondrial encephalomyopathies, in general, at 1:11,000, and an incidence of infantile mitochondrial myopathy with cytochrome C oxidase deficiency at 1:51,000. The authors conclude that "mitochondrial encephalomyopathies are relatively common neurometabolic disorders in childhood" (Darin et al., 2001). A 2015 study in the United Kingdom reports that "the total prevalence of adult mitochondrial disease, including pathogenic mutations of both the mitochondrial and nuclear genomes (≈1 in 4,300), is among the commonest adult forms of inherited neurological disorders" (Gorman et al., 2015). An Australian study estimates a "minimum birth prevalence of 13.1/100 000 or 1/7634 for respiratory chain disorders with onset at any age" (Skladal et al., 2003).

Barca et al. (2020) evaluated the phenotypic and molecular characteristic of 666 participants in the North American Mitochondrial Disease Consortium (NAMDC) aiming to better understand mitochondrial diseases in North America. Multisystemic disorder was the most common diagnosis (113 participants), compared to classical mitochondrial syndrome. Leigh syndrome (97 participants) and MELAS (71 participants) were the most frequent classical syndromes. Pathogenic variants in mtDNA, with the most common variants being *POLG1* and *PDHA1*, were more common than pathogenic gene variants (Barca et al., 2020).

The figure below (taken from O'Brien et al. (2014)) gives examples of classical phenotype mitochondrial diseases along with the clinical features and molecular genetics associated with each disorder.

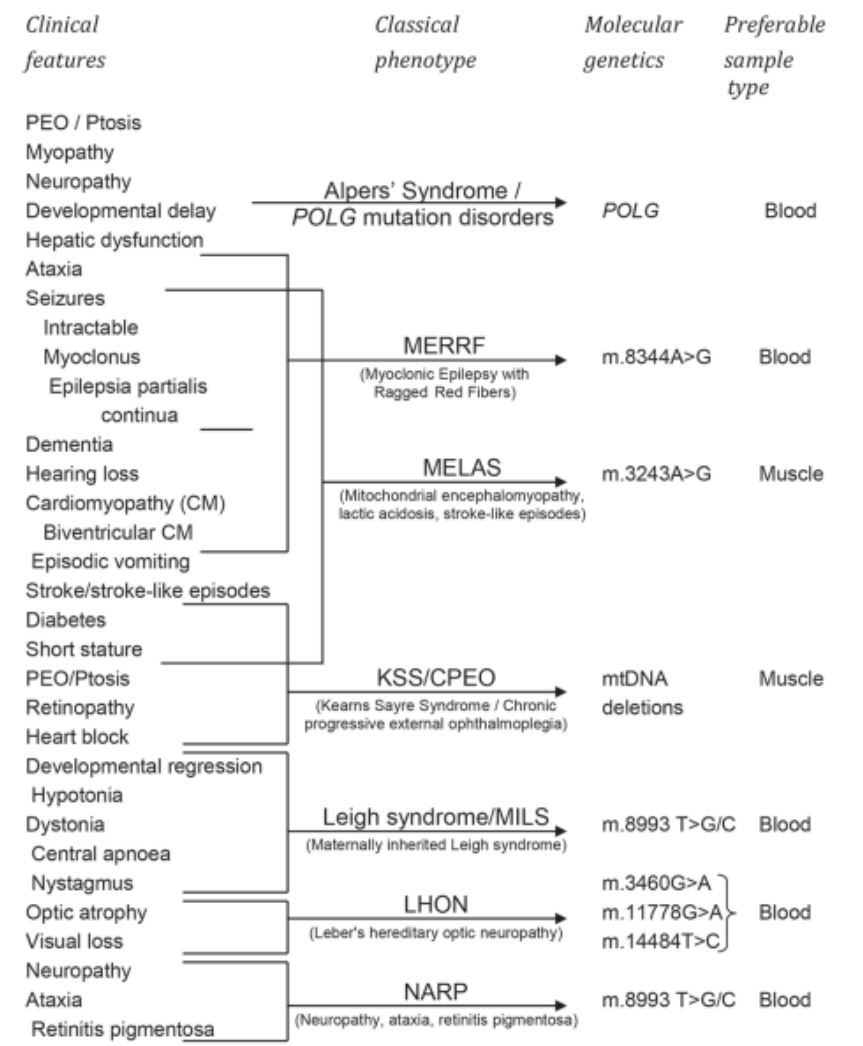


Figure 1. Algorithm for mitochondrial mutation analysis. Key clinical features, although not specific for each disease, can be used to select pertinent genetic tests and hence improve the efficiency of the analysis process.

The evaluation and diagnostic approach for a mitochondrial disorder varies according to age, clinical phenotype, and presumed inheritance pattern. Biochemical testing is indicated for patients who do not have a clear clinical picture of one specific disorder. Measurement of serum lactic acid is often used as a screening test, but the test is neither sensitive nor specific for mitochondrial disorders (Schon et al., 2012). "Identifying causative mutations underlying mitochondrial dysfunction is the ultimate gold standard for the diagnosis. Two mitochondrial diseases (MNGIE and coenzyme Q10 deficiency) are particularly important to identify because of potential treatments" (O'Ferrall, 2024).

Proprietary Testing

Vairantyx provides two separate tests, Exome Plus Analysis and Whole Genome Analysis. While the manufacturer offers two tests, the descriptions of what these two tests are reporting on is nearly identical and, currently, it is unclear as to the difference between them.

Genomic Unity ® Exome Plus Analysis

This test provides a full, phenotypically driven analysis of all relevant genes and variant types. This analysis includes genome-wide sequence analysis, genome-wide structural variant analysis, mitochondrial genome analysis with heteroplasmy (≥ five percent), mitochondrial genome large deletions analysis, short tandem repeat (STR) analysis (AFF2, *AR, ARX, ATN1, ATXN1, ATXN2, ATXN3, ATXN7, ATXN8OS, ATXN10, C9ORF72, CACNA1A, CNBP, CSTB,- DIP2B, DMPK, FGF14, FMR1, FOXL2, FXN, GIPC1, GLS, HTT, JPH3, LRP12, NOP56, NOTCH2NLC, PABPN1, PHOX2B, PPP2R2B, RFC1, TBP, TCF4, VWA1, ZFHX3, ZIC2*). This test has a performance rate of >99.9% sensitivity, >99.9% specificity, 99.8% positive predictive value, and a 96% clinical sensitivity for structural variants (Variantyx, 2024a).

Genomic Unity ® Whole Genome Analysis

Whole Genome Analysis can be ordered as a first-line test or when previous testing was non-diagnostic. This type of analysis includes genome-wide sequence analysis, genome-wide structural variant analysis, mitochondrial genome sequence analysis with heteroplasmy (≥ five percent) and large deletion analysis, STR analysis for adult-onset movement disorder with or without cognitive involvement (*AFF2, AR, ARX, ATN1, ATXN1, ATXN2, ATXN3, ATXN7, ATXN8OS, ATXN10, C9ORF72, CACNA1A, CNBP, CSTB, DIP2B, DMPK, FGF14, FMR1, FOXL2, FXN, GIPC1, GLS, HTT, JPH3, LRP12, NOP56, NOTCH2NLC, PABPN1, PHOX2B, PPP2R2B, RFC1, SOX3, TBP, TCF4, VWA1, ZFHX3, ZIC2*). This test has a performance rate of >99.9% sensitivity, >99.9% specificity, >99.8% positive predictive value, and a 96% clinical sensitivity for structural variants (Variantyx, 2024b).

Clinical Utility and Validity

Clinical utility is high for confirming the diagnosis of mitochondrial disorders in people who have clinical features consistent with a specific mitochondrial disease. In these patients, a positive result on genetic testing can avoid a muscle biopsy and eliminate the need for further clinical workup (Dimmock & Lawlor, 2017). Additionally, genetic testing may impact reproductive decision making when a defined mitochondrial disease is present in the family that is severe enough to cause impairment and/or

disability. If genetic testing is used in this situation, there will be a decreased risk of a mitochondrial disorder in the offspring. Such testing includes whole-exome sequencing, whole-genome sequencing, and whole mitochondrial sequencing.

The most common first-line diagnostic test for a mitochondrial disease is massively parallel sequencing (MPS), also known as Next-Generation Sequencing (NGS); other accepted methods included targeted panels, whole-exome sequencing (WES) and whole-genome sequencing (WGS) (Horvath & Chinnery, 2019). Broad-based exome sequencing has been considered the first-line diagnostic tool for primary mitochondrial disease (PMD) (McCormick et al., 2018), and other researchers have used WES in tandem with rapid mitochondrial genome (mtDNA) sequencing for diagnoses (Akesson et al., 2019).

Whole-exome sequencing is likely to increase the detection rate but will also increase the rate of identifying a variant of uncertain significance (VUS). In one study from the U.K., 53 patients who had biochemical evidence of a mitochondrial disorder but were negative on genetic testing of the primary mitochondrial disorder mutations, underwent whole exome sequencing. Probable pathogenic mutations causative of a mitochondrial disorder was identified in 28 patients (53%), and an additional four patients had variants that were possibly pathogenic (Taylor et al., 2014). "False negative rates vary by genomic region; therefore, genomic testing may not be as accurate as targeted single gene testing or multigene molecular genetic testing panels. Most laboratories confirm positive results using a second, well-established method" (Chinnery, 2014).

Expanded panels are defined as panels that include more genes than are associated with any specific disorder. When these panels are used in individuals with nonspecific signs and symptoms that are not consistent with a "classic" presentation of a mitochondrial disorder, the probability of finding a pathogenic mutation is considerably less. Conversely, the likelihood of a false-positive result and the number of VUS may be substantially increased (O'Brien et al., 2014). Table 1, below, lists examples of commercially available expanded genetic panels for mitochondrial disorders.

Table 1. Examples of Commercially Available Expanded Genetic Panels for Mitochondrial Disorders

Laboratory	Lab Test or Panel	Number of Genes Included on Panel

Gene Dx® (Gaithersburg, MD)	MitoXpanded Panel (GeneDX, 2023b)	~1800
	Combined Mito Genome Plus Mito Focused Nuclear Gene Panel (GeneDX, 2023a)	202
ARUP® (Salt Lake City, UT)	Mitochondrial Disorders Panel (ARUP, 2023)	>150
Baylor® Genetics (Houston, TX)	Mitome200 Nuclear Panel (Baylor, 2023)	164
Medical Neurogenetics® (Atlanta, GA)	Mitochondrial Genome Sequencing & Deletion Analysis (Medical Neurogenetics, 2022)	>300

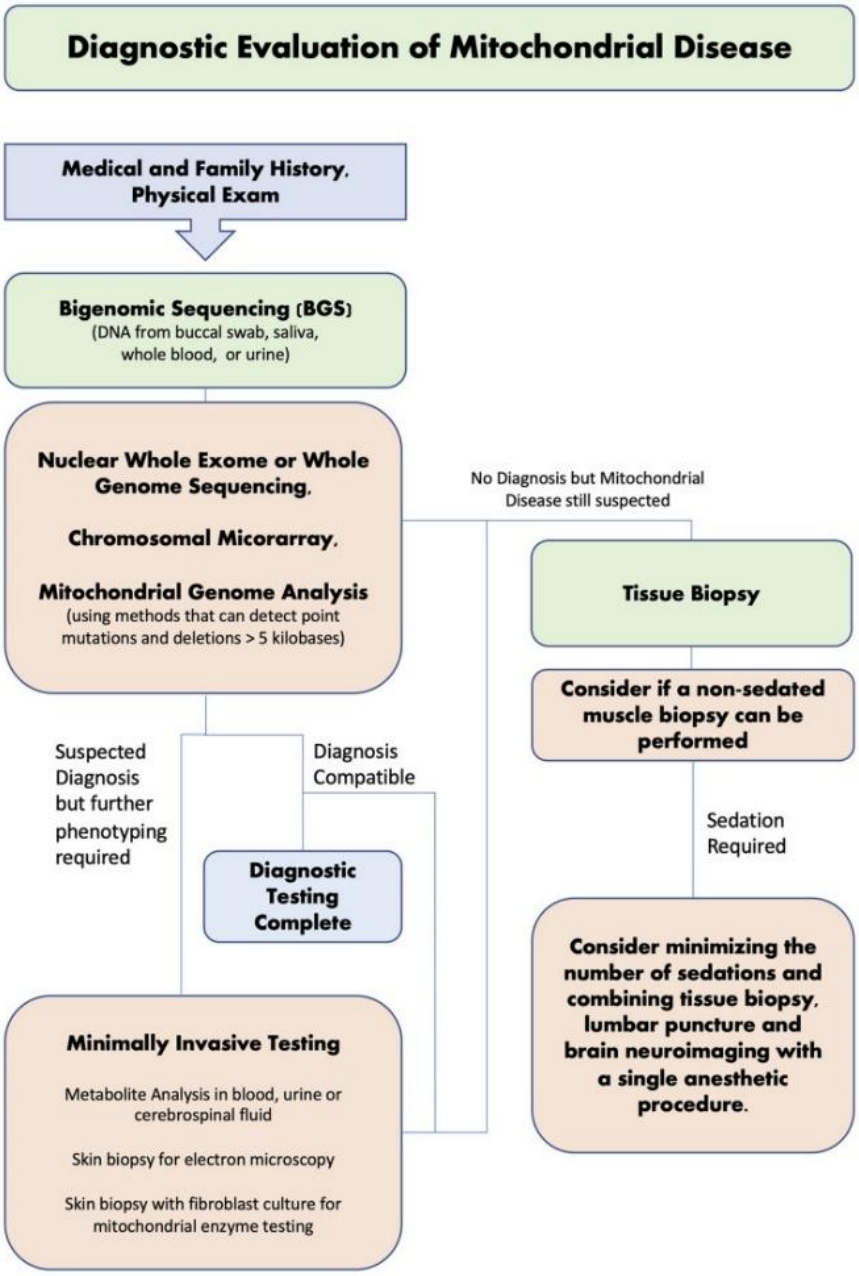
Wood et al. (2019) compared the mtDNA sequencing results of the long-read sequencing technology Oxford Nanopore Technologies (ONT) MinION to the Illumina MiSeq, a NGS platform. A total of 12 patients participated in this study (three healthy controls and nine with known mtDNA deletion disorders). Both of these NGS methods were more efficient than LR-PCR and/or Southern Blotting; further, MinION proved to be just as accurate as the Illumina MiSeq in this study, successfully identifying all mtDNA deletions (Wood et al., 2019). This tool may assist in making the mitochondrial disease diagnostic process more efficient and cost effective in the future.

Wagner et al. (2019) researched the effectiveness of exome sequencing (ES) for mitochondrial disease diagnostics; data was used from 2111 clinical cases. The researchers stated that "ES identified known pathogenic mtDNA point mutations in 38 individuals, increasing the diagnostic yield by nearly two percent. Analysis of mtDNA variants by ES had a high recall rate (96.2 ± 5.6%) and excellent precision (99.5 ± 2.2%) when compared to the gold standard of targeted mtDNA next generation sequencing" (Wagner et al., 2019). These results suggest that ES should be considered as a diagnostic tool for both nDNA and mtDNA testing.

Legati et al. (2016) suggest a two-tiered approach to genetic testing where targeted NGS is used first in cases of suspected mitochondrial disorders followed by WES in patients who have inconclusive results. "Importantly, WES on selected cases has unraveled the presence of pathogenic mutations in genes encoding non-mitochondrial proteins (e.g. the transcription factor E4F1), an observation that further expands the intricate genetics of mitochondrial disease and suggests a new area of investigation in mitochondrial medicine" (Legati et al., 2016).

Pronicka et al. (2016) suggest that WES is a better diagnostic tool than NGS. A total of 113 pediatric Polish patients participated in this study; variants were identified in both nDNA and mtDNA (Pronicka et al., 2016). WES was able to identify "likely causative mutations" in 67 patients, with 50.5% of all detected genetic changes novel variants; further, "In 47 patients, changes in 31 MD-related genes (*ACAD9, ADCK3, AIFM1, CLPB, COX10, DLD, EARS2, FBXL4, MTATP6, MTFMT, MTND1, MTND3, MTND5, NAXE, NDUFS6, NDUFS7, NDUFV1, OPA1, PARS2, PC, PDHA1, POLG, RARS2, RRM2B, SCO2, SERAC1, SLC19A3, SLC25A12, TAZ, TMEM126B, VARS2*) were identified" (Pronicka et al., 2016).

In a study by Kerr et al. (2020), 390 patients were recruited and tested for mitochondrial disease (MD) through traditional diagnostic pathways (metabolite analysis, tissue neuropathology and respiratory chain enzyme activity) and new diagnostic approaches (next-generation sequencing (NGS) and nuclear whole-exome sequencing (nWES)). Testing through traditional diagnostic methods resulted in a mitochondrial diagnosis in 115 out of 390 patients (29.50%). In comparison, 116 out of 390 patients were recruited for NGS, which identified 36 patients (31%) with a mitochondrial diagnosis. To confirm diagnosis, patients were further tested through nWES, which provided a secondary diagnosis in "two cases who already had a pathogenic variant in mtDNA, and a revised diagnosis (*GLUL*) in one case that had abnormal pathology but no pathogenic mtDNA variant" (Kerr et al., 2020). nWES also identified a mitochondrial diagnosis in one patient who tested negative from NGS. The author offers a diagnostic evaluation strategy, as shown in the figure below, and recommends that "a non-invasive, bigenomic sequencing (BGS) approach (using both a nWES and optimized mtDNA analysis to include large deletions) should be the first step in investigating for mitochondrial diseases. There may still be a role for tissue biopsy in unsolved cases or when the diagnosis is still not clear after NGS studies" (Kerr et al., 2020).



Another study clearly demonstrates the heterogeneity of genetic mutations causing mitochondrial disorders. Of the 142 patients with childhood-onset mitochondrial disorders, researchers "identified 37 novel mutations in known mitochondrial disease genes and three mitochondria-related genes (*MRPS23*, *QRSL1*, and *PNPLA4*) as novel causative genes" (Kohda et al., 2016). These researchers utilized whole mtDNA and exome and chromosomal aberration analysis approaches to "enhance the ability to identify pathogenic gene mutations in patients" (Kohda et al., 2016).

A study by Fang et al. (2017) recruited 141 children with suspected mitochondrial disorders and used NGS to identify genetic characteristics. Forty children were gene

confirmed with a known mitochondrial disease; 62.5% of those cases were due to a mtDNA mutation and 37.5% due to a nDNA mutation. This study found the most prevalent disorders to be Leigh syndrome and MELAS (Fang et al., 2017).

Spath et al. (2021) studied parallel preimplantation genetic testing (PGT) for mtDNA disease and aneuploidy on four patients at risk of transmitting mtDNA disease. Aneuploidy is the condition of having an abnormal number of chromosomes. "Half of the embryos tested were shown to be aneuploid (16/33)." Notably, not all the participants had the same mtDNA mutations. Patients A and D had a m.8993T>G mutation; mutations were detected in embryo biopsies from Patient A but not Patient B. Patient C had a m.10191T.G mutation, no mutations were detected in embryo biopsies from Patient C. Patient D had a m3243A>G mutation, mutations were detected in embryo biopsies from Patient D. Patients A and D displayed somatic heteroplasmy for mtDNA mutations, "heteroplasmic women had a higher incidence of affected embryos than those with undetectable somatic mutant mtDNA but were still able to produce mutation-free embryos." The authors concluded that "strategies providing a combination of PGT for mtDNA disease and aneuploidy may be advantageous compared with approaches that consider only mtDNA" (Spath et al., 2021).

Wu et al. (2021) evaluated the cost-efficiency and cost-benefit of genomic sequencing for children presenting clinical indications of mitochondrial diseases compared to the conventional diagnosis pathway in Australia. The authors used a decision tree model approach and a discrete-event simulation approach on data from 78 pediatric-onset patients. The conventional diagnosis pathway refers to a diagnostic workup including metabolic, imaging, and histopathological investigations, and genetic testing. Genomic sequencing was less costly and more effective than conventional care. The authors concluded that "genomic sequencing is cost-saving relative to traditional investigative approaches, while enabling more diagnoses to be made in a timely manner, offering substantial personal benefits to children and their families" (Wu et al., 2021).

Farahvash et al. (2021) did a case study on two siblings with mitochondrial neurogastrointestinal encephalopathy disease (MNGIE). The two patients experienced gastrointestinal and cachexia symptoms for years and underwent numerous exploratory tests and surgeries which did not improve their condition. Eventually, the patients were diagnosed with MNGIE following genetic testing for the TYMP gene. The authors concluded that "MNGIE diagnosis is important to establish to avoid unnecessary invasive testing for gastrointestinal, ophthalmological, and neurological symptoms and to ensure patients receive appropriate nutritional and genetic counselling" (Farahvash et al., 2021).

VI. Guidelines and Recommendations

Mitochondrial Medicine Society

The Mitochondrial Medicine Society published the following consensus recommendations on genetic testing for mitochondrial disorders (Parikh et al., 2015):

1. "Massively parallel sequencing/NGS of the mtDNA genome is the preferred methodology when testing mtDNA and should be performed in cases of suspected mitochondrial disease instead of testing for a limited number of pathogenic point mutations.
2. Patients with a strong likelihood of mitochondrial disease because of a mtDNA mutation and negative testing in blood, should have mtDNA assessed in another tissue to avoid the possibility of missing tissue-specific mutations or low levels of heteroplasmy in blood; tissue-based testing also helps assess the risk of other organ involvement and heterogeneity in family members and to guide genetic counseling.
3. Heteroplasmy analysis in urine can selectively be more informative and accurate than testing in blood alone, especially in cases of MELAS due to the common m. 3243A>G mutation.
4. mtDNA deletion and duplication testing should be performed in cases of suspected mitochondrial disease via NGS of the mtDNA genome, especially in all patients undergoing a diagnostic tissue biopsy.
    a. If a single small deletion is identified using polymerase chain reaction–based analysis, then one should be cautious in associating these findings with a primary mitochondrial disorder.
    b. When multiple mtDNA deletions are noted, sequencing of nuclear genes involved in mtDNA biosynthesis is recommended.
5. When a tissue specimen is obtained for mitochondrial studies, mtDNA content (copy number) testing via real-time quantitative polymerase chain reaction should strongly be considered for mtDNA depletion analysis because mtDNA depletion may not be detected in blood.
    a. mtDNA proliferation is a nonspecific compensatory finding that can be seen in primary mitochondrial disease, secondary mitochondrial dysfunction, myopathy, hypotonia, and as a by-product of regular, intense exercise.
6. When considering nuclear gene testing in patients with likely primary mitochondrial disease, NGS methodologies providing complete coverage of known mitochondrial disease genes is preferred. Single-gene testing should usually be avoided because mutations in different genes can produce the same

phenotype. If no known mutation is identified via known NGS gene panels, then whole exome sequencing should be considered."

The Mitochondrial Medicine Society also commented on the set of mtDNA depletion syndromes, which are "characterized by a significant reduction in mtDNA copy number in affected tissues". They note that diagnosis of these conditions "requires quantification of mtDNA content, typically in affected tissue, with identification of a significant decrease below the mean of normal age, gender, and tissue-specific control when normalized to nDNA tissue content". Since NGS of the mtDNA genome does not identify mtDNA content, a separate quantitative real-time polymerase chain reaction must be used (Parikh et al., 2015).

The Mitochondrial Medicine Society in 2017 released their guidelines regarding patient care standards. Within this set of guidelines, they state, "Pregnancy in mitochondrial disease also elicits the concern of transmission of a genetic disorder. Appropriate preconception genetic counseling and discussion of options of prenatal testing are needed. A fetus affected by mitochondrial disease may also be at higher risk for prenatal morbidity. Finally, premature ovarian failure is a feature of several mitochondrial disorders and affected women should be referred for assisted reproductive technologies if they wish to have children" (Parikh et al., 2017).

Association for Clinical Genomic Science (ACGS)

The ACGS published guidelines for the genetic testing strategies for diagnostic and familial testing, variant interpretation and reporting, and prenatal diagnosis and reproductive options. The guidelines report the minimal level of testing recommended for the most common diagnostic referrals, listed below, noting possible further testing with nuclear DNA testing and whole mtDNA sequencing for all phenotypes.

- Phenotype: ataxia, possible diagnosis of late-childhood or adult-onset peripheral neuropathy, pigmentary retinopathy (NARP) or mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS). Minimum level of testing for: m.8993T>G p.(Leu156Arg), m.8993T>C p.(Leu156Pro)(MT-ATP6), m.3243A>G (MT-TL1).
- Phenotype: cardiomyopathy, familial hypertrophic cardiomyopathy with maternal inheritance. Minimum level of testing for: m.4300A>G (MT-TI), m.3243A>G (MT-TL1).
- Phenotype: diabetes mellitus and sensorineural hearing loss. Minimum level of testing for: m.3243A>G (MT-TL1), m.3243A>G (MT-TL1).
- Phenotype: encephalopathy/seizures with lactic acidosis, possible diagnosis of mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) or infantile onset subacute relapsing encephalopathy,

cerebellar and brain stem signs (MILS). Minimum level of testing for: m.3243A>G, (MT-TL1), m.8993T>G p.(Leu156Arg), m.8993T>C p.(Leu156Pro), (MT-ATP6).

- Phenotype: non-syndromic sensorineural hearing loss, particularly if onset following aminoglycoside exposure. Minimum level of testing for m.1555A>G (MT-RNR1).
- Phenotype: Kearns-Sayre syndrome onset, below the age of 20 years, PEO and pigmentary retinopathy with one of either cardiac conduction block, cerebrospinal fluid protein concentration greater than 100 mg/dL, or cerebellar ataxia. Minimum level of testing for: large-scale mtDNA rearrangements (single and multiple deletions), m.3243A>G (MT-TL1).
- Phenotype: Leber hereditary optic neuropathy, optic atrophy, childhood or midlife (adult-onset) acute or subacute painless bilateral central vision loss. Minimum level of testing for: m.3460G>A p.(Ala52Thr) (MT-ND1), m.11778G>A p.(Arg340His) (MT-ND4), m.14484T>C p.(Met64Val) (MT-ND6).
- Phenotype: mtDNA depletion syndrome, neonatal or infantile hepatocerebral, myopathic, encephalomyopathic or neurogastrointestinal presentations; may also include growth failure, lactic acidosis, and hypoglycemia. Minimum level of testing for: mtDNA copy number analysis.
- Phenotype: myoclonic epilepsy, myoclonus, seizures; cerebellar ataxia; myopathy. Minimum level of testing for: m.8344A>G (MT-TK), m.3243A>G (MT-TL1).
- Phenotype: Pearson syndrome, sideroblastic anemia of childhood; Pancytopenia; Exocrine pancreatic failure. Minimum level of testing for: large-scale mtDNA rearrangements.
- Phenotype: progressive external ophthalmoplegia (PEO), ptosis, typically adult-onset ptosis, paralysis of the extraocular muscles (ophthalmoplegia), oropharyngeal weakness, and variably severe proximal limb weakness. Minimum level of testing for: large-scale mtDNA rearrangements (single and multiple deletions, m.3243A>G (MT-TL1).
- Phenotype: stroke-like episodes, typically before age 40 years. Minimum level of testing for: m.3243A>G (MT-TL1) (Mavraki et al., 2020).

European Academy of Neurology (EAN)

Regarding genetic testing for mitochondrial encephalopathy, lactic acidosis and stroke-like episodes (MELAS), the EAN recommends "Urgent genetic testing for MELAS should be considered in patients presenting with suspected SLE [stroke-like episode]. Search for the m.3243A>G variant (in urine where possible) and, if m.3243A>G variant is negative, POLG sequencing is required. Muscle biopsy should be considered after excluding m.3243A>G and POLG variants." The EAN also notes that valproic acid is "contraindicated, mainly in patients with POLG variants" (Mancuso et al., 2020).

MNGIE International Network

The MNGIE [Mitochondrial neurogastrointestinal encephalomyopathy] International Network recommended *TYMP* sequencing to confirm a MNGIE diagnosis. If a variant of unknown significance or a wild-type variant is found, the Network recommends biochemical testing of thymidine (dThd) and deoxyuridine levels to determine thymidine phosphorylase (TP) activity. The Network also remarks on several treatment options (both short- and long-term), which focus on restoring the biochemical balance of the patient (Hirano et al., 2020).

United Kingdom Best Practice Guidelines

In 2024, a group of clinical scientists part of the "United Kingdom National Health Service Highly Specialised Services for Rare Mitochondrial Disorders" group revised and updated their 2008 guidelines for genetic testing for mitochondrial disease (Mavraki et al., 2023).

In a proband with suspected mitochondrial disease, the group recommends that "first-line targeted testing of blood DNA can be appropriate for routine referrals (where there is not an urgent clinical need to obtain a diagnosis), for clinical presentations which are highly suggestive of a particular variant or gene (e.g., MELAS, MERRF, LHON, Pearson syndrome, POLG-related disorders), and/or where resources are limited. In patients where no pathogenic variant is detected in blood, additional clinical information and samples (e.g., urine) or a muscle biopsy for biochemical and histopathological investigations may be suggested." The group also notes that "alternatively, comprehensive NGS-based testing can be adopted for sensitive and efficient first-line genetic testing. This approach can be followed for all referral indications but is particularly appropriate for more complex phenotypes and/or for urgent referrals" (Mavraki et al., 2023).

The group notes that "specific single gene testing remains a valuable simple and rapid first-line test when the associated disorder is common, for treatable disorders with a characteristic phenotype, when there are common founder variants(s), and/or if biochemical evidence points to a defect in the particular gene" (Mavraki et al., 2023).

For family testing, the group recommends that "following a genetic diagnosis of mitochondrial disease, it is recommended that the proband (and their family) are referred to clinical genetics or a specialist mitochondrial service for appropriate genetic counselling and family follow-up." The group notes that testing of asymptomatic children below the age of 16 with a family history of mtDNA-related mitochondrial disease is challenging, and recommends that "laboratories discuss referrals for genetic testing of asymptomatic children with specialised clinicians or clinical geneticists wherever possible prior to testing" (Mavraki et al., 2023).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81401	Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)
81403	Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)
81404	Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons)

CPT	Code Description
81440	Nuclear encoded mitochondrial genes (eg, neurologic or myopathic phenotypes), genomic sequence panel, must include analysis of at least 100 genes, including BCS1L, C10orf2, COQ2, COX10, DGUOK, MPV17, OPA1, PDSS2, POLG, POLG2, RRM2B, SCO1, SCO2, SLC25A4, SUCLA2, SUCLG1, TAZ, TK2, and TYMP
81460	Whole mitochondrial genome (eg, Leigh syndrome, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes [MELAS], myoclonic epilepsy with ragged-red fibers [MERFF], neuropathy, ataxia, and retinitis pigmentosa [NARP], Leber hereditary optic neuropathy [LHON]), genomic sequence, must include sequence analysis of entire mitochondrial genome with heteroplasmy detection
81465	Whole mitochondrial genome large deletion analysis panel (eg, Kearns-Sayre syndrome, chronic progressive external ophthalmoplegia), including heteroplasmy detection, if performed
81479	Unlisted molecular pathology procedure
0212U	Rare diseases (constitutional/heritable disorders), whole genome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, proband Proprietary test: Genomic Unity® Whole Genome Analysis – Proband Lab/Manufacturer: Variantyx Inc
0213U	Rare diseases (constitutional/heritable disorders), whole genome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, each comparator genome (eg, parent, sibling) Proprietary test: Genomic Unity® Whole Genome Analysis – Comparator- Lab/Manufacturer: Variantyx Inc
0214U	Rare diseases (constitutional/heritable disorders), whole exome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, proband Proprietary test: Genomic Unity® Exome Plus Analysis – Proband Lab/Manufacturer: Variantyx Inc
0215U	Rare diseases (constitutional/heritable disorders), whole exome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and

CPT	Code Description
	categorization of genetic variants, each comparator exome (eg, parent, sibling) Proprietary test: Genomic Unity® Exome Plus Analysis – Comparator Lab/Manufacturer: Variantyx Inc
0417U	Rare diseases (constitutional/heritable disorders), whole mitochondrial genome sequence with heteroplasmy detection and deletion analysis, nuclear-encoded mitochondrial gene analysis of 335 nuclear genes, including sequence changes, deletions, insertions, and copy number variants analysis, blood or saliva, identification and categorization of mitochondrial disorder–associated genetic variants Proprietary test: Genomic Unity® Comprehensive Mitochondrial Disorders Analysis Lab/Manufacturer: Variantyx Inc

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Akesson, L., Eggers, S., Chong, B., Hunter, M., Krzesinski, E., Brown, N., Tan, T. Y., Richmond, C., Thorburn, D., Christodoulou, J., Stark, Z., & Lunke, S. (2019). Rapid mitochondrial genome (MTDNA) sequencing: facilitating rapid diagnosis of mitochondrial diseases in paediatric acute care. *51*, 118–S119. https://doi.org/10.1016/j.pathol.2018.12.339

ARUP. (2023). *Mitochondrial Disorders Panel (mtDNA and Nuclear Genes)*. ARUP Laboratories. https://ltd.aruplab.com/Tests/Pub/3001959

Barca, E., Long, Y., Cooley, V., Schoenaker, R., Emmanuele, V., DiMauro, S., Cohen, B. H., Karaa, A., Vladutiu, G. D., Haas, R., Van Hove, J. L. K., Scaglia, F., Parikh, S., Bedoyan, J. K., DeBrosse, S. D., Gavrilova, R. H., Saneto, R. P., Enns, G. M., Stacpoole, P. W., . . . Hirano, M. (2020). Mitochondrial diseases in North America: An analysis of the NAMDC Registry. *Neurol Genet*, *6*(2), e402. https://doi.org/10.1212/nxg.0000000000000402

Baylor. (2023). *Providing insights into unclear health conditions through Mitochondrial testing*. Baylor Genetics https://www.baylorgenetics.com/mitochondrial/

Chinnery, P. F. (2014). Mitochondrial Disorders Overview. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews((R))* (Vol. 2014 update). University of Washington, Seattle. https://www.ncbi.nlm.nih.gov/books/NBK1224/

Darin, N., Oldfors, A., Moslemi, A. R., Holme, E., & Tulinius, M. (2001). The incidence of mitochondrial encephalomyopathies in childhood: clinical features and morphological, biochemical, and DNA abnormalities. *Ann Neurol*, *49*(3), 377-383. https://pubmed.ncbi.nlm.nih.gov/11261513/

Dimmock, D. P., & Lawlor, M. W. (2017). Presentation and Diagnostic Evaluation of Mitochondrial Disease. *Pediatr Clin North Am*, *64*(1), 161-171. https://doi.org/10.1016/j.pcl.2016.08.011

Fang, F., Liu, Z., Fang, H., Wu, J., Shen, D., Sun, S., Ding, C., Han, T., Wu, Y., Lv, J., Yang, L., Li, S., Lv, J., & Shen, Y. (2017). The clinical and genetic characteristics in children with mitochondrial disease in China. *Sci China Life Sci*, *60*(7), 746-757. https://doi.org/10.1007/s11427-017-9080-y

Farahvash, A., Kassardjian, C. D., & Micieli, J. A. (2021). Mitochondrial Neurogastrointestinal Encephalopathy Disease: A Rare Disease Diagnosed in Siblings with Double Vision. *Case Rep Ophthalmol*, *12*(1), 174-181. https://doi.org/10.1159/000514098

GeneDX. (2023a). *Combined Mito Genome Plus Mito Focused Nuclear Gene Panel*. GeneDx, Inc. https://www.genedx.com/tests/detail/combined-mito-genome-plus-mito-focused-nuclear-gene-panel-736

GeneDX. (2023b, August 2021). *MitoXpanded Panel*. GeneDx, Inc. https://providers.genedx.com/tests/detail/mitoxpanded-panel-891

Gorman, G. S., Schaefer, A. M., Ng, Y., Gomez, N., Blakely, E. L., Alston, C. L., Feeney, C., Horvath, R., Yu-Wai-Man, P., Chinnery, P. F., Taylor, R. W., Turnbull, D. M., & McFarland, R. (2015). Prevalence of nuclear and mitochondrial DNA mutations related to adult mitochondrial disease. *Ann Neurol*, *77*(5), 753-759. https://doi.org/10.1002/ana.24362

Hatakeyama, H., & Goto, Y. (2016). Concise Review: Heteroplasmic Mitochondrial DNA Mutations and Mitochondrial Diseases: Toward iPSC-Based Disease Modeling, Drug Discovery, and Regenerative Therapeutics. *Stem Cells*, *34*(4), 801-808. https://doi.org/10.1002/stem.2292

Hirano, M., Carelli, V., De Giorgio, R., Pironi, L., Accarino, A., Cenacchi, G., D'Alessandro, R., Filosto, M., Martí, R., Nonino, F., Pinna, A. D., Baldin, E., Bax, B. E., Bolletta, A., Bolletta, R., Boschetti, E., Cescon, M., D'Angelo, R., Dotti, M. T., . . . Zeviani, M. (2020). Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE): Position paper on diagnosis, prognosis, and treatment by the MNGIE International Network. *J Inherit Metab Dis*. https://doi.org/10.1002/jimd.12300

Horvath, R., & Chinnery, P. (2019). *Diagnostic Approach to Mitochondrial Diseases*. https://doi.org/10.1007/978-3-030-05517-2_17

Kerr, M., Hume, S., Omar, F., Koo, D., Barnes, H., Khan, M., Aman, S., Wei, X.-C., Alfuhaid, H., McDonald, R., McDonald, L., Newell, C., Sparkes, R., Hittel, D., & Khan, A. (2020). MITO-FIND: A study in 390 patients to determine a diagnostic strategy for

mitochondrial disease. *Molecular Genetics and Metabolism*. https://doi.org/10.1016/j.ymgme.2020.08.009

Kohda, M., Tokuzawa, Y., Kishita, Y., Nyuzuki, H., Moriyama, Y., Mizuno, Y., Hirata, T., Yatsuka, Y., Yamashita-Sugahara, Y., Nakachi, Y., Kato, H., Okuda, A., Tamaru, S., Borna, N. N., Banshoya, K., Aigaki, T., Sato-Miyata, Y., Ohnuma, K., Suzuki, T., . . . Okazaki, Y. (2016). A Comprehensive Genomic Analysis Reveals the Genetic Landscape of Mitochondrial Respiratory Chain Complex Deficiencies. *PLoS Genet*, *12*(1), e1005679. https://doi.org/10.1371/journal.pgen.1005679

Legati, A., Reyes, A., Nasca, A., Invernizzi, F., Lamantea, E., Tiranti, V., Garavaglia, B., Lamperti, C., Ardissone, A., Moroni, I., Robinson, A., Ghezzi, D., & Zeviani, M. (2016). New genes and pathomechanisms in mitochondrial disorders unraveled by NGS technologies. *Biochim Biophys Acta*, *1857*(8), 1326-1335. https://doi.org/10.1016/j.bbabio.2016.02.022

Lightowlers, R. N., Taylor, R. W., & Turnbull, D. M. (2015). Mutations causing mitochondrial disease: What is new and what challenges remain? *Science*, *349*(6255), 1494-1499. https://doi.org/10.1126/science.aac7516

Ling, T.-k., Law, C.-y., Ko, C.-h., Fong, N.-c., Wong, K.-c., Lee, K.-l., Chiu-wing Chu, W., Brea-Calvo, G., & Lam, C.-w. (2019). A common COQ4 mutation in undiagnosed mitochondrial disease: a local case series. *51*, S112–S113. https://doi.org/10.1016/j.pathol.2018.12.317

Mancuso, M., Arnold, M., Bersano, A., Burlina, A., Chabriat, H., Debette, S., Enzinger, C., Federico, A., Filla, A., Finsterer, J., Hunt, D., Lesnik Oberstein, S., Tournier-Lasserve, E., & Markus, H. S. (2020). Monogenic cerebral small-vessel diseases: diagnosis and therapy. Consensus recommendations of the European Academy of Neurology. *Eur J Neurol*, *27*(6), 909-927. https://doi.org/10.1111/ene.14183

Mascialino, B., Leinonen, M., & Meier, T. (2012). Meta-analysis of the prevalence of Leber hereditary optic neuropathy mtDNA mutations in Europe. *Eur J Ophthalmol*, *22*(3), 461-465. https://doi.org/10.5301/ejo.5000055

Mavraki, E., Labrum, R., Sergeant, K., Alston, C., Woodward, C., Smith, C., Watson, E. L., Polke, J., Taylor, R. W., & Fratter, C. (2020). Best Practice Guidelines for the Molecular Diagnosis of Mitochondrial Disease. https://www.acgs.uk.com/media/11935/bpg-for-the-molecular-diagnosis-of-mitochondrial-disease_ratified-november-2020.pdf

Mavraki, E., Labrum, R., Sergeant, K., Alston, C. L., Woodward, C., Smith, C., Knowles, C. V., Patel, Y., Hodsdon, P., & Baines, J. P. (2023). Genetic testing for mitochondrial disease: the United Kingdom best practice guidelines. *European Journal of Human Genetics*, *31*(2), 148-163.

McCormick, E. M., Zolkipli-Cunningham, Z., & Falk, M. J. (2018). Mitochondrial disease genetics update: recent insights into the molecular diagnosis and expanding phenotype of primary mitochondrial disease. *Curr Opin Pediatr*, *30*(6), 714-724. https://doi.org/10.1097/mop.0000000000000686

Medical Neurogenetics, L. (2022). *Mitochondrial Genome Sequencing + Deletion Analysis*. Medical Neurogenetics, LLC. https://mnglabs.labcorp.com/tests/MOL189/mitochondrial-genome-sequencing-deletion-analysis

Meyers, D. E., Basha, H. I., & Koenig, M. K. (2013). Mitochondrial cardiomyopathy: pathophysiology, diagnosis, and management. *Tex Heart Inst J*, *40*(4), 385-394. https://pubmed.ncbi.nlm.nih.gov/24082366/

Ng, Y. S., Alston, C. L., Diodato, D., Morris, A. A., Ulrick, N., Kmoch, S., Houstek, J., Martinelli, D., Haghighi, A., Atiq, M., Gamero, M. A., Garcia-Martinez, E., Kratochvilova, H., Santra, S., Brown, R. M., Brown, G. K., Ragge, N., Monavari, A., Pysden, K., . . . McFarland, R. (2016). The clinical, biochemical and genetic features associated with RMND1-related mitochondrial disease. *J Med Genet*, *53*(11), 768-775. https://doi.org/10.1136/jmedgenet-2016-103910

O'Brien, M., Cryan, J., Brett, F., Howley, R., & Farrell, M. (2014). Ten years on: genetic screening for mitochondrial disease in Ireland. *Clin Neuropathol*, *33*(4), 279-283. https://doi.org/10.5414/np300722

O'Ferrall, E. (2024). *Mitochondrial myopathies: Clinical features and diagnosis*. Wolters Kluwer. https://www.uptodate.com/contents/mitochondrial-myopathies-clinical-features-and-diagnosis

Parikh, S., Goldstein, A., Karaa, A., Koenig, M. K., Anselm, I., Brunel-Guitton, C., Christodoulou, J., Cohen, B. H., Dimmock, D., Enns, G. M., Falk, M. J., Feigenbaum, A., Frye, R. E., Ganesh, J., Griesemer, D., Haas, R., Horvath, R., Korson, M., Kruer, M. C., . . . Chinnery, P. F. (2017). Patient care standards for primary mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society. *Genet Med*, *19*(12). https://doi.org/10.1038/gim.2017.107

Parikh, S., Goldstein, A., Koenig, M. K., Scaglia, F., Enns, G. M., Saneto, R., Anselm, I., Cohen, B. H., Falk, M. J., Greene, C., Gropman, A. L., Haas, R., Hirano, M., Morgan, P., Sims, K., Tarnopolsky, M., Van Hove, J. L., Wolfe, L., & DiMauro, S. (2015). Diagnosis and management of mitochondrial disease: a consensus statement from the Mitochondrial Medicine Society. *Genet Med*, *17*(9), 689-701. https://doi.org/10.1038/gim.2014.177

Pronicka, E., Piekutowska-Abramczuk, D., Ciara, E., Trubicka, J., Rokicki, D., Karkucinska-Wieckowska, A., Pajdowska, M., Jurkiewicz, E., Halat, P., Kosinska, J., Pollak, A., Rydzanicz, M., Stawinski, P., Pronicki, M., Krajewska-Walasek, M., & Ploski, R. (2016). New perspective in diagnostics of mitochondrial disorders: two years' experience with whole-exome sequencing at a national paediatric centre. *J Transl Med*, *14*(1), 174. https://doi.org/10.1186/s12967-016-0930-9

Schon, E. A., DiMauro, S., & Hirano, M. (2012). Human mitochondrial DNA: roles of inherited and somatic mutations. *Nat Rev Genet*, *13*(12), 878-890. https://doi.org/10.1038/nrg3275

Skladal, D., Halliday, J., & Thorburn, D. R. (2003). Minimum birth prevalence of mitochondrial respiratory chain disorders in children. *Brain*, *126*(Pt 8), 1905-1912. https://doi.org/10.1093/brain/awg170

Sofou, K., de Coo, I. F. M., Ostergaard, E., Isohanni, P., Naess, K., De Meirleir, L., Tzoulis, C., Uusimaa, J., Lonnqvist, T., Bindoff, L. A., Tulinius, M., & Darin, N. (2018). Phenotype-genotype correlations in Leigh syndrome: new insights from a multicentre study of 96 patients. *J Med Genet*, *55*(1), 21-27. https://doi.org/10.1136/jmedgenet-2017-104891

Spath, K., Babariya, D., Konstantinidis, M., Lowndes, J., Child, T., Grifo, J. A., Poulton, J., & Wells, D. (2021). Clinical application of sequencing-based methods for parallel preimplantation genetic testing for mitochondrial DNA disease and aneuploidy. *Fertil Steril*, *115*(6), 1521-1532. https://doi.org/10.1016/j.fertnstert.2021.01.026

Taylor, R. W., Pyle, A., Griffin, H., Blakely, E. L., Duff, J., He, L., Smertenko, T., Alston, C. L., Neeve, V. C., Best, A., Yarham, J. W., Kirschner, J., Schara, U., Talim, B., Topaloglu, H., Baric, I., Holinski-Feder, E., Abicht, A., Czermin, B., . . . Chinnery, P. F. (2014). Use of whole-exome sequencing to determine the genetic basis of multiple mitochondrial respiratory chain complex deficiencies. *Jama*, *312*(1), 68-77. https://doi.org/10.1001/jama.2014.7184

Thompson, K., Collier, J., Glasgow, R., Robertson, F., Pyle, A., Blakely, E., Alston, C., Olahova, M., McFarland, R., & Taylor, R. W. (2019). Recent advances in understanding the molecular genetic basis of mitochondrial disease. https://doi.org/10.1002/jimd.12104

Variantyx. (2024a). *Genomic Unity® Exome Plus Analysis*. https://www.variantyx.com/products-services/rare-disorder-genetics/comprehensive-analyses/genomic-unity-exome-plus-analysis/

Variantyx. (2024b). *Genomic Unity® Whole Genome Analysis*. https://www.variantyx.com/products-services/rare-disorder-genetics/comprehensive-analyses/genomic-unity-whole-genome-analysis/

Wagner, M., Berutti, R., Lorenz-Depiereux, B., Graf, E., Eckstein, G., Mayr, J. A., Meitinger, T., Ahting, U., Prokisch, H., Strom, T. M., & Wortmann, S. B. (2019). Mitochondrial DNA mutation analysis from exome sequencing-A more holistic approach in diagnostics of suspected mitochondrial disease. *J Inherit Metab Dis*, *42*(5), 909-917. https://doi.org/10.1002/jimd.12109

Wood, E., Parker, M., Dunning, M., Hesketh, S., Wang, D., Pink, R., & Fratter, C. (2019). Clinical long-read sequencing of the human mitochondrial genome for mitochondrial disease diagnostics. https://doi.org/10.1101/597187

Wu, Y., Balasubramaniam, S., Rius, R., Thorburn, D. R., Christodoulou, J., & Goranitis, I. (2021). Genomic sequencing for the diagnosis of childhood mitochondrial disorders: a health economic evaluation. *Eur J Hum Genet*. https://doi.org/10.1038/s41431-021-00916-8

a) Testing restricted to the known familial *NOTCH3* likely pathogenic or pathogenic variant.

b) Comprehensive *NOTCH3* sequencing only if the specific familial likely pathogenic or pathogenic variant is unknown.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

3) For all other situations not discussed above, genetic testing for CADASIL syndrome **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: First-degree relatives include parents, full siblings, and children of the individual. Second-degree relatives include grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings of the individual.

IV. Table of Terminology

Term	Definition
AD	Alzheimer disease
AHA	American Heart Association
ASA	American Stroke Association
CADASIL	Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
cSVD	Cerebral small-vessel disease
CT	Computed tomography
EAN	European Academy of Neurology
EFNS	European Federation of Neurological Studies
EGFr	Epidermal growth factor-like repeat
EMR	Electronic medical records
FDA	Food and Drug Administration
GOM	Granular osmiophilic material
HTRA1	Serine protease
LDT	Laboratory-developed test
MCI	Mild cognitive impairment
MRI	Magnetic resonance imaging

NGS	Next-generation sequencing
NORD	National Organization for Rare Disorders
NOTCH3	*Notch receptor 3*
PV	Pathogenic variant
SVD	Small vessel disease
SVaD	Subcortical vascular dementia
T2	Transverse relaxation time
TIA	Transient ischemic attack
USPSTF	United States Preventive Services Task Force
VCI-SVD	Vascular cognitive impairment secondary to small vessel disease
VSMCs	Vascular smooth muscle cells
WES	Whole-exome sequencing
WMHs	White matter hyperintensities

V. Scientific Background

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the most common hereditary small vessel disease and is characterized by granular osmiophilic material deposits surrounding blood vessels, a prominent thickening of the vessel wall by extracellular matrix accumulation, and a progressive loss of vascular smooth muscle cells (VSMCs) (Fernandez-Susavila et al., 2018; Ferrante et al., 2019; Monet-Lepretre et al., 2013). Small vessel diseases such as this are an important cause of stroke and vascular cognitive decline in adults (Chabriat et al., 2009). VSMC dysfunction may be caused by mutations in the *NOTCH3* gene, leading to irregularities in VSMC proliferation, cell cycle affliction, senescence, and cellular apoptosis (Dziewulska et al., 2018).

Individual symptoms, onset, and disease severity span a wide spectrum (Wang, 2018). Thus, descriptions of hereditary multi-infarct dementia, chronic familial vascular encephalopathy, and familial subcortical dementia, originally thought to be separate disorders, represent early reports of this condition (Dichgans, 2024). CADASIL usually presents with one or more of the following: dementia, psychiatric disturbances, migraine, and recurrent strokes (Chabriat et al., 2009; Dichgans et al., 1998; M.Wang, 2018). Rarer symptoms include lumbago, humpback, and Parkinson syndrome (Lim et al., 2019). Migraine with aura occurs in 55% of CADASIL cases and is often the initial manifestation of the disease (Di Donato et al., 2017). Subcortical ischemic attacks begin at a mean age of 47 years and present as lacunar syndromes (Adib-Samii et al., 2010; Dichgans et al., 1998). Accumulation of lacunae, which impact executive performance and function independence, strongly correlate to clinical severity (Ling et al., 2017). Cognitive impairment associated with CADASIL is progressive; a profile of frontal lobe dysfunction, declarative memory impairment suggestive of a retrieval deficit, and

relatively preserved language is often evident with this disease (Harris & Filley, 2001). A concurrent stepwise deterioration due to recurrent strokes is also common (Rutten & Lesnik Oberstein, 2016). Mood disturbances are reported in approximately 30% of individuals (Adib-Samii et al., 2010; Dichgans et al., 1998). Further, apathy, which may be independent of depression, is reported in 40% of individuals (Reyes et al., 2009).

Genetic linking of the disorder to chromosome 19 was first recognized in 1993, and the identification of the *NOTCH3* gene from the CADASIL mapped region was later discovered in 1996 (Ping & Zhao, 2018). While CADASIL was originally diagnosed via neuroimaging techniques, such as magnetic resonance imaging (MRI), the identification of the distinctive missense mutations in *NOTCH3* has allowed genetic testing to debut as the current gold standard for CADASIL diagnostics (Rutten & Lesnik Oberstein, 2016). However, MRI testing for the detection of cerebral white matter changes in the brain is still used to assist in CADASIL diagnoses; most often, MRI imaging is used as a diagnostic measure before symptoms present (Ferrante et al., 2019).

Missense mutations in the *NOTCH3* gene typically lead to the gain or loss of a cysteine, therefore resulting in an unpaired number of cysteine residues in one of 34 highly conserved epidermal growth factor-like repeat (EGFr) domains (Joutel et al., 1996; Papakonstantinou et al., 2019; Rutten et al., 2014). This leads to an increased multimerization tendency of mutant NOTCH3 (Duering et al., 2011), toxic accumulation of the protein and extracellular matrix in disulfide cross-linked detergent-insoluble aggregates (Monet-Lepretre et al., 2013), altered neurovascular coupling (Huneau et al., 2018), and ultimately reduced cerebral blood flow, recurrent stroke, and vascular dementia (Rutten et al., 2016). However, certain *NOTCH3* mutations do not present with a cysteine change; this type of non-cysteine mutation can cause a great loss of structure in the NOTCH3 protein (Papakonstantinou et al., 2019).

More than 200 *NOTCH3* mutations have been reported since its original discovery in the development of CADASIL syndrome in 1996; some of these mutations result in a phenotypic change while some present as a silent mutation. A few prevalent *NOTCH3* variants include the 34 identified in EGFr. EGFr 1–6 pathogenic variants are more common in the CADASIL population than EGFr 7–34 pathogenic variants; unfortunately, patients with EGFr 1–6 variants tend to present with more severe symptoms and phenotypes (Papakonstantinou et al., 2019; Rutten et al., 2018). These severe symptoms include stroke onset an average of 12 years earlier and overall lower survival rates (Papakonstantinou et al., 2019).

The prevalence of the disease has been estimated to be at 0.8 to five per 100,000 individuals (Moreton et al., 2014; Narayan et al., 2012; Razvi et al., 2005). However,

many suspect that these numbers are underestimates. A more recent investigation of the frequency of the characteristic missense CADASIL mutations in a public database found a total prevalence of 3.4/1000 (Rutten et al., 2016).

Currently, no efficient treatment options to cure or prevent CADASIL syndrome are available (Hack et al., 2019; NORD, 2019); however, recent studies have shown proof of concept for a novel application of exon skipping and are a first step towards the development of a rational therapeutic approach to treat up to 94% of CADASIL-causing mutations (Rutten et al., 2016). Further, neurofilament light chains have now been identified as a promising CADASIL biomarker and can be detected in the serum of affected patients (Ferrante et al., 2019).

Analytical Validity

There are no established diagnostic criteria for CADASIL. The phenotype is highly variable, and although imaging may be suggestive, no characteristic is pathognomonic; genetic testing remains the gold standard for diagnosis (Rutten & Lesnik Oberstein, 2016; Wang, 2018). As a heterozygous pathogenic variant in the NOTCH3 protein coding gene is well established as a main reason for CADASIL development, a CADASIL diagnosis is generally delivered based on molecular genetic testing or electron microscopy and immunohistochemistry results. Molecular genetic testing approaches may include both gene-targeted testing and in-depth genomic testing, such as exome sequencing and genome sequencing (Hack et al., 2019; Papakonstantinou et al., 2019).

Immunohistochemistry combined with electron microscopy of skin biopsy can be useful when molecular testing is not definitive (Rutten & Lesnik Oberstein, 2016). Immunohistochemistry assay of a skin biopsy sample for the accumulation of NOTCH3 protein in the walls of small blood vessels (Joutel et al., 2001) has an estimated sensitivity and specificity at 85-90% and 95-100%, respectively (Lesnik Oberstein et al., 2003). Detection of granular osmiophilic material deposits (GOM) containing the ectodomain of the *NOTCH3* gene by electron microscopy (del Rio-Espinola et al., 2009; Muqtadar & Testai, 2012) had a sensitivity of 45% and a specificity of 100% (Brulin et al., 2002; Malandrini et al., 2007; Markus et al., 2002).

Magnetic resonance imaging (MRI) is useful to demonstrate radiologic features of CADASIL, including recent lunar infarctions and white matter hyperintensities. Computed tomography (CT) scans are less sensitive than MRI in this regard (Dichgans, 2024). MRI may also provide prognostic information. Brain lesions in CADASIL patients tend to precede symptoms by 10 to 15 years; however, a normal MRI in the fourth decade of life should not automatically rule out CADASIL syndrome even though most patients exhibit an abnormal MRI by age 35 (Samoes et al., 2016). White matter hyperintensities on MRI can be visualized in those aged 21 years and older, and lesion

volume correlates with the level of disability and three-year clinical course of CADASIL (Jouvent et al., 2016). Isolated T2 hyperintensities involving the temporal poles can differentiate CADASIL from chronic microvascular ischemia due to hypertension with a sensitivity and specificity of 95% and 80%, respectively (O'Sullivan et al., 2001). Cerebral microbleeds visible on T2 weighted MRI images detected in 36% of patients with CADASIL were independently associated with an increased risk of incident ischemic stroke and may be a marker for a subgroup of patients with CADASIL who have a more severe or advanced form of the disease (Puy et al., 2017).

Guo et al. (2021) studied the role of *NOTCH3* gene mutations and variants in Alzheimer Disease (AD) and subcortical vascular dementia (SVaD). CADASIL is a common etiology of SVaD. A total of 667 AD patients, 96 SVaD patients, and 365 healthy control participants, all recruited from the Southern Han Chinese population, were included in the study. The authors performed targeted capture sequencing on *NOTCH3* and adjacent intron regions. "Five known pathogenic variants (p.R182C, p.C201S, p.R544C, p.R607C, and p.R1006C) and two novel likely pathogenic variants (p.C201F and p.C1061F) were detected in 16 SVaD patients."

No pathogenic variants were found in AD patients. The authors concluded that the "findings broaden the mutational spectrum of NOTCH3 and validate the pathogenic role of *NOTCH3* mutations in SVaD, but do not support the notion that *NOTCH3* variation influences the risk of AD" (Guo et al., 2021).

Cho et al. (2021) performed an analysis on whole-exome sequencing data from 200,632 participants in the UK Biobank. The authors note that CADASIL is considered rare, but there is a higher frequency of cysteine-altering *NOTCH3* variants which could increase risk of apparently sporadic lacunar stroke. The authors compared frequency of stroke, vascular dementia, clinical features of CADASIL, and MRI white matter hyperintensity volume between carriers and non-carriers of 67 cysteine-altering *NOTCH3* variants. "*NOTCH3* variant carriers had increased risk of stroke (OR: 2.33, $p=0.0004$) and vascular dementia (OR: 5.00, $p=0.007$), and increased white matter hyperintensity volume (standardised difference: 0.52, $p<0.001$) and white matter ultrastructural damage on diffusion MRI (standardised difference: 0.72, $p<0.001$)." The authors concluded that "cysteine-changing *NOTCH3* variants are more common in the general population than expected from CADASIL prevalence and are risk factors for apparently 'sporadic' stroke and vascular dementia" (Cho et al., 2021).

Gravesteijn et al. (2021) studied the effect of *NOTCH3* variant position on NOTCH3 protein aggregation load. Vascular NOTCH3 aggregation was measured in skin biopsies and brain tissue from CADASIL patients. "CADASIL patients with an EGFr 7-34 variant have significantly less vascular NOTCH3 aggregation than patients with an EGFr

1-6 variant." The authors concluded that *NOTCH3* variant position may be a factor that underlies differences in CADASIL disease severity (Gravesteijn et al., 2021).

Clinical Utility and Validity

One study has reported that the sequence analysis of *NOTCH3* is 95-100% sensitive and 100% specific to establish the diagnosis of CADASIL (Dotti et al., 2005; Peters et al., 2005; Tikka et al., 2009; Yin et al., 2015). A preliminary scale was proposed to screen for patients who should undergo *NOTCH3* gene analysis with a sensitivity of 96.7% and a specificity of 74.2% (Pescini et al., 2012). Another study of Russian patients with clinically suspected CADASIL concluded that careful assessment of genealogical, clinical, and neuroimaging data in patients with lacunar stroke can help select patients with a high probability of finding mutations on genetic screening (Abramycheva et al., 2015). In the absence of clinical features suggestive of CADASIL, screening of patients with lacunar stroke, leukoarosis, and migraine have low yield (de Vries et al., 2009; Dong et al., 2003).

As individual symptoms and disease severity span a wide spectrum, it must be noted that symptom onset alone cannot warrant a CADASIL syndrome diagnosis. Researchers previously screened 123 patients who exhibited two common CADASIL symptoms: lacunar stroke and transient ischemic attack. These participants were genetically tested for CADASIL; it was determined that only 12.5% had a *NOTCH3* mutation, showing that common CADASIL symptoms are shared with many other disorders (Bersano et al., 2018). This highlights the importance of genetic testing as a diagnostic measure. Further, three features were found to be significantly associated with a CADASIL diagnosis: "A family history of stroke, the presence of dementia and external capsule lesions on MRI" (Bersano et al., 2018).

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) was first diagnosed by visualizing granular osmiophilic material (GOM) in the tunica media of small arteries through light microscopy. Although GOM deposit is the pathological hallmark of CADASIL, *NOTCH3* genetic sequencing is the confirmative diagnostic tool. While most genetic tests use Sanger sequencing methods to target specific *NOTCH3* exons, next-generation sequencing (NGS) and whole exome sequencing (WES) have proven to deliver greater efficacy. One study has reported that NGS and WES have increased sensitivity to detect low frequency variants of *NOTCH3* mutations compared to Sanger sequencing. Through Sanger sequencing, 10.8% of tests were able to identify *NOTCH3* mutations compared to 15.8% of tests identifying mutations through next-generation sequencing. With NGS, the results were in concordance with Sanger sequencing, but it extended the capacity to detect mutations and previously unreported variants. As

diagnostic sequencing techniques continue to advance, NGS and WES may play an important role in identifying other genes involved with CADASIL (Dunn et al., 2020).

Rutten et al. (2018) analyzed the effect of *NOTCH3* pathogenic variant (PV) location on CADASIL disease variability. The authors correlated PV position with brain MRI lesion load, age of first stoke, and survival on 664 European CADASIL patients. "CADASIL patients with an EGFr 1–6 pathogenic variant have a 12-year earlier onset of stroke than those with an EGFr 7–34 pathogenic variant, lower survival, and higher white matter hyperintensity volumes." The authors concluded that *NOTCH3* PV location is "the most important determinant of CADASIL disease severity"(Rutten et al., 2018).

Mukai et al. (2020) correlated genotypes and phenotypes of 179 Japanese CADASIL probands. The authors identified 68 mutations, "p.Cys388Arg, p.Cys435Phe, p.Gly481Cys, p.Cys743Tyr, and p.Cys1009Phe were novel ones." The authors then analyzed genotype-phenotype correlations on the three most common mutations. "p.Arg141Cys showed typical CADASIL phenotypes, whereas p.Arg75Pro showed mild and atypical phenotypes, a low frequency of stroke/TIA [transient ischemic attack], high frequency of hypertension, and low frequency of temporal pole lesions. p.Arg182Cys showed various initial symptoms other than stroke/TIA." The authors also studied mutation location and the age of stroke/TIA onset, and found that mutations of EGFr 1-6 (excluding p.Arg75Pro) were significantly correlated with a younger age of stroke/TIA onset than mutations in EGFr 7-43. The authors concluded that the data clarified genotype-phenotype correlations and the effect of mutation location on the age of stroke/TIA onset in Japanese CADASIL probands (Mukai et al., 2020).

Hack et al. (2020) performed a cross-sectional study using 118 participants with a *NOTCH3* cysteine altering variant and 184 age- and sex-matched control participants. Clinical, neuroimaging, and whole-exome data was compared. There was no difference in dementia, mild cognitive impairment, migraine with aura, or depression prevalence. Participants with a *NOTCH3* cysteine altering variant had a higher had a higher risk of stroke, white matter hyperintensity, and lacunas after age 65. The authors note that the classic mid-adult onset CADASIL phenotype was not reported, suggesting "*NOTCH3* variants do not only cause the rate and more severe hereditary CADASIL but are much more commonly associated with a milder [cerebral small vessel disease] SVD phenotype, specifically when these variants are located in EGFr 7 to 35" (Hack et al., 2020).

Liu et al. (2021) tracked clinical and MRI data of three patients from a family in China over seven years. Genetic tests confirmed CADASIL diagnosis on all three participants, including a novel mutation of p.C533S on exon 10 of *NOTCH3*. The same heterozygous mutations were detected across family members. The authors conclude that there is

"distinct heterogeneity of CADASIL patients in the same family with the same mutation" (Liu et al., 2021).

Chen et al. (2021) assessed the diagnostic utility of using NGS and MRI data for the diagnosis of adult onset leukodystrophy. The authors used a panel of 200 neurodegeneration-related genes and an MRI brain-based diagnostic algorithm from 45 patients with young-onset cognitive impairment with leukodystrophy. All the patients with an established genetic diagnosis had MRI brain patterns consistent with their diagnosis. A total of 51.4% of patients with MRI changes consistent with vascular cognitive impairment secondary to small vessel disease (VCI-SVD) had pathogenic variants (89.5% of which were pathogenic *NOTCH3* and 11.5% of which were *HTRA1* variants). The authors concluded that the results "demonstrated a high diagnostic utility incorporating a targeted neurodegeneration gene panel and MRI-based diagnostic algorithms in young-onset cognitive impairment patients with leukodystrophy" (Chen et al., 2021).

Anisetti et al. (2023) gathered the electronic medical records (EMR) of adult patients with confirmed CADASIL disease to analyze the use of a recently proposed grading system. A grade of zero (asymptomatic), Grade One (migraine only), Grade Two (stroke, TIA, or MCI), Grade Three (gait assistance or dementia) or Grade four (bedbound or end-stage) was given to each patient. The inter-rater reliability of grading was also assessed with an 81.8% agreement on ratings. Results showed that those patients who received a lower grade on the CADASIL scale were younger (49.5 vs. 61.9 years) and were less likely to have hypertension and/or diabetes mellitus. Higher ratings were correlated with increased vascular risk factors. The authors concluded that a CADASIL grading system based on symptoms was a reliable categorization of patients to assess higher vascular risk factor burden (Anisetti et al., 2023).

Boston et al. (2024) completed a systematic review on the most common *NOTCH3* mutations that cause CADASIL and CADASIL-like cerebral small vessel disease. The authors were aiming to investigate the association between phenotypes and genotypes across the most common *NOTCH3* mutations in CADASIL patients. "The six most common NOTCH3 missense mutations globally were the p.R75P, p.R133C, p.R141C, p.R169C, p.R182C, and p.R544C, of which p.R133C was described to occur most often." p.R75P, p.R141C, p.R182C and p.R544C genotypes were "highly congruent" with white matter hyperintensities on MRI. P.R141C genotype was associated with decreased disease severity. Although there were some associations, overall, "statistical analysis showed there were no overall differences between the phenotypic characteristics of the two common mutations, p.R141C and p.R544C" (Boston et al., 2024)

Predictive Testing of At-Risk Family Members

For an asymptomatic individual, knowledge of mutation status will generally not lead to any management changes that can prevent or delay the onset of the disorder. Avoiding tobacco use may be a factor that delays onset of disease, but this is a general recommendation that is not altered by genetic testing. Goldman (2015) has suggested that asymptomatic family members follow the guidelines for presymptomatic testing for Huntington disease (HDSA, 2016).

Genetic testing for CADASIL may assist decision making in areas such as employment choices and reproductive decision making. However, the impact of these decisions on health outcomes is uncertain. Further, the testing of asymptomatic at-risk individuals with nonspecific or equivocal symptoms is not useful in predicting age of onset, severity, type of symptoms, or rate of progression in asymptomatic individuals (Rutten & Lesnik Oberstein, 2016). Initial data from Reyes et al. (2012) show that predictive testing is rarely requested and has a high dropout rate.

Di Donato et al. (2017) state that the MRI of an unaffected family member could have a similar impact to a genetic test because MRIs are able to accurately predict CADASIL disease development before symptoms present. Therefore, the potential implications of MRI testing should be shared before this type of testing is completed.

Akrich et al. (2024) studied the population at risk of CADASIL who had not completed diagnostic procedures, aiming to understand the hesitation. The study included a questionnaire survey with 40 questions about why people choose to undergo or not undergo a genetic test, and what lead to that decision. The questionnaire was sent to 883 people, and 359 replied. Of the 359 replies, 197 were from *NOTCH3*-mutation carriers, 81 were from close relatives, and 81 were from individuals at risk of CADASIL. "Results suggest that, far from being a simple, unequivocal path, the decision-making process leading to the choice of diagnosis is initially slowed down by the need to distance oneself from the disease so that it doesn't take over one's life, and then evolves under the influence of a complex tangle between advancing age, the presence of early symptoms, and the personal relationship with uncertainty" (Akrich et al., 2024).

VI. Guidelines and Recommendations

American Heart Association (AHA) and American Stroke Association (ASA)

The American Heart Association and American Stroke Association provide suggestions on when rare genetic causes could be suspected. They suggest that the diagnosis could be made based on testing for mutations in the *NOTCH3* gene (Kleindorfer et al., 2021; Powers et al., 2019; Smith et al., 2017).

In 2023, the AHA released a scientific statement about the management of inherited central nervous system small vessel diseases, specifically CADASIL. The AHA recommends: "Consider gene testing in patients with small vessel stroke before 55 y of age with a paucity of vascular risk factors (eg, normotensive, nondiabetic, nonsmoker) or positive family history of CADASIL." The AHA also notes that "one should distinguish diagnostic testing in individuals who have clinical manifestations of disease from predictive or presymptomatic testing. In general, children, unless emancipated minors, should not undergo predictive testing because this robs them of the choice of knowing or not knowing their status. The penetrance of *NOTCH3* variants is incomplete and highly variable. Testing and finding mutations in *NOTCH3* can lead to pessimistic prognostication and bias of detecting asymptomatic brain lesions with MRI. This can have life-changing, negative psychological consequences" (Meschia et al., 2023).

European Federation of Neurological Societies (EFNS)

The European Federation of Neurological Societies guideline on the molecular diagnosis of channelopathies, epilepsies, migraine, stroke, and dementias notes that most *NOTCH3* mutations occur within exons three and four and suggests direct sequencing of these two exons if clinical suspicion is high (Burgunder et al., 2010).

United States Preventive Services Task Force (USPSTF)

As of September 19, 2023, the USPSTF has not published guidelines for the genetic testing of CADASIL patients.

European Academy of Neurology (EAN)

The European Academy of Neurology (EAN) released guidelines for monogenic cerebral small-vessel disease (cSVD), including diagnosis and management of CADASIL. EAN suggests that the first line diagnosis for CADASIL should be genetic testing, but diagnosis can also be established by skin biopsy with electron microscopy revealing granular osmiophilic material (GOM). Most *NOTCH3* variants causing CADASIL are due to a loss or gain of a cysteine in the EGFR repeats. Some non-cysteine changing variants have been reported, but most of these non-cysteine changing variants do not lead to a diseased state. If genetic testing reveals a non-cysteine changing variant, electron microscopy to visualize GOM is a useful tool to confirm CADASIL diagnosis. If the *NOTCH3* variant is of unknown significance, CADASIL diagnosis can be established with skin biopsy via electron microscopy or immunohistochemistry of the NOTCH3 extracellular domain. The guideline recommends "considering" a CADASIL diagnosis in any patient with "unexplained symmetrical periventricular WMHs [white matter hyperintensities] and a positive family history of migraine with aura, stroke, mood disorders or dementia." The guideline also

notes that CADASIL cannot be ruled out in the presence of "common cerebrovascular risk factors and extensive WMHs" or in "the absence of a medical or family history of migraine with aura." The guideline remarks that "although most patients have a family history, if the clinical and imaging phenotype is consistent with CADASIL the diagnosis should be considered" (Mancuso et al., 2020).

Overall, the EAN remarks that "CADASIL can only be definitively confirmed by genetic testing, revealing a *NOTCH3* mutation altering the number of cysteines in one of the 34 EGFr domains of the NOTCH3 protein" (Mancuso et al., 2020).

National Organization for Rare Diseases (NORD)

The National Organization for Rare Diseases released diagnosis guidelines on the disease. "CADASIL is based on symptoms, family history, and brain MRI lesions compatible with the disease. The CADASIL diagnosis can only be confirmed by DNA testing of blood samples for characteristic mutations in the *NOTCH3* gene or by identifying granular osmiophilic material (GOM) inclusions on a skin biopsy" (Orjuela, 2019).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description

81403	Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons)

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Abramycheva, N., Stepanova, M., Kalashnikova, L., Zakharova, M., Maximova, M., Tanashyan, M., Lagoda, O., Fedotova, E., Klyushnikov, S., Konovalov, R., Sakharova, A., & Illarioshkin, S. (2015). New mutations in the Notch3 gene in patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL). *J Neurol Sci*, *349*(1-2), 196-201. https://doi.org/10.1016/j.jns.2015.01.018

Adib-Samii, P., Brice, G., Martin, R. J., & Markus, H. S. (2010). Clinical spectrum of CADASIL and the effect of cardiovascular risk factors on phenotype: study in 200 consecutively recruited individuals. *Stroke*, *41*(4), 630-634. https://doi.org/10.1161/STROKEAHA.109.568402

Akrich, M., Rabeharisoa, V., Paterson, F., & Chabriat, H. (2024). Genetic diagnosis of individuals at risk of CADASIL: prospect for future therapeutic development. *J Neurol*. https://doi.org/10.1007/s00415-024-12640-6

Anisetti, B., Greco, E., Stojadinovic, E., Goldstein, E. D., Sakusic, A., Badi, M. K., Liu, M. D., Lin, M. P., Chiang, C. C., Elahi, F. M., Worrall, B. B., Petrosian, D., Ross, O., & Meschia, J. F. (2023). Novel grading system for CADASIL severity: A multicenter cross-sectional study. *Cereb Circ Cogn Behav*, *5*, 100170. https://doi.org/10.1016/j.cccb.2023.100170

Bersano, A., Bedini, G., Markus, H. S., Vitali, P., Colli-Tibaldi, E., Taroni, F., Gellera, C., Baratta, S., Mosca, L., Carrera, P., Ferrari, M., Cereda, C., Grieco, G., Lanfranconi, S., Mazucchelli, F., Zarcone, D., De Lodovici, M. L., Bono, G., Boncoraglio, G. B., . . . Candelise, L. (2018). The role of clinical and neuroimaging features in the diagnosis of CADASIL. *J Neurol*, *265*(12), 2934-2943. https://doi.org/10.1007/s00415-018-9072-8

Boston, G., Jobson, D., Mizuno, T., Ihara, M., & Kalaria, R. N. (2024). Most common NOTCH3 mutations causing CADASIL or CADASIL-like cerebral small vessel disease: A systematic review. *Cereb Circ Cogn Behav*, *6*, 100227. https://doi.org/10.1016/j.cccb.2024.100227

Brulin, P., Godfraind, C., Leteurtre, E., & Ruchoux, M. M. (2002). Morphometric analysis of ultrastructural vascular changes in CADASIL: analysis of 50 skin biopsy specimens and pathogenic implications. *Acta Neuropathol*, *104*(3), 241-248. https://doi.org/10.1007/s00401-002-0530-z

Burgunder, J. M., Finsterer, J., Szolnoki, Z., Fontaine, B., Baets, J., Van Broeckhoven, C., Di Donato, S., De Jonghe, P., Lynch, T., Mariotti, C., Schols, L., Spinazzola, A., Tabrizi, S. J., Tallaksen, C., Zeviani, M., Harbo, H. F., & Gasser, T. (2010). EFNS guidelines on the molecular diagnosis of channelopathies, epilepsies, migraine, stroke, and dementias. *Eur J Neurol*, *17*(5), 641-648. https://doi.org/10.1111/j.1468-1331.2010.02985.x

Chabriat, H., Joutel, A., Dichgans, M., Tournier-Lasserve, E., & Bousser, M. G. (2009). Cadasil. *Lancet Neurol*, *8*(7), 643-653. https://doi.org/10.1016/S1474-4422(09)70127-9

Chen, Z., Tan, Y. J., Lian, M. M., Tandiono, M., Foo, J. N., Lim, W. K., Kandiah, N., Tan, E. K., & Ng, A. S. L. (2021). High Diagnostic Utility Incorporating a Targeted Neurodegeneration Gene Panel With MRI Brain Diagnostic Algorithms in Patients With Young-Onset Cognitive Impairment With Leukodystrophy. *Front Neurol*, *12*, 631407. https://doi.org/10.3389/fneur.2021.631407

Cho, B. P. H., Nannoni, S., Harshfield, E. L., Tozer, D., Gräf, S., Bell, S., & Markus, H. S. (2021). NOTCH3 variants are more common than expected in the general population and associated with stroke and vascular dementia: an analysis of 200 000 participants. *J Neurol Neurosurg Psychiatry*, *92*(7), 694-701. https://doi.org/10.1136/jnnp-2020-325838

de Vries, B., Frants, R. R., Ferrari, M. D., & van den Maagdenberg, A. M. (2009). Molecular genetics of migraine. *Hum Genet*, *126*(1), 115-132. https://doi.org/10.1007/s00439-009-0684-z

del Rio-Espinola, A., Mendioroz, M., Domingues-Montanari, S., Pozo-Rosich, P., Sole, E., Fernandez-Morales, J., Fernandez-Cadenas, I., & Montaner, J. (2009). CADASIL management or what to do when there is little one can do. *Expert Rev Neurother*, *9*(2), 197-210. https://doi.org/10.1586/14737175.9.2.197

Di Donato, I., Bianchi, S., De Stefano, N., Dichgans, M., Dotti, M. T., Duering, M., Jouvent, E., Korczyn, A. D., Lesnik-Oberstein, S. A. J., Malandrini, A., Markus, H. S., Pantoni, L., Penco, S., Rufa, A., Sinanović, O., Stojanov, D., & Federico, A. (2017). Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) as a model of small vessel disease: update on clinical, diagnostic, and management aspects. *BMC Med*, *15*. https://doi.org/10.1186/s12916-017-0778-8

Dichgans, M. (2024, Jun 24, 2024). *Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)*. https://www.uptodate.com/contents/cerebral-autosomal-dominant-arteriopathy-with-subcortical-infarcts-and-leukoencephalopathy-cadasil

Dichgans, M., Mayer, M., Uttner, I., Bruning, R., Muller-Hocker, J., Rungger, G., Ebke, M., Klockgether, T., & Gasser, T. (1998). The phenotypic spectrum of CADASIL: clinical findings in 102 cases. *Ann Neurol*, *44*(5), 731-739. https://doi.org/10.1002/ana.410440506

Dong, Y., Hassan, A., Zhang, Z., Huber, D., Dalageorgou, C., & Markus, H. S. (2003). Yield of screening for CADASIL mutations in lacunar stroke and leukoaraiosis. *Stroke*, *34*(1), 203-205. https://www.ncbi.nlm.nih.gov/pubmed/12511775

Dotti, M. T., Federico, A., Mazzei, R., Bianchi, S., Scali, O., Conforti, F. L., Sprovieri, T., Guidetti, D., Aguglia, U., Consoli, D., Pantoni, L., Sarti, C., Inzitari, D., & Quattrone, A. (2005). The spectrum of Notch3 mutations in 28 Italian CADASIL families. *J Neurol Neurosurg Psychiatry*, *76*(5), 736-738. https://doi.org/10.1136/jnnp.2004.048207

Duering, M., Karpinska, A., Rosner, S., Hopfner, F., Zechmeister, M., Peters, N., Kremmer, E., Haffner, C., Giese, A., Dichgans, M., & Opherk, C. (2011). Co-aggregate formation of CADASIL-mutant NOTCH3: a single-particle analysis. *Hum Mol Genet*, *20*(16), 3256-3265. https://doi.org/10.1093/hmg/ddr237

Dunn, P. J., Maksemous, N., Smith, R. A., Sutherland, H. G., Haupt, L. M., & Griffiths, L. R. (2020). Investigating diagnostic sequencing techniques for CADASIL diagnosis. *Hum Genomics*, *14*(1), 2. https://doi.org/10.1186/s40246-019-0255-x

Dziewulska, D., Nycz, E., Rajczewska-Oleszkiewicz, C., Bojakowski, J., & Sulejczak, D. (2018). Nuclear abnormalities in vascular myocytes in cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). *Neuropathology*, *38*(6), 601-608. https://doi.org/10.1111/neup.12519

Fernandez-Susavila, H., Mora, C., Aramburu-Nunez, M., Quintas-Rey, R., Arias, S., Collado, M., Lopez-Arias, E., Sobrino, T., Castillo, J., Dell'Era, P., & Campos, F. (2018). Generation and characterization of the human iPSC line IDISi001-A isolated from blood cells of a CADASIL patient carrying a NOTCH3 mutation. *Stem Cell Res*, *28*, 16-20. https://doi.org/10.1016/j.scr.2018.01.023

Ferrante, E. A., Cudrici, C. D., & Boehm, M. (2019). CADASIL: new advances in basic science and clinical perspectives. *Curr Opin Hematol*, *26*(3), 193-198. https://doi.org/10.1097/moh.0000000000000497

Goldman, J. S. (2015). Genetic testing and counseling in the diagnosis and management of young-onset dementias. *Psychiatr Clin North Am*, *38*(2), 295-308. https://doi.org/10.1016/j.psc.2015.01.008

Gravesteijn, G., Hack, R. J., Mulder, A. A., Cerfontaine, M. N., van Doorn, R., Hegeman, I. M., Jost, C. R., Rutten, J. W., & Lesnik Oberstein, S. A. J. (2021). NOTCH3 variant position is associated with NOTCH3 aggregation load in CADASIL vasculature. *Neuropathol Appl Neurobiol*. https://doi.org/10.1111/nan.12751

Guo, L., Jiao, B., Liao, X., Xiao, X., Zhang, W., Yuan, Z., Liu, X., Zhou, L., Wang, X., Zhu, Y., Yang, Q., Wang, J., Tang, B., & Shen, L. (2021). The role of NOTCH3 variants in

Alzheimer's disease and subcortical vascular dementia in the Chinese population. *CNS Neurosci Ther*, *27*(8), 930-940. https://doi.org/10.1111/cns.13647

Hack, R., Rutten, J., & Lesnik Oberstein, S. A. J. (2019). CADASIL. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews((R))*. University of Washington, Seattle.

Hack, R. J., Rutten, J. W., Person, T. N., Li, J., Khan, A., Griessenauer, C. J., Abedi, V., Lesnik Oberstein, S. A. J., & Zand, R. (2020). Cysteine-Altering NOTCH3 Variants Are a Risk Factor for Stroke in the Elderly Population. *Stroke*, *51*(12), 3562-3569. https://doi.org/10.1161/strokeaha.120.030343

Harris, J. G., & Filley, C. M. (2001). CADASIL: neuropsychological findings in three generations of an affected family. *J Int Neuropsychol Soc*, *7*(6), 768-774. https://www.ncbi.nlm.nih.gov/pubmed/11575598

HDSA. (2016). *HDSA Genetic Testing Protocol for HD* http://hdsa.org/wp-content/uploads/2015/02/HDSA-Gen-Testing-Protocol-for-HD.pdf

Huneau, C., Houot, M., Joutel, A., Beranger, B., Giroux, C., Benali, H., & Chabriat, H. (2018). Altered dynamics of neurovascular coupling in CADASIL. *Ann Clin Transl Neurol*, *5*(7), 788-802. https://doi.org/10.1002/acn3.574

Joutel, A., Corpechot, C., Ducros, A., Vahedi, K., Chabriat, H., Mouton, P., Alamowitch, S., Domenga, V., Cecillion, M., Marechal, E., Maciazek, J., Vayssiere, C., Cruaud, C., Cabanis, E. A., Ruchoux, M. M., Weissenbach, J., Bach, J. F., Bousser, M. G., & Tournier-Lasserve, E. (1996). Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature*, *383*(6602), 707-710. https://doi.org/10.1038/383707a0

Joutel, A., Favrole, P., Labauge, P., Chabriat, H., Lescoat, C., Andreux, F., Domenga, V., Cecillon, M., Vahedi, K., Ducros, A., Cave-Riant, F., Bousser, M. G., & Tournier-Lasserve, E. (2001). Skin biopsy immunostaining with a Notch3 monoclonal antibody for CADASIL diagnosis. *Lancet*, *358*(9298), 2049-2051. https://doi.org/10.1016/S0140-6736(01)07142-2

Jouvent, E., Duchesnay, E., Hadj-Selem, F., De Guio, F., Mangin, J. F., Herve, D., Duering, M., Ropele, S., Schmidt, R., Dichgans, M., & Chabriat, H. (2016). Prediction of 3-year clinical course in CADASIL. *Neurology*, *87*(17), 1787-1795. https://doi.org/10.1212/WNL.0000000000003252

Kleindorfer, D. O., Towfighi, A., Chaturvedi, S., Cockroft, K. M., Gutierrez, J., Lombardi-Hill, D., Kamel, H., Kernan, W. N., Kittner, S. J., Leira, E. C., Lennon, O., Meschia, J. F., Nguyen, T. N., Pollak, P. M., Santangeli, P., Sharrief, A. Z., Smith, S. C., Jr., Turan, T. N., & Williams, L. S. (2021). 2021 Guideline for the Prevention of Stroke in Patients With Stroke and Transient Ischemic Attack: A Guideline From the American Heart Association/American Stroke Association. *Stroke*, *52*(7), e364-e467. https://doi.org/10.1161/str.0000000000000375

Lesnik Oberstein, S. A., van Duinen, S. G., van den Boom, R., Maat-Schieman, M. L., van Buchem, M. A., van Houwelingen, H. C., Hegeman-Kleinn, I. M., Ferrari, M. D., Breuning, M. H., & Haan, J. (2003). Evaluation of diagnostic NOTCH3 immunostaining in CADASIL. *Acta Neuropathol*, *106*(2), 107-111. https://doi.org/10.1007/s00401-003-0701-6

Lim, H. K., Millar, Z. A., & Zaman, R. (2019). CADASIL and Bipolar Affective Disorder. *Psychiatr Danub*, *31*(Suppl 3), 591-594.

Ling, Y., De Guio, F., Duering, M., Jouvent, E., Herve, D., Godin, O., Dichgans, M., & Chabriat, H. (2017). Predictors and Clinical Impact of Incident Lacunes in Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy. *Stroke*, *48*(2), 283-289. https://doi.org/10.1161/strokeaha.116.015750

Liu, Y., Huang, S., Yu, L., Li, T., Diao, S., Chen, Z., Zhou, G., Sheng, X., Xu, Y., & Fang, Q. (2021). A Chinese CADASIL Family with a Novel Mutation on Exon 10 of Notch3 Gene. *J Stroke Cerebrovasc Dis*, *30*(8), 105674. https://doi.org/10.1016/j.jstrokecerebrovasdis.2021.105674

M.Wang, A. l. o. o. p. (2018). *Handbook of Clinical Neurology* (Vol. 148). https://www.sciencedirect.com/science/article/pii/B9780444640765000478

Malandrini, A., Gaudiano, C., Gambelli, S., Berti, G., Serni, G., Bianchi, S., Federico, A., & Dotti, M. T. (2007). Diagnostic value of ultrastructural skin biopsy studies in CADASIL. *Neurology*, *68*(17), 1430-1432. https://doi.org/10.1212/01.wnl.0000264018.46335.c8

Mancuso, M., Arnold, M., Bersano, A., Burlina, A., Chabriat, H., Debette, S., Enzinger, C., Federico, A., Filla, A., Finsterer, J., Hunt, D., Lesnik Oberstein, S., Tournier-Lasserve, E., & Markus, H. S. (2020). Monogenic cerebral small-vessel diseases: diagnosis and therapy. Consensus recommendations of the European Academy of Neurology. *Eur J Neurol*, *27*(6), 909-927. https://doi.org/10.1111/ene.14183

Markus, H. S., Martin, R. J., Simpson, M. A., Dong, Y. B., Ali, N., Crosby, A. H., & Powell, J. F. (2002). Diagnostic strategies in CADASIL. *Neurology*, *59*(8), 1134-1138. https://www.ncbi.nlm.nih.gov/pubmed/12395806

Meschia, J. F., Worrall, B. B., Elahi, F. M., Ross, O. A., Wang, M. M., Goldstein, E. D., Rost, N. S., Majersik, J. J., & Gutierrez, J. (2023). Management of Inherited CNS Small Vessel Diseases: The CADASIL Example: A Scientific Statement From the American Heart Association. *Stroke*, *54*(10), e452-e464. https://doi.org/10.1161/str.0000000000000444

Monet-Lepretre, M., Haddad, I., Baron-Menguy, C., Fouillot-Panchal, M., Riani, M., Domenga-Denier, V., Dussaule, C., Cognat, E., Vinh, J., & Joutel, A. (2013). Abnormal recruitment of extracellular matrix proteins by excess Notch3 ECD: a new pathomechanism in CADASIL. *Brain*, *136*(Pt 6), 1830-1845. https://doi.org/10.1093/brain/awt092

Moreton, F. C., Razvi, S. S., Davidson, R., & Muir, K. W. (2014). Changing clinical patterns and increasing prevalence in CADASIL. *Acta Neurol Scand*, *130*(3), 197-203. https://doi.org/10.1111/ane.12266

Mukai, M., Mizuta, I., Watanabe-Hosomi, A., Koizumi, T., Matsuura, J., Hamano, A., Tomimoto, H., & Mizuno, T. (2020). Genotype-phenotype correlations and effect of mutation location in Japanese CADASIL patients. *J Hum Genet*, *65*(8), 637-646. https://doi.org/10.1038/s10038-020-0751-9

Muqtadar, H., & Testai, F. D. (2012). Single gene disorders associated with stroke: a review and update on treatment options. *Curr Treat Options Cardiovasc Med*, *14*(3), 288-297. https://doi.org/10.1007/s11936-012-0179-4

Narayan, S. K., Gorman, G., Kalaria, R. N., Ford, G. A., & Chinnery, P. F. (2012). The minimum prevalence of CADASIL in northeast England. *Neurology*, *78*(13), 1025-1027. https://doi.org/10.1212/WNL.0b013e31824d586c

NORD. (2019). *CADASIL*. https://rarediseases.org/rare-diseases/cadasil/

O'Sullivan, M., Jarosz, J. M., Martin, R. J., Deasy, N., Powell, J. F., & Markus, H. S. (2001). MRI hyperintensities of the temporal lobe and external capsule in patients with CADASIL. *Neurology*, *56*(5), 628-634. https://www.ncbi.nlm.nih.gov/pubmed/11245715

Opherk, C., Peters, N., Herzog, J., Luedtke, R., & Dichgans, M. (2004). Long-term prognosis and causes of death in CADASIL: a retrospective study in 411 patients. *Brain*, *127*(Pt 11), 2533-2539. https://doi.org/10.1093/brain/awh282

Orjuela, K. (2019). *CADASIL*. National Organization for Rare Diseases Retrieved Oct. 11, 2022 from https://rarediseases.org/rare-diseases/cadasil/

Papakonstantinou, E., Bacopoulou, F., Brouzas, D., Megalooikonomou, V., D'Elia, D., Bongcam-Rudloff, E., & Vlachakis, D. (2019). NOTCH3 and CADASIL syndrome: a genetic and structural overview. *EMBnet J*, *24*. https://doi.org/10.14806/ej.24.0.921

Pescini, F., Nannucci, S., Bertaccini, B., Salvadori, E., Bianchi, S., Ragno, M., Sarti, C., Valenti, R., Zicari, E., Moretti, M., Chiti, S., Stromillo, M. L., De Stefano, N., Dotti, M. T., Federico, A., Inzitari, D., & Pantoni, L. (2012). The Cerebral Autosomal-Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy (CADASIL) Scale: a screening tool to select patients for NOTCH3 gene analysis. *Stroke*, *43*(11), 2871-2876. https://doi.org/10.1161/STROKEAHA.112.665927

Peters, N., Opherk, C., Bergmann, T., Castro, M., Herzog, J., & Dichgans, M. (2005). Spectrum of mutations in biopsy-proven CADASIL: implications for diagnostic strategies. *Arch Neurol*, *62*(7), 1091-1094. https://doi.org/10.1001/archneur.62.7.1091

Ping, S., & Zhao, L.-R. (2018). *Current Understanding of Pathology and Therapeutic Status for CADASIL*. https://link.springer.com/chapter/10.1007/978-3-319-90194-7_12

Powers, W. J., Rabinstein, A. A., Ackerson, T., Adeoye, O. M., Bambakidis, N. C., Becker, K., Biller, J., Brown, M., Demaerschalk, B. M., Hoh, B., Jauch, E. C., Kidwell, C. S., Leslie-Mazwi, T. M., Ovbiagele, B., Scott, P. A., Sheth, K. N., Southerland, A. M., Summers, D.

V., & Tirschwell, D. L. (2019). Guidelines for the Early Management of Patients With Acute Ischemic Stroke: 2019 Update to the 2018 Guidelines for the Early Management of Acute Ischemic Stroke: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. *Stroke*, *50*(12), e344-e418. https://doi.org/doi:10.1161/STR.0000000000000211

Puy, L., De Guio, F., Godin, O., Duering, M., Dichgans, M., Chabriat, H., & Jouvent, E. (2017). Cerebral Microbleeds and the Risk of Incident Ischemic Stroke in CADASIL (Cerebral Autosomal Dominant Arteriopathy With Subcortical Infarcts and Leukoencephalopathy). *Stroke*, *48*(10), 2699-2703. https://doi.org/10.1161/strokeaha.117.017839

Razvi, S. S., Davidson, R., Bone, I., & Muir, K. W. (2005). The prevalence of cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) in the west of Scotland. *J Neurol Neurosurg Psychiatry*, *76*(5), 739-741. https://doi.org/10.1136/jnnp.2004.051847

Reyes, S., Kurtz, A., Herve, D., Tournier-Lasserve, E., & Chabriat, H. (2012). Presymptomatic genetic testing in CADASIL. *J Neurol*, *259*(10), 2131-2136. https://doi.org/10.1007/s00415-012-6468-8

Reyes, S., Viswanathan, A., Godin, O., Dufouil, C., Benisty, S., Hernandez, K., Kurtz, A., Jouvent, E., O'Sullivan, M., Czernecki, V., Bousser, M. G., Dichgans, M., & Chabriat, H. (2009). Apathy: a major symptom in CADASIL. *Neurology*, *72*(10), 905-910. https://doi.org/10.1212/01.wnl.0000344166.03470.f8

Rutten, J. W., Dauwerse, H. G., Gravesteijn, G., van Belzen, M. J., van der Grond, J., Polke, J. M., Bernal-Quiros, M., & Lesnik Oberstein, S. A. (2016). Archetypal NOTCH3 mutations frequent in public exome: implications for CADASIL. *Ann Clin Transl Neurol*, *3*(11), 844-853. https://doi.org/10.1002/acn3.344

Rutten, J. W., Haan, J., Terwindt, G. M., van Duinen, S. G., Boon, E. M., & Lesnik Oberstein, S. A. (2014). Interpretation of NOTCH3 mutations in the diagnosis of CADASIL. *Expert Rev Mol Diagn*, *14*(5), 593-603. https://doi.org/10.1586/14737159.2014.922880

Rutten, J. W., & Lesnik Oberstein, S. A. J. (2016). *Cadasil* https://www.ncbi.nlm.nih.gov/pubmed/20301673

Rutten, J. W., Van Eijsden, B. J., Duering, M., Jouvent, E., Opherk, C., Pantoni, L., Federico, A., Dichgans, M., Markus, H. S., Chabriat, H., & Lesnik Oberstein, S. A. J. (2018). The effect of NOTCH3 pathogenic variant position on CADASIL disease severity: NOTCH3 EGFr 1-6 pathogenic variant are associated with a more severe phenotype and lower survival compared with EGFr 7-34 pathogenic variant. *Genet Med*. https://doi.org/10.1038/s41436-018-0088-3

Samoes, R., Alves, J. E., Taipa, R., Silva, J., Melo Pires, M., & Pereira-Monteiro, J. M. (2016). CADASIL: MRI may be normal in the fourth decade of life - a case report. *Cephalalgia*, *36*(11), 1082-1085. https://doi.org/10.1177/0333102415618613

Smith, E. E., Saposnik, G., Biessels, G. J., Doubal, F. N., Fornage, M., Gorelick, P. B., Greenberg, S. M., Higashida, R. T., Kasner, S. E., Seshadri, S., American Heart Association Stroke, C., Council on Cardiovascular, R., Intervention, Council on Functional, G., Translational, B., & Council on, H. (2017). Prevention of Stroke in Patients With Silent Cerebrovascular Disease: A Scientific Statement for Healthcare Professionals From the American Heart Association/American Stroke Association. *Stroke*, *48*(2), e44-e71. https://doi.org/10.1161/STR.0000000000000116

Tikka, S., Mykkanen, K., Ruchoux, M. M., Bergholm, R., Junna, M., Poyhonen, M., Yki-Jarvinen, H., Joutel, A., Viitanen, M., Baumann, M., & Kalimo, H. (2009). Congruence between NOTCH3 mutations and GOM in 131 CADASIL patients. *Brain*, *132*(Pt 4), 933-939. https://doi.org/10.1093/brain/awn364

Wang, M. (2018). Cadasil. *Handb Clin Neurol*, *148*, 733-743. https://doi.org/10.1016/B978-0-444-64076-5.00047-8

Yin, X., Wu, D., Wan, J., Yan, S., Lou, M., Zhao, G., & Zhang, B. (2015). Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy: Phenotypic and mutational spectrum in patients from mainland China. *Int J Neurosci*, *125*(8), 585-592. https://doi.org/10.3109/00207454.2014.951929

Zhu, S., & Nahas, S. J. (2016). CADASIL: Imaging Characteristics and Clinical Correlation. *Curr Pain Headache Rep*, *20*(10), 57. https://doi.org/10.1007/s11916-016-0584-6

Genomic Testing for Hematopoietic Neoplasms

Policy Number: AHS – M2182 – Genomic Testing for Hematopoietic Neoplasms	Prior Policy Name and Number, as applicable:
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Hematologic cancers impact the normal production and function of blood cells. These cancers often begin in bone marrow, where stem cells develop into white blood cells, red blood cells, or platelets. Hematologic cancers such as myeloid and lymphoid neoplasms occur when there is an uncontrolled growth of abnormal cells which overtake the development of normal blood cells. An overabundance of abnormal cells interferes with the regular functions of normal cells (ACCC, 2022). Myeloid neoplasms occur when there is a proliferation of myeloid cells that have originated from a primitive stem cell (NCBI, 2023b). In contrast, lymphoid neoplasms are composed of a lymphocytic cell population which is usually malignant (clonal) by molecular genetic and/or immunophenotypic analysis (NCBI, 2023a).

For guidance on flow cytometry in hematopoietic neoplasms, please refer to AHS-F2019-Flow Cytometry. For guidance on minimal/measurable residual disease (MRD) in hematopoietic neoplasms, please refer to AHS-M2175-Minimal Residual Disease.

II. Related Policies

Policy Number	Policy Title
AHS-F2019	Flow Cytometry
AHS-M2101	Mutation Analysis in Myeloproliferative Neoplasms
AHS-M2175	Minimal Residual Disease

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For the initial diagnosis and profiling of hematopoietic neoplasms, the following testing **MEETS COVERAGE CRITERIA**:
   a) Bone marrow cytogenetics **and/or** fluorescent in situ hybridization (FISH) of bone marrow samples.
   b) Multigene panel testing.

2) For the differential diagnosis of chronic myeloid leukemia (CML) or acute lymphoblastic leukemia (ALL) or for optimal risk stratification and treatment planning for individuals diagnosed with B-cell ALL (B-ALL), one time qualitative or quantitative RT-PCR testing on blood or bone marrow for identification of the *BCR-ABL1* fusion gene transcript type, including determination of transcript size (e.g., p190 vs. p210), **MEETS COVERAGE CRITERIA.**

3) For individuals diagnosed with acute lymphoblastic leukemia (ALL) or pediatric ALL (PEDALL) who are under surveillance, the following tests **MEET COVERAGE CRITERIA** once every 3 to 6 months for at least 5 years:
   a) Bone marrow cytogenetics.
   b) Bone marrow FISH.
   c) Multigene panel testing.

4) For all individuals with Philadelphia chromosome positive ALL (Ph+ ALL), *BCR-ABL1* transcript-specific quantification **MEETS COVERAGE CRITERIA** at the following frequency:
   a) Once per month for individuals who have detectable levels of *BCR-ABL1* transcript.
   b) Once every three months for individuals who have undetectable levels of *BCR-ABL1* transcript.

5) For individuals diagnosed with acute myeloid leukemia (AML) who had cytogenetic abnormalities at diagnosis, cytogenetic or FISH testing **MEETS COVERAGE CRITERIA** at the following time points:
   a) Fourteen to twenty-one days after the start of therapy to document hypoplasia.

i) When hypoplasia is documented, repeat analysis seven to fourteen days later to clarify persistence of leukemia.

ii) When hypoplasia is not documented, repeat analysis at the time of hematologic recovery to document remission.

b) By 42 days post-treatment with either standard-dose or high-dose cytarabine induction for poor-risk AML, regardless of the degree of hematologic recovery.

6) For individuals with AML who have experienced relapsed/refractory disease after the completion of post-remission therapy, multigene panel testing **MEETS COVERAGE CRITERIA**.

7) For optimal risk stratification and treatment planning for individuals diagnosed with chronic lymphocytic leukemia (CLL) or small lymphocytic leukemia (SLL), one time testing with any of the following tests (when not already performed during initial diagnosis) **MEETS COVERAGE CRITERIA:**

a) FISH to detect +12; del(11q); del(13q); del(17p).

b) *TP53* mutation testing.

c) CpG-stimulated metaphase karyotype for complex karyotype.

d) Immunoglobulin heavy chain variable region gene (IGHV) mutation testing.

8) For individuals diagnosed with chronic myeloid leukemia (CML), quantitative reverse transcription polymerase chain reaction (qPCR) testing for the *BCR-ABL1* fusion gene transcript **MEETS COVERAGE CRITERIA** at the following frequency:

a) To establish baseline levels at diagnosis.

b) For individuals undergoing TKI therapy:

i) Every 3 months after initiation of therapy until major molecular response (MMR) (*BCR-ABL1* (IS) < 1% (>0.1%-1%)) has been achieved.

ii) Every 3 months for 2 years and every 3-6 months thereafter.

iii) If there is a 1-log increase in *BCR-ABL1* transcript levels with MMR, repeat in 1-3 months.

9) For individuals diagnosed with CML who are pursuing or who are actively undergoing TKI therapy, bone marrow cytogenetics **MEETS COVERAGE CRITERIA** at the following time points:

a) To establish cytogenetic changes.

b) When there is a failure to reach response milestones.

c) When there is any sign of loss of hematologic response.

d) When there is any sign of loss of complete cytogenetic response (CCyR) or its molecular response correlate, defined as an increase in *BCR-ABL1* transcript to >1%.

10) Evaluation of BCR-ABL kinase domain point mutations in patients with CML **MEETS COVERAGE CRITERIA** at the following time points:

a) When the individual has chronic phase CML.

b) When there is failure to reach response milestones.

c) When there is any sign of loss of hematologic response.

d) When there is any sign of loss of CCyR or its molecular response correlate, defined as an increase in *BCR-ABL1* transcript to 1%.

e) When there is a 1-log increase in *BCR-ABL1* transcript levels and a loss of MMR.

f) When the disease progresses to accelerated or blast phase.

11) For individuals with CML who are undergoing treatment discontinuation with TKI therapy and who remain in MMR after discontinuation of therapy, qPCR on blood or bone marrow for the *BCR-ABL1* fusion gene transcription **MEETS COVERAGE CRITERA** at the following frequency:

a) Every month for the first 6 months following discontinuation.

b) Once every two months in months 7-12 following discontinuation.

c) Once every three months beginning 12 months following discontinuation of therapy, as long as the individual remains in MMR.

12) For individuals with CML, the use of FISH to monitor response to TKI therapy **DOES NOT MEET COVERAGE CRITERIA**.

13) Simultaneous testing of both bone marrow and blood for monitoring purposes **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

14) For the diagnosis or prognosis of individuals with confirmed acute leukemia, global/gene specific methylation, microRNA (miRNA) expression, **or** gene expression analysis **DOES NOT MEET COVERAGE CRITERIA**.

15) For diagnosis or prognosis of myeloid or lymphoid neoplasms, all other testing not addressed above **DOES NOT MEET COVERAGE CRITERIA.**

NOTES:

Note: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
ABL1	*Abelson murine leukemia viral oncogene homolog 1*
AML	Acute myeloid leukemia
ANKRD26	*Ankyrin repeat domain containing 26*
APL	Acute promyelocytic leukemia
ASCO	American Society of Clinical Oncology
ASH	American Society of Hematology
ASXL1	*ASXL transcriptional regulator 1*
ASXL2	*ASXL transcriptional regulator 2*
BAALC	Brain and acute leukemia cytoplasmic
BCR-ABL1	*BCR activator of RhoGEF and GTPase- ABL proto-oncogene 1, non-receptor tyrosine kinase*
BCSH	British Committee for Standards in Haematology
c-KIT	Commonly used alias for *KIT proto-oncogene, receptor tyrosine kinase*
CAP	College of American Pathologists
CBF	Core-binding factor
CALR	*Calreticulin*
CBFA2	*CBFA2/RUNX1 partner transcriptional co-repressor 2*
CEBPA	*CCAAT/enhancer binding protein alpha*
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CLL	Chronic lymphocytic leukemia
CMS	Centers for Medicare and Medicaid
CML	Chronic myeloid leukemia
CN	Normal karyotypes
CT	Computed tomography
CR	Complete hematologic remission
DDX41	*DEAD-box helicase 41*
DNA	Deoxyribonucleic acid
DNMT3A	*Deoxyribonucleic acid methyltransferase 3A*

ELN	European LeukemiaNet
EMSO	European Society For Medical Oncology
ERG	ETS transcription factor ERG
EVI1	Ecotropic virus integration site 1 protein homolog
EZH2	*Enhancer of zeste 2 polycomb repressive complex 2 subunit*
FDA	Food and Drug Administration
FDG-PET	Fluorodeoxyglucose positron emission tomography
FISH	Fluorescence in situ hybridization
FLT3	*FMS-like tyrosine kinase 3*
GATA2	GATA binding protein 2
IDH1	*Isocitrate dehydrogenase 1*
IDH2	*Isocitrate dehydrogenase 2*
IGHV	Immunoglobulin heavy chain variable region gene
ITD	Internal tandem duplications
iwCLL	International Workshop on Chronic Lymphocytic Leukemia
JAK2	*Janus kinase 2*
KIT	*KIT proto-oncogene, receptor tyrosine kinase*
KRAS	*KRAS proto-oncogene, GTPase*
LTD	Laboratory-developed test
MBNL1	*Muscleblind like splicing regulator 1*
MECOM	MDS1 and EVI1 complex locus
MEIS1	Muscleblind like splicing regulator 1
miRNA	Micro ribonucleic acid
Mkneg	Non-chromosomal karyotype
MLL-PTD	*Mll -Partial Tandem Duplication*
MPL	*MPL proto-oncogene, thrombopoietin receptor*
MRD	Minimal residual disease
MYH11	*Myosin heavy chain 11*
NCCN	National Comprehensive Cancer Network
NGS	Next generation sequencing
NPM1	*Nucleophosmin 1*
*NPM1*mut	*NPM1* mutations
NRAS	*NRAS proto-oncogene, GTPase*
PCR	Polymerase chain reaction
PMF	Polyamine modulated factor 1
PML-RAR	*Promyelocytic leukemia-retinoic acid receptor*
RNA	Ribonucleic acid
RT-PCR	Reverse transcription- polymerase chain reaction
RUNX1	*RUNX family transcription factor 1*

RUNX1T1	*RUNX1 partner transcriptional co-repressor 1*
SPI1	Spi-1 proto-oncogene
SF3B1	*Splicing factor 3b subunit 1*
SRSF2	*Serine and arginine rich splicing factor 2*
SLL	Small lymphocytic leukemia
TET2	*Tet methylcytosine dioxygenase 2*
TKD	*Tyrosine kinase domain*
TP53	*Tumor protein 53*
WHO	World Health Organization
WT1	*Wilms' tumor 1*

V. Scientific Background

Acute Lymphoblastic Leukemia (ALL)

Acute lymphocytic leukemia occurs when a bone marrow cell develops a mutation in its genetic material. ALL can be of B-cell precursor (BCP) lineage (BCP-ALL) or, less commonly, T-cell precursor lineage (T-ALL). However, "Both comprise multiple subtypes commonly defined by structural chromosomal alterations that are initiating lesions, with secondary somatic (tumor-acquired) DNA copy number alterations and sequence mutations that contribute to leukemogenesis. Chromosomal alterations include aneuploidy and chromosomal rearrangements that result in oncogene deregulation or expression of chimeric fusion genes. The prevalence of these alterations varies according to age and identification is important for diagnosis, risk classification, and, for some lesions, targeted therapy. Common genomic features of *BCR-ABL1*–like ALL are alterations of B-lymphoid transcription factor genes (particularly *IKZF1* deletions) and genetic alterations deregulating cytokine receptor and tyrosine kinase signaling. These include rearrangements and mutation of *CRLF2* (approximately 50%), rearrangements of *ABL*-class tyrosine kinase genes (12%), rearrangements of *JAK2* (7%) and the erythropoietin receptor gene (*EPOR*; three to ten percent), mutations activating JAK-STAT signaling (11%) and Ras signaling (*NRAS*, *KRAS*, *PTPN11*, and *NF1*; six percent), and less common kinase alterations (*FLT3*, *NTRK3*, *BLNK, TYK2*, and *PTK2B*)" (Iacobucci & Mullighan, 2017).

Acute myeloid leukemia (AML)

Acute myeloid leukemia is the most common acute leukemia in adults (80%), with a median age at diagnosis of 65 years. An AML diagnosis in adults is generally associated with a poor prognosis. This disease is much less common in children younger than ten years of age, as less than ten percent of acute leukemias are

diagnosed as AML in this age group (Schiffer & Gurbuxani, 2024; Siegel et al., 2017; Yamamoto & Goodman, 2008).

A clinical presentation of AML includes symptoms related to complications of pancytopenia, including weakness and fatigability, infections of variable severity, and hemorrhagic findings (Schiffer & Gurbuxani, 2024). Analysis of gene sequencing of AML cases generally reveal more than ten significant gene mutations; many of which are thought to participate in leukemogenesis (CGARN, 2013). The most common gene mutations are as follows: *FLT3* (28%), *NPM1* (27%), *DNMT3A* (26%), *IDH1* or *IDH2* (20%), *NRAS* or *KRAS* (12%), *RUNX1* (10%), *TET2* (8%), *TP53* (8%), *CEBPA* (6%), and *WT1* (6%). Mutations impacting signal activation are frequent (60% of cases); the most common of which are mutations in *FLT3* (Stock & Thirman, 2022).

The second most common mutation in AML (27%) is of nucleophosmin (NPM1), a ubiquitously expressed phosphoprotein that normally shuttles between the nucleus and cytoplasm (Falini et al., 2005; Stock & Thirman, 2022). *NPM1* mutations are associated with improved outcomes although the mechanism is not known. Concurrent mutations (such as an *FTL3* mutation) may influence prognosis, but generally *NPM1* patients without concurrent mutations have better prognoses (Schiffer & Uy, 2024).

The *CCAAT/enhancer binding protein alpha* (*CEBPA*) gene mutation is also common in AML. *CEBPA* encodes a transcription factor necessary for myeloid differentiation. This mutation is one of the two mutations associated with familial leukemia and consists of about ten percent of AML cases. Familial AML with a *CEBPA* mutation has a phenotype similar to those of "sporadic AML with biallelic *CEBPA* mutations," but most of the current data revolves around the assessment of *CEBPA* double mutations. *CEBPA* single mutations and hypermethylated *CEBPA* requires further study (Schiffer & Uy, 2024).

Isocitrate dehydrogenase (IDH) 1 and 2 mutations comprise approximately 15% of AML cases. These mutations are mutually exclusive with *Tet Methylcytosine Dioxygenase 2* (*TET2*) and *Wilms' tumor 1* (*WT1*) mutations but are commonly seen with *NPM1* and *DNA methyltransferase 3A* (*DNMT3A*) mutations. Data on the prognoses of these mutations is varied (Schiffer & Uy, 2024).

The *KIT* mutations also comprise about six percent of AML cases. *KIT* encodes the receptor for a stem cell factor, and prognoses are varied (Castells & Akin, 2021; Schiffer & Uy, 2024). Some researchers suggest that of all *KIT* mutations, the D816 mutation is the most unfavorable prognostic factor in AML patients (Yui et al., 2017).

Approximately eight percent of AML cases consist of *WT1* gene mutations. *WT1* encodes a transcriptional regulator for genes involved in maturation and growth.

Again, the prognosis of this mutation is mixed (Schiffer & Uy, 2024), although some researchers strongly support the theory that *WT1* mutations are associated with poor AML prognoses (Hou et al., 2010). The *WT1* mutation status of AML patients may also change during disease progression. New research has suggested that after allogenic stem cell transplantation, AML relapse could be due to a gain in *WT1* gene alterations and a "high mutation load" (Vosberg et al., 2018).

The *ASXL Transcriptional Regulator 1 (ASXL1)* and *ASXL Transcriptional Regulator 2* (*ASXL2)* may also be mutated in AML cases. *ASXL1* has an unclear function, but it is speculated to be related to histone post-translational modifications. The frequency of *ASXL1* is varied, as estimates range from six percent to 30%. Furthermore, *ASXL1* mutations are mutually exclusive with *NPM1* mutations, and *ASXL2* mutations are associated with *RUNX1* mutations (also known as *AML1* or *CBFA2*) (Schiffer & Uy, 2024).

The *DNMT3A* gene amounts to 20%-22% of AML cases. This gene plays a role in epigenetic modifications for development and differentiation. Mutations in this gene affect hematopoietic stem cell differentiation. Prognoses of this gene mutation have been mixed (Schiffer & Uy, 2024).

Tumor protein 53 (*TP53*) and RAS and may also be present in AML cases and may be accompanied by other genetic abnormalities. RAS regulates cell signal transduction, and its mutation leads to a constitutionally active growth stimulus whereas *TP53* encodes a transcriptional activator of growth inhibitory genes (Frucht & Lucas, 2023; Rai & Stilgenbauer, 2023).

Gene expression profiling and microRNA expression profiling may also contribute to assessment and management of AML. Gene expression profiling has been used to differentiate between risk groups based on cytogenetic evaluation whereas microRNA profiling evaluates the regulation of gene expression. However, neither technique is used regularly in clinical practice as these techniques have yet to be widely validated (Schiffer & Uy, 2024).

Chronic Lymphoblastic Leukemia (CLL)

In Western countries, CLL is the most common form of leukemia; in the United States, it accounts for 25 to 30 percent of all leukemias (Rai et al., 2024). CLL and SLL (small lymphocytic leukemia) are mature B cell neoplasms characterized by a progressive accumulation of monoclonal B lymphocytes. It should be noted that "CLL is considered to be identical (ie, one disease with different manifestations) to the non-Hodgkin

lymphoma SLL. The malignant cells seen in CLL and SLL have identical pathologic and immunophenotypic features. The term CLL is used when the disease manifests primarily in the blood, whereas the term SLL is used when involvement is primarily nodal" (Rai et al., 2024). It is also worth mentioning that "In less than 10 percent of patients, CLL/SLL presents primarily in the lymph nodes as a non-Hodgkin lymphoma (ie, SLL)" and that "this presentation accounts for less than 5 percent of all non-Hodgkin lymphomas" (Rai et al., 2024).

The CLL exomes carry approximately "5–20 somatically acquired mutated genes per individual case, much fewer than many other solid cancers. Importantly, the somatically acquired mutation status of sequence variants in genes, like other studies of genomic changes in cancer, necessitates confirmation of the absence of these candidate mutations in paired non-tumorous DNA (T-cell–, buccal cell– or skin-derived DNA). Mutations in *TP53* are of major clinical relevance, are often associated with del17p and gain in frequency over time. *TP53* mutated and associated del17p states substantially lower response rates, remission duration, and survival in CLL. Mutations in *NOTCH1* and *SF3B1* are recurrent, often associated with progressive CLL" (Amin & Malek, 2016).

Chronic myeloid leukemia (CML)

The first human malignancy in which a specific chromosomal defect, known as the minute or Philadelphia (Ph) chromosome, could be linked to pathogenetic events of leukemogenesis (Nowell & Hungerford, 1960). The Ph chromosome translocation (t(9;22)(q34;q11.2)) fuses the breakpoint cluster region protein (*BCR*) gene from chromosome 22 with the Abelson murine leukemia viral oncogene homolog 1 (*ABL1*) proto-oncogene from chromosome nine in a head-to-tail manner to form the transcriptionally active *BCR-ABL* fusion gene (Schrijver & Zehnder, 2022). The fusion of *BCR* at the 5' side in *ABL* alters the tightly regulated function of the Src homology 3 (SH3) domain, disabling control over the tyrosine kinase enzyme. The resulting chimeric BCR-ABL protein has constitutively elevated tyrosine phosphokinase activity (Kurzrock et al., 2003) that activates a number of downstream signaling molecules, including PI3K, AKT, JNK, RAS and STAT5 (Ren, 2005). This then disrupts cellular signal transduction pathways, leading to issues in the regulation of both apoptosis and cell proliferation (Warmuth et al., 1999), ultimately leading to factor-independent and leukemogenic cell growth (Van Etten, 2023).

Detection of the Ph chromosome is the hallmark of CML and is found in up to 95 percent of patients (Leoni & Biondi, 2015). In approximately five percent of CML cases, the Ph chromosome cannot be detected, and *BCR-ABL1* formation is attributed to microscopically undetectable translocations or variant complex translocations involving

a third chromosome (Schrijver & Zehnder, 2022). Independent of which other chromosomes are involved in variant translocations, the generation of the *BCR-ABL* fusion gene is the "fundamental cause of Ph-positive leukemias" (Van Etten, 2023), as the 210-KDa fusion protein BCR-ABL is essential for initiation, maintenance, and progression of CML (Ren, 2005). Testing for BCR-ABL1 detects both the Ph chromosome and fusion gene or its transcripts. The BCR-ABL1 transcript is the RNA copy made by the cell.

The FMS-like tyrosine kinase 3 (FLT3) is a transmembrane tyrosine kinase receptor that stimulates cell proliferation upon activation. Both internal tandem duplications (ITDs) of different lengths and point mutations in the activating loop of the kinase domain result in ligand-independent activation of the FLT3 receptor and a proliferative signal. A *FLT3-ITD* mutation has been shown to have a poor prognosis in contrast to *FTL3* point mutations in the activation loop of the kinase domain. Higher ratios of mutated alleles compared to wild-type alleles confer worse prognoses (Schiffer & Uy, 2024).

The *BCR-ABL1*, refers to the fusion gene resulting from a reciprocal translocation that joins the *ABL1* gene from chromosome 9 to the *BCR* gene on chromosome 22 and is necessary for the development of chronic myeloid leukemia (Tachibana et al., 2022) (Van Etten, 2023). This reciprocal translocation also generates a shortened derivative chromosome 22, known as the Philadelphia (Ph) chromosome (Schrijver & Zehnder, 2022). The Ph chromosome is a diagnostic hallmark, present in 95% of people with CML and approximately three to five percent children and 25%–40% adults with acute lymphoblastic leukemia (ALL) (Leoni & Biondi, 2015). An aggressive form of cancer resulting from the neoplastic transformation of lymphoid precursors characterized by the presence of too many lymphoblasts or lymphocytes in the bone marrow and peripheral blood (PDQ, 2023). Predominately a childhood disease, approximately 60% of cases were diagnosed in patients younger than 20 years of age (Pui, 2011).

Analytic Validity

There is very limited published literature on the analytic validity and clinical validity of genetic testing for *FLT3* and *NPM1* mutations in AML. However, the analytic validity of PCR in general is extremely high (Leonard, 2016). Other tools, such as flow cytometry and next generation sequencing (NGS) have also been used for AML prognostic and diagnostic purposes.

Ampasavate et al. (2019) have developed a quantitative protocol and flow cytometry-based method for monitoring an anti-FLT3 interaction. The FLT3 biomarker has been previously identified as a poor prognostic marker for AML patients. This method can rapidly identify intact FLT3 on the leukemic cell surface. "The results demonstrated good linearity ($r2 > 0.99$)"; further, "when compared with Western blotting results, FLT3

protein expression levels in leukemia patient's bone marrow samples were demonstrated in the same trend" (Ampasavate et al., 2019). The researchers state that this technique is reliable, rapid, effective and "provided a practical analysis of FLT-3 biomarker levels which is valuable for physician decision in acute leukemia treatment" (Ampasavate et al., 2019).

Alonso et al. (2019) have researched the utility of a 19-gene NGS panel for AML diagnostic purposes. This targeted NGS panel was studied in a cohort of 162 patients with AML. The authors note that "The assay yielded a median read depth >2000×, with 88% of on-target reads and a mean uniformity >93% without significant global strand bias. The method was sensitive and specific, with a valid performance at the clinical variant allele frequency cutoff of 3% for point mutations and five percent for insertions or deletions." The researchers conclude that this is a "reliable and reproducible method" for AML diagnoses (Alonso et al., 2019).

Molecular testing for the diagnosis of CML confirms typical findings in the blood and bone marrow by the demonstration of the Ph chromosome, the *BCR-ABL1* fusion gene or the BCR-ABL1 fusion mRNA. Molecular testing techniques include conventional cytogenetics, fluorescence in situ hybridization (FISH) analysis and reverse transcription polymerase chain reaction (RT-PCR) (Van Etten, 2022). Conventional cytogenetic karyotyping is no longer the diagnostic modality of choice due to its requirements for a highly skilled staff, culturing of cells, long turnaround time, and lower sensitivity (5-10%). Despite this, conventional cytogenetics are still the gold standard, and "should be performed" especially at diagnosis to detect additional clonal abnormalities (Yeung et al., 2016). FISH is more sensitive (0.1-5%) than karyotyping and can be performed on peripheral blood in addition to bone marrow and tissue. FISH can detect certain very rare translocations not usually detectable by the vast majority of commercial and laboratory-developed RT-PCR assays, but FISH is highly specific to the targeted region and may miss other chromosomal changes. Quantitative RT-PCR is the most sensitive technique currently available (0.001-0.01% sensitivity).

On July 22, 2016 the FDA approved the QuantideX qPCR BCR-ABL IS Kit as an *in vitro* nucleic acid amplification test for the quantitation of BCR-ABL1 and ABL1 transcripts in total RNA from whole blood of diagnosed t(9;22) positive Chronic Myeloid Leukemia (Tachibana et al.) patients expressing BCR-ABL1 fusion transcripts type e13a2 and/or e14a2 as a class II device with special controls (FDA, 2016). Brown et al. (2019) performed a study to describe the analytical validation of this kit. They were able to find that "the test has a limit of detection of MR molecular response] 4.7 (0.002% IS) and a linear range from MR0.3 (50%IS) to MR4.7 (0.002%IS) for both Major transcripts. Single-site and multisite precision studies demonstrated a maximum SD of 0.13 MR (30% CV within the assay range between MR0.7 and MR3.7)."

Clinical Utility and Validity

The clinical utility of testing fallows for further risk stratification, prognostication, and guide management decisions in patients with AML. Several studies have concluded that *FLT3* and *NPM1* mutation testing in cytogenetically normal AML is useful for prognosis and treatment decision making (DeZern et al., 2011; Pastore et al., 2014; Willemze et al., 2014).

Devillier et al. (2015) sought to identify biological and prognostic subgroups based on genetic mutations in AML patients. A total of 125 AML patients with myelodysplasia-related changes ("MRC") were evaluated. The authors focused on the 26 patients with *ASXL1* mutations and 28 with *TP53* mutations. The *ASXL1* mutation cohort was found to have a higher proportion of marrow dysgranulopoiesis and an overall survival (OS) rate that was below average for wild-types (14% for *ASXL1* mutants, 37% for wild-types). The *TP53* cohort was found to have a "complex karyotype" and predicted a poor outcome with unfavorable cytogenetic risk AML. Both mutations were found to be an independent factor associated with shorter OS (Devillier et al., 2015).

Bolouri et al. (2018) examined 993 children's genetic data from the Children's Oncology Group (Khoury et al.) AML trials to characterize the molecular landscape of AML. The authors found that certain somatic variants, such as *MBNL1,* were "disproportionately prevalent" in children compared to adults. However, certain variants common in adults such as *TP53*, were not found in children. Other mutations such as *NRAS* and *KRAS* were "frequent" in pediatric AML. The authors concluded that their results "highlight the need for and facilitate the development of age-tailored targeted therapies for the treatment of pediatric AML" (Bolouri et al., 2018).

Jongen-Lavrencic et al. (2018) conducted a study of 482 patients 18 to 65 years with newly diagnosed AML. Targeted next-generation sequencing (NGS) was carried out at diagnosis and after induction therapy (during complete remission). At least one mutation was detected in 430 (89.2%) patients, and mutations persisted in 51.4% of those patients during complete remission. The detection of minimal residual disease was associated with a significantly increased relapse rate than no detection. Persistent DTA mutations (mutations in *DNMT3A*, *TET2*, and *ASXL1*) were not correlated with an increased relapse rate. Overall, the authors concluded, "A comparison of sequencing with flow cytometry for the detection of residual disease showed that sequencing had significant additive prognostic value. Among patients with AML, the detection of molecular minimal residual disease during complete remission had significant independent prognostic value with respect to relapse and survival rates, but the detection of persistent mutations that are associated with clonal hematopoiesis did not have such prognostic value within a 4-year time frame" (Jongen-Lavrencic et al., 2018).

Kuwatsuka et al. (2018) evaluated the genetic background of 103 young adults and their subsequent clinical outcomes. The 103 cases included mutations in *FLT3-ITD, KIT, CEBPA, NRAS, KRAS, WT1, MLL-PTD*, and *NPM1*. Overall, *FLT3-ITD* and *NPM1* mutations were associated with a greater mortality risk. *NPM1* mutations conferred a 100% survival rate in the absence of *FLT3-ITD* mutations, but *FLT3-ITD* conferred only a 35% survival without *NPM1* mutations (Kuwatsuka et al., 2018).

Zhu et al. (2017) assessed the effect of gene mutations on the subsequent cytogenetic aberrations. A total of 560 patients were enrolled, and the authors examined the following alterations: "*CEBPA*, *NPM1, FLT3, C-KIT, NRAS, WT1, DNMT3A, MLL-PTD* and *IDH1/2*, as well as expression levels of MECOM, ERG, GATA2, WT1, BAALC, MEIS1 and SPI1." The investigators found that the expression levels of MECOM, MEIS1, and BAALC influenced cytogenetic aberration. Further, *FLT3*, *C-KIT*, and *NRAS* mutations all contained a "conversed" expression profile of MEIS1, WT1, GATA2, and BAALC expression. The investigators also noted "*FLT3, DNMT3A, NPM1* and biallelic *CEBPA* represented the mutations associated with the prognosis of AML in our group" (Zhu et al., 2017).

Papaemmanuil et al. (2016) examined the relationship between genotype and pathophysiology in AML. A total of 1540 patients with 5234 driver mutations across 76 genes were studied. The authors found three genomic subcategories in addition to the currently defined AML subgroups: mutations in genes encoding chromatin, RNA splicing regulators or both (such as *ASXL1* or *RUNX1*), *TP53* mutations, chromosomal aneuploidies or both (unusual karyotypes and *TP53*), and *IDH2* mutations. The authors noted that "patients with chromatin–spliceosome and *TP53*–aneuploidy AML had poor outcomes." The *NPM1* cohort was the largest of the sample (27%, 436 patients) and 319 of those patients also carried a DNA methylation or hydroxylmethylation gene, such as *IDH1/2* or *TET2*. The authors also noted that *NPM1-DNMT3A–NRASG12/13* had an "unexpectedly benign" prognosis, and the *NPM1* subgroup's prognoses were largely determined by the context in which the NPM1 mutation occurred (such as in *NRAS, IDH*, and so on) (Papaemmanuil et al., 2016).

Sperr et al. (2016) evaluated the effect of a genetic mutation and karyotype on the efficacy of treatment for elderly patients. A total of 192 patients over 60 years old were enrolled, and 115 of these patients achieved "complete hematologic remission (CR)." The authors stated that *NPM1* mutations (*NPM1*mut) and karyotype were the only independent predictors of survival, also noting that *NPM1*mut showed a prognostic impact on both normal (CN) and non-chromosomal (Mkneg) karyotypes. The authors concluded that "elderly patients with CN/Mkneg-*NPM1*mut or core binding factor AML can achieve long term median continuous CR when treated with intensive induction and consolidation therapy whereas most elderly patients with CN/Mkneg-NPM1wild-

type or CN/Mkpos AML may not benefit from intensive chemotherapy" (Sperr et al., 2016).

Heiblig et al. (2019) assessed the impact of *NPM1* subtypes on treatment outcomes. One hundred seventy-five patients were examined. The authors found that out of the *NPM1* AML cases, 73% (128) were "Type A" mutations (TCTG at exon 12) and 27% (47) were "Non Type-A mutations" (Type B: CATG and Type D: CGTG). The Type-A mutations were found to achieve minimal residual disease (MRD) earlier than non Type-A mutations. However, non-type A mutations achieved better rates of medial survival (Heiblig et al., 2019).

D'Adda et al. (2019) evaluated the effect of the BCR-ABL transcript on efficacy of TKIs. The *BCR*-ABL1 fusion gene may cause CML pathogenesis due to several breakpoints; the most common occur around exon 13 and 14 of the *BCR* gene and cause the formation of e13a2 and e14a2 transcripts (Greenfield et al., 2019). Out of 173 sampled patients, 67 had the e13a2 transcript, and 106 had the e14a2 version. The patients with the e14a2 version were more likely to achieve a deep molecular response to TKIs (sustained or otherwise). After 68 months, the sustained deep molecular response (sDMR) rate was 39.6% for e14a2 patients compared to 19.6% for e13a2 patients. Overall, the maximum rate of sDMR for e13a2 patients was 37%, after 60 months. Furthermore, only two patients (three percent) with the e13a2 transcript achieved treatment-free remission (TFR) whereas 25 of e14a2 patients achieved TFR (23%) (D'Adda et al., 2019).

Xu et al. (2020) have analyzed data from 220 normal karyotype AML pediatric patients. Participants were selected from the Cancer Genome Atlas database. It was found that 12.7% of these patients had *WT1* mutations, and that "the *WT1*-mutated group suffered lower rates of complete remission (CR) ($P < 0.001$ and $P < 0.001$, respectively) but higher rates of minimal residual disease (MRD) ($P = 0.003$ and $P = 0.021$, respectively) after both one and two courses of induction chemotherapy" (Xu et al., 2020). Patients with *WT1* mutations also had significantly worse event-free and overall survival rates ($P=0.007$ and $P<0.001$, respectively) (Xu et al., 2020).

Sasaki et al. (2020) studied the impact of *ASXL1, DNMT3A, JAK2, TET2*, and *TP53* mutations on survival in newly diagnosed acute myeloid leukemia (AML) patients. The 421 bone marrow aspirates were studied using NGS for these mutations with a minimum variant allele frequency (VAF) of 5%. "A total of 71 patients (17%) had *ASXL1* mutations, 104 patients (25%) had *DNMT3A* mutations, 16 patients (4%) had *JAK2* mutations, 82 patients (20%) had *TET2* mutations, and 86 patients (20%) had *TP53* mutations." The median VAF of *ASXL1* was 34.31% (range, 1.17%-58.62%), *DNMT3A* was 41.76% (range, 1.02%-91.66%), *JAK2* was 46.70% (range, 10.4%-71.7%), *TET2* was

42.78% (range, 2.26%-95.32%), and *TP53* was 45.47% (range, 1.15%-93.74%). In patients with these mutations, the median overall survival was 11 months. In patients without these mutations, the overall survival was 27 months. The authors conclude that "The VAF of *ASXL1, DNMT3A, JAK2, TET2, TP53*, and *NPM1* mutations is associated with worse prognosis in patients with newly diagnosed AML" (Sasaki et al., 2020).

Duncavage et al. (2021) investigated the clinical utility and accuracy of whole-genome sequencing (WGS) for the purpose of risk stratification in patients with acute myeloid leukemia (AML) or myelodysplastic syndromes (MDS). The results from 263 patients were compared with findings from cytogenetic analysis and targeted sequencing. When conducting the WGS, they found that all 40 recurrent translocations and 91 copy-number alterations found on cytogenetic analysis were identified on WGS. There were also new clinically reportable genomic events among 17.0% of the patient sample. The standard AML risk groups, "as defined by sequencing results instead of cytogenetic analysis, correlated with clinical outcomes." WGS was also able to classify patients with inconclusive cytogenetic analysis results into risk groups. This demonstrates that WGS could increase the diagnostic yield of AML and MDS and supplement the accuracy of cytogenetic analysis (Duncavage et al., 2021).

In a retrospective study, Yang reported the clinical significance of *BCR-ABL1* mutations in patients with Philadelphia chromosome-positive CML who underwent allogeneic hematopoietic cell transplantation. Of 315 patients in this study, point mutations were detected in 152 patients. One hundred and one of these 152 patients (66%) had T315I mutations and 51 had mutations other than T315I. The patients were followed 38 months later and overall survival at three years was worse in patients with the mutations than the non-mutation group, especially in the chronic phase of CML. Overall survival in the non-T315I group was significantly worse than that in the no-mutation group. The authors conclude that "mortality risk was significantly higher in patients with the BCR-ABL1 mutation than in patients without the mutation" (Tachibana et al., 2022).

VI. Guidelines and Recommendations

World Health Organization (WHO)

In a revision of the fourth edition on Classification of Tumors of Hematopoietic and Lymphoid Tissues, World Health Organization (WHO) incorporated new molecular genetic findings and clinical data into its classification of acute leukemias. WHO expanded on the prognostic significance of various gene mutations for each AML subtype. For example, for the AML with recurrent genetic abnormalities, inv(3) (q21.3; q26.2) does not represent a fusion gene, but rather a repositioning of a distal GATA2 enhancer leading to activation of MECOM (EVI1) expression and GATA2

haploinsufficiency. The AML with *CEBPA* mutation is defined based on biallelic mutation instead of single mutations because of prognostic significance. The provisional two categories are also added such as AML with *RUNX1* for de novo AML without preexisting cytogenetic abnormalities associated with MDS and AML with *BCR-ABL1* fusion gene. AML with *NPM1* or biallelic *CEBPA* mutations and multilineage dysplasia are now considered separately instead of being a part of AML with myelodysplasia-related changes because of a lack of prognostic significance. The complete list for acute myeloid neoplasms 2016 WHO classification is shown in Figure 1 (Arber et al., 2016).

In the absence of *JAK2, CALR,* and *MPL* mutations, the presence of another clonal marker is included as one of the major diagnostic criteria for PMF. Additional mutation in *ASXL1, EZH2, TET2, IDH1, IDH2, SRSF2* and *SF3B1* genes are noted to be of use in determining the clonal nature of the disease.

In the fifth edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms, WHO places greater emphasis in "the separation of AML with defining genetic abnormalities from AML defined by differentiation". The latter eschews the previously ambiguous categorical term AML NOS, and other notable changes include the "elimination of the 20% blast requirement for AML types with defining genetic abnormalities (with the exception of AML with BCR::ABL1 fusion and AML with CEBPA mutation)" and "the introduction of a section on AML with other defined genetic alterations, a landing spot for new and/or uncommon AML subtypes that may (or may not) become defined types in future editions of the classification" (Khoury et al., 2022). Their list of acute myeloid leukemias is presented below.

Acute myeloid leukaemia with defining genetic abnormalities
Acute promyelocytic leukaemia with *PML::RARA* fusion
Acute myeloid leukaemia with *RUNX1::RUNX1T1* fusion
Acute myeloid leukaemia with *CBFB::MYH11* fusion
Acute myeloid leukaemia with *DEK::NUP214* fusion
Acute myeloid leukaemia with *RBM15::MRTFA* fusion
Acute myeloid leukaemia with *BCR::ABL1* fusion
Acute myeloid leukaemia with *KMT2A* rearrangement
Acute myeloid leukaemia with *MECOM* rearrangement
Acute myeloid leukaemia with *NUP98* rearrangement
Acute myeloid leukaemia with *NPM1* mutation
Acute myeloid leukaemia with *CEBPA* mutation
Acute myeloid leukaemia, myelodysplasia-related
Acute myeloid leukaemia with other defined genetic alterations

Acute myeloid leukaemia, defined by differentiation
Acute myeloid leukaemia with minimal differentiation
Acute myeloid leukaemia without maturation
Acute myeloid leukaemia with maturation
Acute basophilic leukaemia
Acute myelomonocytic leukaemia
Acute monocytic leukaemia
Acute erythroid leukaemia
Acute megakaryoblastic leukaemia

(Khoury et al., 2022)

Moreover, in defining acute myeloid leukemia, *myelodysplasia-related* (AML-MR), WHO offers the following abnormalities and mutations of interest:

Cytogenetic and molecular abnormalities defining acute myeloid leukaemia, myelodysplasia-related.

Defining cytogenetic abnormalities	Defining somatic mutations
Complex karyotype (≥3 abnormalities)	*ASXL1*
5q deletion or loss of 5q due to unbalanced translocation	*BCOR*
Monosomy 7, 7q deletion, or loss of 7q due to unbalanced translocation	*EZH2*
11q deletion	*SF3B1*
12p deletion or loss of 12p due to unbalanced translocation	*SRSF2*
Monosomy 13 or 13q deletion	*STAG2*
17p deletion or loss of 17p due to unbalanced translocation	*U2AF1*
Isochromosome 17q	*ZRSR2*
idic(X)(q13)	

(Khoury et al., 2022).

The WHO published guidelines on the classification of myeloid neoplasms and acute leukemia. In the recent revision of the fourth edition on Classification of Tumors of Hematopoietic and Lymphoid issues, World Health Organization (WHO) incorporated new molecular genetic findings and clinical data into its classification of acute leukemias (Arber et al., 2016).

They state that: "With regard to chronic myeloid leukemia , BCR-ABL1^{+}, most cases of CML in chronic phase can be diagnosed from peripheral blood (Papaemmanuil et al.) findings combined with detection of t(9;22)(q34.1;q11.2) or, more specifically, *BCR-ABL1* by molecular genetic techniques. However, a bone marrow (BM) aspirate is essential to ensure sufficient material for a complete karyotype and for morphologic evaluation to confirm the phase of disease. In the era of tyrosine-kinase inhibitor (TKI) therapy, newly diagnosed patients may have a nearly normal lifespan, but regular monitoring for *BCR-ABL1* burden and for evidence of genetic evolution and development of resistance to TKI therapy is essential to detect disease progression" (Arber et al., 2016).

They also introduced a provisional classification of ALL: B-ALL with translocations involving tyrosine kinases or cytokine receptors ("BCR-ABL1–like ALL"). They note that "This newly recognized entity is assuming increasing importance because of its association with an adverse prognosis and responses of some cases to TKI therapies; however, it has been difficult to define in the clinical setting. It was originally described separately by different groups who demonstrated a series of cases of poor-prognosis

childhood ALL with gene expression profiles similar to those seen in cases of ALL with BCR-ABL1, though different algorithms applied to the same sets of cases did not classify all cases the same way.

The cases with translocations involving tyrosine kinase genes involve many different genes including ABL1 (with partners other than BCR), as well as other kinases including ABL2, PDGFRB, NTRK3, TYK2, CSF1R, and JAK2. Over 30 different partner genes have been described. Some patients, especially those with EBF1-PDGFRB translocations, have shown remarkable responses to TKI therapy, even after failing conventional therapy" (Arber et al., 2016).

In the 5th Edition of the World Health Organization Classification of Hematolymphoid Tumors, the WHO updated their cytogenetic and molecular genetic information regarding AML. They highlight the following in their update:

- "Newly discovered recurrent cytogenetic abnormalities listed in *AML with other defined genetic abnormalities* include *RUNX1T3* (*CBFA2T3)::GLIS, KAT6A::CREBBP, FUS::ERG, MNX1::ETV6*, and *NPM1::MLF1*. Those listed in "B-LBL/L with other defined genetic abnormalities"
  include *DUX4* rearrangement, *MEF2D* rearrangement, *ZNF384* rearrangement, *NU TM1* rearrangements, *MYC* rearrangement, *PAX5alt* or *PAX5 p.P80R*. With more data accumulated, neoplasms with these new genetic abnormalities will very likely become separated subtypes in the next edition, and there will be more newly discovered genetic alterations listed here. Biallelic *TP53* alterations have been frequently found in MDNs, acute erythroid leukemia, and myeloid neoplasms post cytotoxic therapy, and they usually predict a worse prognosis"
- "Gene mutations in the MAPK pathway are commonly seen in histiocytic neoplasms. *CXCR4* mutations are detected in a significant proportion of lymphoplasmacytic lymphoma cases, mostly concurrent with *MYD88* mutations, and the presence of them is associated with resistance to ibrutinib therapy (32). The mutational profiles of extranodal MZL (EMZL) and nodal MZL differ, and there are significant genetic differences among EMZLs arising in different anatomic sites"
- "*STAT3* mutation is commonly seen in CD8+ T-LGLL and gamma/delta T-LGLL, and it is associated with neutropenia and unfavorable overall survival;
  while *STAT5b* mutation is associated with a poor prognosis only in CD8+ T-LGLL but has no prognostic impact in CD4+ T-LGLL and gamma/delta T-LGLL" (Li, 2022).

Though the WHO did not take any specific stance with these in their new version, these mutations and pathways may be worth keeping abreast of for the future.

College of American Pathologists (CAP) and American Society of Hematology (ASH)

Following recent progress in molecular genetic findings and 2016 WHO classification of acute leukemias, the College of American Pathologists (CAP) and the American Society of Hematology (ASH) have formed an expert panel to review and establish guidelines for appropriate laboratory testing. The published guideline provides twenty-seven guideline statements ranging from recommendations on what testing is appropriate for the diagnostic and prognostic evaluation of leukemias to where the testing should be performed and how results should be reported.

There, the societies recommend that "The pathologist should review recent or concurrent CBC counts and leukocyte differentials and evaluate a peripheral blood (PB) smear (*Strong recommendation*)." Moreover, they note that "In addition to performing morphologic assessment (blood and BM), the pathologist or treating clinician should obtain sufficient samples and perform conventional cytogenetic analysis (ie, karyotype), appropriate molecular genetic and/or FISH testing, and FCI. The flow cytometry panel should be sufficient to distinguish AML (including APL), including early T-ALL, B-ALL, and AL of ambiguous lineage in all patients diagnosed with AL. Molecular genetic and/or FISH testing does not, however, replace conventional cytogenetic analysis (*Strong recommendation*)" (de Haas et al., 2019).

In those patients with suspected or confirmed AL, "the pathologist may request and evaluate cytochemical studies to assist in the diagnosis and classification of AML *(Expert consensus opinion)*". Furthermore, "For patients with suspected or confirmed AL, the pathologist may use flow cytometry in the evaluation of CSF (*Recommendation)*" and that "the pathologist or treating clinician should ensure that flow cytometry analysis or molecular characterization is comprehensive enough to allow subsequent detection of MRD (*Strong recommendation*)." It is also mentioned that "For patients with AL other than those with ALL who are receiving intrathecal therapy, the treating clinician may, under certain circumstances, obtain a CSF sample when there is no clinical contraindication. The treating clinician or pathologist should ensure that a cell count is performed and that examination and enumeration of blasts on a cytocentrifuge preparation are performed and are reviewed by the pathologist *(Expert consensus opinion)*" (de Haas et al., 2019).

Many genes should be tested for patients with suspected or confirmed B-ALL. In pediatric patients, "the pathologist or treating clinician should ensure that testing for

t(12;21)(p13.2;q22.1); *ETV6-RUNX1*, t(9;22)(q34.1;q11.2); *BCR-ABL1*, *KMT2A* (*MLL*) translocation, *iAMP21*, and trisomy 4 and 10 is performed (*Strong recommendation*)", while for adult patients, they assert "the pathologist or treating clinician should ensure that testing for t(9;22)(q34.1;q11.2); *BCR-ABL1* is performed. In addition, testing for *KMT2A* (*MLL*) translocations may be performed. (*Strong recommendation* for testing for t(9;22)(q34.1;q11.2) and *BCR-ABL1*; *Recommendation* for testing for *KMT2A* (*MLL*) translocations)" (de Haas et al., 2019).

The CAP and the ASH also propound that "For patients with suspected or confirmed ALL, the pathologist or treating clinician may order appropriate mutational analysis for selected genes that influence diagnosis, prognosis, and/or therapeutic management, which include, but are not limited to, *PAX5, JAK1, JAK2,* and/or *IKZF1* for B-ALL and *NOTCH1* and/or *FBXW7* for T-ALL. Testing for overexpression of CRLF2 may also be performed for B-ALL (*Recommendation*)."

The appropriate molecular genetic testing for AML is discussed starting from 16th guideline statement.

The expert panel strongly recommends testing for *FLT3-ITD* in adult and pediatric patients with suspected or confirmed AML of any type. They also recommend testing for other mutational analysis that could include, but not limited to, *IDH1, IDH2, TET2, WT1, DNMT3A*, and/or *TP53* for prognostic and/or therapeutic purposes (Statement 16).

In the 17th guideline statement, expert panel strongly recommends testing for *KIT* mutation in adult patients with confirmed core-binding factor (CBF) AML (AML with t(8;21)(q22;q22.1); RUNX1-RUNX1T1 or inv(16)(p13.1q22) /t(16;16)(p13.1;q22); CBFB-MYH11). It is only an expert consensus opinion for testing *KIT* mutation in pediatric patients with confirmed core binding factor AML (AML with t(8;21)(q22;q22.1); RUNX1-RUNX1T1 or inv(16)(p13.1q22) /t(16;16)(p13.1;q22); CBFB-MYH11) which is not a strong recommendation (Statement 17).

The strong recommendation is also given for patients other than those with confirmed core binding factor AML, APL, or AML with myelodysplasia-related cytogenetic abnormalities that testing is needed for mutational analysis for *NPM1*, *CEBPA*, and *RUNX1* (Statement 19).

In the 20th guideline statement, expert panel is providing no recommendation on either for or against the use of global/gene specific methylation, microRNA (miRNA) expression, or gene expression analysis for diagnosis or prognosis in patients with confirmed acute leukemia.

Finally, in their last statement, expert panel strongly recommends the use of current WHO terminology for the final diagnosis and classification of acute leukemias (Arber et al., 2017).

American Society of Clinical Oncology (ASCO)

The ASCO fully endorses the 2017 CAP and ASH guideline regarding the initial diagnostic work-up of acute leukemia (de Haas et al., 2019). The ASCO supports all twenty-seven guideline statements. The statements relevant to this policy are noted in the 2017 CAP and ASH guidelines above.

National Comprehensive Cancer Network (NCCN)

Acute myeloid leukemia

For the evaluation of AML in individuals 18 years of age or older, the NCCN guidelines recommends a history and a physical, complete blood count (CBC), platelets, differential, comprehensive metabolic panel (CMP), uric acid, lactate dehydrogenase (LDH), B12 and folic acid evaluation, prothrombin time (PT), partial thromboplastin time (PTT), and fibrinogen as part of the workup. Moreover, they recommend bone marrow core biopsy and aspirate analyses (including immunophenotyping by immunohistochemistry stains plus flow cytometry) and analysis for chromosomal structural variations using cytogenetics, FISH, or whole genome sequencing.

In terms of molecular analyses, they list "(*ASXL1*, *c-KIT*, *FLT3* [ITD (internal tandem duplication) and TKD (tyrosine kinase domain)], *NPM1*, *CEBPA*, *IDH1, IDH2*, *RUNX1*, *TP53*, and other mutations" as targets. A "Comprehensive pathology report, including diagnosis of AML (acute myeloid leukemia) with recurrent cytogenetics vs. AML not otherwise specified (NOS), blast count, cellularity, morphologic dysplasia, and mutation status if available" is also mentioned, and the NCCN "Recommend additional molecular and genetic testing for heritable hematologic malignancy predisposition in a subset of patients, particularly in patients <50 years". For patients with potential hematopoietic cell transplantation (HCT) in the future, HLA typing is also noted, "(except for patients with a major contraindication to HCT) and/or early referral to transplant center" (NCCN, 2024d). The NCCN also highlights that "A variety of gene mutations are associated with specific prognoses (category 2A) and may guide medical decision-making (category 2B). Other genetic lesions may have therapeutic significance. The field of genomics in myeloid malignancies and related implications in AML are evolving rapidly. Mutations should be tested in all patients. Multiplex gene panels and targeted next-generation sequencing (NGS) analysis are recommended for the ongoing management of AML and various phases of treatment" (NCCN, 2024d).

The NCCN cautions that the "importance of obtaining adequate samples of marrow or peripheral blood at diagnosis for full karyotyping and FISH cytogenetic analysis for the most common abnormalities cannot be overemphasized. Although FISH studies for common cytogenetic abnormalities may allow for rapid screening to identify either favorable- or unfavorable-risk groups, additional tests are needed to provide a full picture of the genetic factors that contribute to risk" (NCCN, 2024d).

As a part of monitoring during therapy for individuals with AML, the NCCN recommends a bone marrow aspirate/biopsy 14 to 21 days after the "start of therapy to document hypoplasia. If hypoplasia is not documented or indeterminate, repeat biopsy within 7 days to clarify persistence of leukemia. If hypoplasia, then repeat biopsy at time of hematologic recovery to document remission. If cytogenetics were initially abnormal, include cytogenetics as part of the remission documentation." Furthermore, as a follow-up to both standard-dose cytarabine induction therapy or high-dose cytarabine induction for poor-risk AML, the NCCN recommends that all patients should have bone marrow aspirate and biopsy by day 42 post-treatment, "regardless of the degree of hematologic recovery. When performed, BM aspirate and biopsy should include cytogenetic and molecular studies, as appropriate" (NCCN, 2024d).

As a part of surveillance and therapy for individuals with relapsed/refractory disease after the completion of consolidation, the NCCN recommends comprehensive genomic profiling to determine mutation status of actionable genes.

For the evaluation or workup for blastic plasmacytoid dendritic cell neoplasm in individuals 18 years of age or older, the NCCN recommends cytogenetic analysis (karyotype and/or FISH) and molecular analysis "(most common aberrations include: most common aberrations include: *TET2*, *ASXL1*, *ZRSR2*, *SRSF2*, *TP53*, *NRAS*, *IDH2*, and *ETV6*)" (NCCN, 2024d).

Chronic Lymphocytic Leukemia (CLL) and Small Lymphocytic Leukemia (SLL)

For the diagnosis of chronic lymphocytic leukemia and small lymphocytic leukemia, the NCCN notes the following components as essential:

- "Hematopathology review of peripheral blood smear and all slides with at least one paraffin block representative of the tumor, if the diagnosis was made on a lymph node or bone marrow biopsy.
- Flow cytometry of blood is adequate for the diagnosis of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL)."

- "CLL diagnosis requires presence of monoclonal B lymphocytes ≥5 x 109 /L in peripheral blood[b]
- Clonality of B cells should be confirmed by flow cytometry"
- Adequate immunophenotyping to establish diagnosis by flow cytometry using cell surface markers:[c] kappa/lambda, CD19, CD20, CD5, CD23, CD10, CD200; if flow cytometry is used to establish diagnosis, also include cytospin for cyclin D1 or fluorescence in situ hybridization (FISH) for t(11;14); t(11q;v)."
- "SLL diagnosis requires presence of lymphadenopathy and/or splenomegaly with monoclonal B lymphocytes ≤5 x 109 /L in peripheral blood
- SLL diagnosis should be confirmed by histopathology evaluation of lymph node biopsy"

- "Biopsy is generally not required. If diagnosis is not established by flow cytometry, then proceed with lymph node biopsy. Bone marrow aspirate with biopsy can be pursued if peripheral blood and lymph node biopsy material are nondiagnostic. Fine-needle aspiration (FNA) or core needle biopsy alone is not generally suitable for the initial diagnosis of CLL/SLL. In certain circumstances, when a lymph node is not easily accessible for excisional or incisional biopsy, a combination of core needle biopsy and FNA biopsy in conjunction with appropriate ancillary techniques for the differential diagnosis (ie, immunohistochemistry [IHC], flow cytometry) may be sufficient for diagnosis."
  - "Adequate immunophenotyping to establish diagnosis by IHC[c]: CD3, CD5, CD10, CD20, CD23, cyclin D1, LEF1, SOX11[d]"
- "Absolute monoclonal B lymphocyte count[b]" (NCCN, 2024a).

In superscript b, the NCCN cautions that "Absolute monoclonal B lymphocyte count <5000/mm^3 that persists more than 3 months in the absence of palpable adenopathy or other clinical features of lymphoproliferative disorder is MBL. Cells of the same phenotype may be seen in reactive lymph nodes; therefore, diagnosis of SLL should only be made when effacement of lymph node architecture is seen. Bone marrow examination is not helpful for the diagnosis of MBL." Moreover, they note in superscript c that "Typical immunophenotype: CD5+, CD23+, CD43+/-, CD10-, CD19+, CD20 dim, sIg dim+, and cyclin D1-. Note: Some cases may be sIg bright+ or CD23- or dim; some MCL may be CD23+; cyclin D1 immunohistochemistry or FISH for t(11;14) should be considered in all cases, especially for those with an atypical immunophenotype (ie, CD23 dim or negative, CD20 bright, sIg bright). CD200 positivity may distinguish CLL from mantle cell lymphoma (MCL), which is usually CD200-" (NCCN, 2024a).

Other analyses that may be "informative for prognostic and/or therapy determination" include

- "FISH to detect: +12; del(11q); del(13q); del(17p)
- *TP53* sequencing
- CpG-stimulated metaphase karyotype for complex karyotype (CK)
- Molecular analysis to detect: Immunoglobulin heavy chain variable region gene (IGHV) mutation status
- Beta-2-microglobulin" (NCCN, 2024a).

During workup, history and physical exam including measurement of size of liver and spleen and palpable lymph nodes, performance status, B symptoms, complete blood count (CBC) with differential, comprehensive metabolic panel, and beta-2-microglobulin are essential according to the NCCN. They also note that the following tests may be "useful under certain circumstances": quantitative immunoglobulins; reticulocyte count, haptoglobin, and direct antiglobulin test (Coombs) ; chest/abdominal/pelvic CT with contrast of diagnostic quality, if clinically indicated; uric acid; lactate dehydrogenase (LDH); unilateral bone marrow aspirate and biopsy (may be informative for the diagnosis of immune-mediated or disease-related cytopenias); hepatitis B and C testing if treatment is contemplated; pregnancy testing in patients of childbearing age if systemic therapy or RT is planned; discussion of fertility preservation; and FDG-PET/CT scan to direct nodal biopsy, if histologic transformation is suspected (NCCN, 2024a).

Chronic Myeloid Leukemia

The NCCN's recommendations for CML include the following table:

Monitoring Response to TKI Therapy and Mutational Analysis	
Test	**Recommendation**
Bone marrow cytogenetics[1]	At diagnosis Response milestones not reached Any signs of loss of hematologic response Any sign of loss of CCyR or its molecular response correlate (MR2.0: BCR::ABL1 [IS] ≤1%) - defined as an increase in *BCR::ABL1* transcript to >1%
qPCR using IS [International Scale]	At diagnosis Every 3 months after initiating treatment. After *BCR::ABL1* (IS) ≤1%[2] (MR2.0)[2] has been achieved, every 3 months for 2 years and every 3-6 months thereafter

	If there is 1-log increase in *BCR::ABL1* transcript levels with MMR, qPCR should be repeated in 1-3 months
***BCR::ABL* kinase domain mutation analysis**	CP-CML Response milestones not reached ○ Any sign of loss of hematologic response ○ Any sign of loss of CCyR or its molecular response correlate (MR2.0: BCR::ABL1 [IS] ≤1%) – defined as an increase in *BCR::ABL1* transcript to >1% ○ 1-log increase in *BCR::ABL1* transcript levels and loss of MMR Disease progression to AP-CML or BP-CML
[1]FISH has been inadequately studied for monitoring response to treatment. [2]CCyR correlates with *BCR-ABL1* (IS) ≤1% (MR2.0). [3]Consider myeloid mutation panel to identify *BCR::ABL1*–independent resistance mutations in patients with no *BCR::ABL1* kinase domain mutations	

The NCCN (2023) also states that for the diagnosis and workup of CML-1:

- "Initial evaluation should consist of a history and physical exam, including palpation of spleen, complete blood count (CBC) with differential, chemistry profile, and hepatitis B panel. Bone marrow aspirate and biopsy for morphologic and cytogenetic evaluation and quantitative reverse transcriptase polymerase chain reaction (RT-PCR) to establish the presence of quantifiable *BCR::ABL1* mRNA transcripts at baseline are recommended to confirm the diagnosis of CML.
- Bone marrow cytogenetics with a minimum of 20 metaphases is useful to detect additional chromosomal abnormalities (ACAs) in Ph-positive cells, also known as clonal cytogenetic evolution (discussed below).9-13 If bone marrow evaluation is not feasible, fluorescence in situ hybridization (FISH) on the bone marrow or a peripheral blood specimen with dual probes for BCR and ABL1 genes can be used to confirm the diagnosis of CML."
- "Quantitative RT-PCR (qPCR) should be done at initial workup to establish the presence of quantifiable BCR::ABL1 mRNA transcripts."
- "Qualitative RT-PCR for detecting atypical BCR::ABL1 transcripts should be considered if there is discordance between FISH and qPCR results." (NCCN, 2023).

The NCCN provides the following early treatment response milestones for those with CML:

EARLY TREATMENT RESPONSE MILESTONES
CRITERIA FOR RESPONSE AND RELAPSE

BCR::ABL1 (IS)	3 months	6 months	12 months[m]
>10%[n]	YELLOW	RED	
>1%–10%	GREEN		YELLOW
>0.1%–1%	GREEN		LIGHT GREEN
≤0.1%	GREEN		

COLOR	CONCERN	CLINICAL CONSIDERATIONS[p]	RECOMMENDATIONS[p]
RED	TKI-resistant disease[o]	• Evaluate patient adherence and drug interactions • Consider *BCR::ABL1* kinase domain mutational analysis[q] • Consider bone marrow cytogenetic analysis to assess additional chromosomal abnormalities (ACAs)	Switch to alternate TKI (CML-5) (other than imatinib) and evaluate for allogeneic HCT
YELLOW	Possible TKI resistance[o]	• Evaluate patient adherence and drug interactions • Consider *BCR::ABL1* kinase domain mutational analysis[q] • Consider bone marrow cytogenetic analysis to assess for MCyR at 3 mo or CCyR at 12 mo	Switch to alternate TKI (CML-5) or Continue same TKI (CML-G)[r] and Consider evaluation for allogeneic HCT
LIGHT GREEN	TKI-sensitive disease	• Evaluate patient adherence and drug interactions • If treatment goal is long-term survival: ≤1% optimal • If treatment goal is treatment-free remission: ≤0.1% optimal	• If optimal: continue same TKI (CML-G) • If not optimal: shared decision-making with patient[o,s]
GREEN	TKI-sensitive disease	• Monitor response (CML-E) • Evaluate patient adherence and drug interactions	Continue same TKI (CML-G)[t]

(NCCN, 2023)

For those discontinuing TKI therapy, the NCCN recommends molecular monitoring every one to two months in the first six months following discontinuation, bimonthly monitoring during months seven to twelve, and quarterly monitoring thereafter (indefinitely) in individuals who remain in MMR (MR3; *BCR::ABL1* ≤0.1% IS) (NCCN, 2023).

Acute Lymphoblastic Leukemia (ALL)

Regarding ALL, the NCCN recommends testing of marrow or peripheral blood lymphoblasts using various techniques. The guidelines state the following:

"The diagnosis of ALL generally requires demonstration of ≥20% bone marrow lymphoblasts" predicated "on hematopathology review of bone marrow aspirate and biopsy materials, which includes:

- Morphologic assessment of Wright-Giemsa-stained bone marrow aspirate smears, and hematoxylin and eosin (H&E)-stained core biopsy and clot sections
- Comprehensive flow cytometric immunophenotyping
- Baseline flow cytometric and/or molecular characterization of leukemic clone to facilitate subsequent minimal/measurable residual disease (MRD) analysis
- Karyotyping of G-banded metaphase chromosomes" (NCCN, 2024c).

Regarding molecular characterization of ALL, is is stated that

- "Cytogenetic and molecular prognostic risk stratification for B-cell ALL (B-ALL)
- Optimal risk stratification and treatment planning require testing marrow or peripheral blood lymphoblasts for specific recurrent genetic abnormalities using:

- Interphase fluorescence in situ hybridization (FISH) testing, including probes capable of detecting the major recurrent genetic abnormalities
- Reverse transcriptase polymerase chain reaction (RT-PCR) testing BCR::ABL1 in B-ALL (quantitative or qualitative) including determination of transcript size (ie, p190 vs. p210)
- Comprehensive testing by next-generation sequencing (NGS) for gene fusions and pathogenic mutations is recommended.

- Additional optional tests include:
  - Assessment with chromosomal microarray (CMA)/array comparative genomic hybridization (cGH) in cases of aneuploidy or inadequate karyotype" (NCCN, 2024c).

According to the NCCN, "*BCR::ABL1*–like or Ph–like ALL is a subgroup of B-cell lineage ALL associated with unfavorable prognosis", and it should be noted that "Although this subgroup is Ph-negative, there is an otherwise similar genetic profile to the Ph-positive ALL subgroup including mutation of the *IKZF1* gene." Nevertheless, "this subtype is typically associated with gene fusions and mutations that activate tyrosine kinase pathways as the common mechanism of transformation", and these gene fusions and mutations include *ABL1, ABL2, CRLF2, CSF1R, EPOR, JAK1, JAK2, JAK3, TYK2, PDGFRβ, PDGFRα, FGFR, EBF1, FLT3, IL7R, NTRK3, PTL2B*, and *SH2B3* genes" (NCCN, 2024c).

As a part of surveillance for individuals with ALL, the NCCN highlights that "An assessment of bone marrow aspirate can be considered as clinically indicated at a frequency of 3 to 6 months for the first 5 years; if a bone marrow aspirate is performed, flow cytometry with additional studies that may include comprehensive cytogenetics, FISH, molecular tests, and MRD assessments should be carried out. While there is insufficient evidence to guide MRD monitoring for patients with Ph-negative disease following completion of maintenance therapy, the approval of blinatumomab, and potentially future therapies for MRD-positive relapse, may warrant testing in this regard." The NCCN also states that "Periodic MRD assessments should be considered (no more than every 3 months) for patients who have achieved a complete molecular remission (undetectable levels)". However, "the frequency may be increased if MRD levels are detectable" (NCCN, 2023, 2024c).

Pediatric Acute Lymphoblastic Leukemia (PEDALL)

The NCCN also produces guidelines focusing on Pediatric Acute Lymphoblastic Leukemia (PEDALL). For PEDALL, the NCCN provides the following recommendation for genetic characterization.

"GENETIC CHARACTERIZATION

Optimal risk stratification and treatment planning require testing marrow or peripheral blood lymphoblasts for specific recurrent genetic abnormalities using:

- Karyotyping of G-banded metaphase chromosomes
- Interphase fluorescence in situ hybridization (FISH) testing, including probes capable of detecting the major recurrent genetic abnormalities
- Reverse transcriptase-polymerase chain reaction (RT-PCR) testing for *BCR::ABL1* in B-ALL (quantitative or qualitative) including determination of transcript size (i.e., p190 vs. p210)
    - If *BCR::ABL1* negative: encourage testing for gene fusions and mutations associated with *BCR::ABL1*–like (Ph-like) ALL
- Assessment of various potentially actionable or prognostic mutations and gene fusions via next-generation sequencing (NGS) or alternative methods
- Additional optional tests include:
    - Additional assessment (eg, microarray comparative genomic hybridization [CGH] and/or NGS) in cases of aneuploidy or inadequate karyotype
    - Whole transcriptome sequencing to identify B-lymphoblastic leukemia/lymphoma (B-ALL/LL) subtypes defined by gene expression profile (ie, *ETV6::RUNX1*-like, *PAX5alt*, *MYCr*)" (NCCN, 2024b).

As a part of surveillance under their "Procedures and Molecular Testing" of individuals with PEDALL, the NCCN recommends testing of "Bone marrow aspirate and cerebrospinal fluid (CSF) for suspected relapse". They note that "If bone marrow aspirate is done: Flow cytometry with additional studies that may include comprehensive cytogenetics, FISH, molecular testing, and MRD testing" and suggest that one can "Consider periodic *BCR::ABL1* transcript-specific quantification (Ph+ ALL)" (NCCN, 2024b).

The guidelines also state that "The *BCR-ALB1*-like (Ph-like) phenotype is associated with recurrent gene fusions and mutations that activate tyrosine kinase pathways and includes gene fusions involving *ABL1, ABL2, CRLF2, CSF1R, EPOR, JAK2 or PDGFRB* and mutations involving *CRLF2, FLT3, IL7R, SH2B3, JAK1, JAK3, and JAK2* (in combination with *CRLF2* gene functions). Testing of these abnormalities at diagnosis may aid in risk stratification" (NCCN, 2024b).

European LeukemiaNet (ELN) Working Party

In 2022, an international expert panel on behalf of the ELN published recommendations for the diagnosis and management of AML.

ELN remarks that "Germline predisposition risk should be considered for all patients diagnosed with a hematopoietic malignancy regardless of age, because some germline predisposition alleles, like those in DDX41, can drive hematopoietic malignancies in older age" and notes key features of clinical presentation warranting consideration of clinical testing for a germline predisposition allele(s), including

"Personal history of ≥ two cancers, one of which is a hematopoietic malignancy (order does not matter)

Personal history of a hematopoietic malignancy plus:

- Another relative within two generations with another hematopoietic malignancy, or
- Another relative within two generations with a solid tumor diagnosed at age 50 or younger, or
- Another relative within two generations with other hematopoietic abnormalities

Presence of a deleterious gene variant in tumor profiling that could be a germline allele, especially if that variant is present during remission*

Age of diagnosis of hematopoietic malignancy at an earlier age than average (eg, MDS diagnosed ≤ 40 y)

Germline status of a variant is confirmed by:

Its presence in DNA derived from a tissue source not likely to undergo somatic mutation frequently (eg, cultured skin fibroblasts or hair follicles) AND at a variant allele frequency consistent with the germline (generally considered between 30-60%), or

Its presence in at least two relatives at a variant allele frequency consistent with the germline" (Döhner et al., 2022). It is also noted that "Certain gene alleles (eg, *CHEK2* I200T and truncating *DDX41* variants) are overwhelmingly likely to be germline and should prompt consideration of germline testing when identified even once" (Döhner et al., 2022).

Furthermore, ELN lists tests and procedures recommended to be performed at diagnosis for a patient with AML in the Table 4, captured below.

Table 4. Tests and procedures at diagnosis for a patient with AML

Tests and procedures		
Tests to establish the diagnosis		**Additional tests and procedures**
Complete blood count and differential count*		Complete physical examination[c]
Bone marrow aspirate†		Performance status (ECOG/WHO score)
Bone marrow trephine biopsy‡		Geriatric assessment[d] (optional)
Immunophenotyping by flow cytometry (see Table 5)		Biochemistry, coagulation tests[e]
Genetic analyses	Results preferably available within	Hepatitis A, B, C; HIV-1 testing; CMV, EBV, HSV, VZV
Cytogenetics§	• 5-7 d	Serum pregnancy test[f]
Screening for gene mutations required for establishing the diagnosis and to identify actionable therapeutic targets#		Eligibility assessment for allogeneic HCT (incl. HLA-typing)[g]
• *FLT3*,¶ *IDH1*, *IDH2*	• 3-5 d	Chest x-ray, 12-lead electrocardiogram, echocardiography or MUGA (on indication)
• *NPM1*	• 3-5 d	Computed tomography of the chest (on indication)[h]
• *CEBPA*,# *DDX41*, *TP53*; *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, *ZRSR2*	• 1st cycle	Lumbar puncture (on indication)[i]
Screening for gene rearrangements**		Information on oocyte and sperm cryopreservation[j]
• *PML::RARA*, *CBFB::MYH11*, *RUNX1::RUNX1T1*, *KMT2A* rearrangements, *BCR::ABL1*, other fusion genes (if available)	• 3-5 d	Biobanking[k]
Additional genes recommended to test at diagnosis††		
• *ANKRD26*, *BCORL1*, *BRAF*, *CBL*, *CSF3R*, *DNMT3A*, *ETV6*, *GATA2*, *JAK2*, *KIT*, *KRAS*, *NRAS*, *NF1*, *PHF6*, *PPM1D*, *PTPN11*, *RAD21*, *SETBP1*, *TET2*, *WT1*		
Medical history		
Demographics and medical history‡‡		
Detailed family history[a]		
Patient bleeding history[b]		
Analysis of comorbidities		

(Döhner et al., 2022).

According to ELN, "Conventional cytogenetic analysis is mandatory in the evaluation of AML. If conventional cytogenetics fails, fluorescence in situ hybridization is an alternative to detect specific abnormalities like RUNX1::RUNX1T1, CBFB::MYH11, KMT2A (MLL), and MECOM (EVI1) gene fusions, or myelodysplasia-related chromosome abnormalities, eg, loss of chromosome 5q, 7q, or 17p material". ELN also asserts that "Molecular genetic testing should screen for all the genetic abnormalities that define disease and risk categories or that are needed for targeted treatment modalities" listed above in their Table 4, and that for "patients with mutant NPM1 and core-binding factor (CBF)- AML, it is recommended to perform baseline molecular assessment by quantitative polymerase chain reaction (qPCR) or droplet digital PCR (dPCR) to facilitate MRD monitoring after treatment" (Döhner et al., 2022). ELN recognizes that "Immunophenotyping by multiparameter flow cytometry (MFC) is required to diagnose AML accurately by identifying cell surface and intracellular markers" and that "It is also important to identify leukemia-associated

immunophenotypes (LAIP) for subsequent MRD monitoring by MFC. In cases where an aspirate is unobtainable and circulating blasts are absent, myeloid phenotype may be confirmed on a core biopsy using immunohistochemistry" (Döhner et al., 2022). Cell-surface and cytoplasmic markers of interest for the diagnosis of AML and MPAL as reported by ELN is shown below (Table 5). Lastly, this ELN special report included an updated table of risk classifications at initial diagnosis based on new data (Table 6).

Table 5. Expression of cell-surface and cytoplasmic markers for the diagnosis of AML and MPAL

Diagnosis of AML	
Diagnosis of AML	
Precursor marker	CD34, CD117, HLA-DR
Myeloid markers	Cytoplasmic MPO, CD33, CD13
Myeloid maturation markers	CD11b, CD15, CD64, CD65
Monocytic markers	CD14, CD36, CD64, CD4, CD38, CD11c
Megakaryocytic markers	CD41 (glycoprotein IIb/IIIa), CD61 (glycoprotein IIIa), CD36
Erythroid markers	CD235a (glycophorin A), CD71, CD36
Diagnosis of MPAL	
Myeloid lineage	MPO (flow cytometry, immunohistochemistry or cytochemistry), or monocytic differentiation (at least 2 of the following: non-specific esterase cytochemistry, CD11c, CD14, CD64, lysozyme), or at least two myeloid markers, ie, CD177, CD33, CD13
T-lineage	Strong cytoplasmic CD3 (with antibodies to CD3 ε chain) or surface CD3
B-lineage[9]	Strong CD19 with at least one of the following strongly expressed: cytoplasmic CD79a, cCD22 or CD10, or weak CD19 with at least two of the following strongly expressed: CD79a, cCD22 or CD10
Core MRD markers	CD34, CD117, CD45, CD33, CD13, CD56, CD7, HLA-DR If monocytic: CD64, CD11b, CD4 (in addition)

Table 6. 2022 ELN risk classification by genetics at initial diagnosis*

Risk category†	Genetic abnormality
Favorable	• t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1*†,‡ • inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB*::*MYH11*†,‡ • Mutated *NPM1*†,§ without *FLT3*-ITD • bZIP in-frame mutated *CEBPA*‖
Intermediate	• Mutated *NPM1*†,§ with *FLT3*-ITD • Wild-type *NPM1* with *FLT3*-ITD (without adverse-risk genetic lesions) • t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A*†,¶ • Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse	• t(6;9)(p23.3;q34.1)/*DEK*::*NUP214* • t(v;11q23.3)/*KMT2A*-rearranged# • t(9;22)(q34.1;q11.2)/*BCR*::*ABL1* • t(8;16)(p11.2;p13.3)/*KAT6A*::*CREBBP* • inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2, MECOM(EVI1)* • t(3q26.2;v)/*MECOM*(*EVI1*)-rearranged • −5 or del(5q); −7; −17/abn(17p) • Complex karyotype,** monosomal karyotype†† • Mutated *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, and/or ZRSR2*‡‡ • Mutated *TP53*[a]

An ELN expert panel has released guidelines for the assessment of measurable residual disease (MRD) of AML that were updated in 2021. First, for molecular MRD recommendations, "techniques for molecular MRD assessment should reach an LOD [limit of detection] of 10^{-3} or lower. To achieve this LOD, qPCR, dPCR, or error-corrected NGS using UMIs [unique molecular identifiers] is recommended (Grade of Recommendation: B)."

Other recommendations for molecular MRD testing include

- "For patients with mutant *NPM1*, CBF AML (*RUNX1-RUNX1T1* or *CBFB-MYH11*), or APL (*PML-RARA*), we recommend molecular MRD assessment by qPCR or dPCR. (Grade of Recommendation: A)"
- "Leukemia-specific PCR assays (eg, for *NPM1, PML-RARA,* or *CBF* AML) are preferred over fewer specific markers, such as *WT1* or *EV11* expression (Grade of Recommendation: B)"
- "Targeted NGS-MRD using specific mutations identified at diagnosis vs agnostic panel approaches has different strengths and limitations, but both approaches can be considered, depending on sensitivity, turnaround time, resource use, setting (research, clinical trial, or clinical routine), and ability to standardize methodology and reporting. (Grade of Recommendation: B)"
- "If a panel approach is used for NGS-MRD, emerging variants not found at diagnosis should be reported only if confidently detected above background noise. (Grade of Recommendation: B)"
- "For NGS-MRD, we recommend considering all detected mutations as potential MRD markers, with the limitations detailed in recommendations B9 to B11 (listed below) (Grade of Recommendation: B)"
    - "Germline mutations (VAF of ~50 in genes *ANKRD26*, *CEBPA*, *DDX41*, *ETV6*, *GATA2*, *RUNX1*, and *TP53*) should be excluded as NGS-MRD markers, as they are noninformative for MRD. (Grade of Recommendation: A)"
    - "Mutations in *DNMT3A*, *TET2*, and *ASXL1* (DTA) can be found in age-related clonal hematopoiesis and should be excluded from MRD analysis. (Grade of Recommendation: A)"
    - "Mutations in signaling pathway genes (*FLT3-ITD*, *FLT3-TKD*, *KIT*, and *RAS*, among others) most likely represent residual AML when detected, but are often subclonal and have a low negative predictive value. These mutations are best used in combination with additional MRD markers. (Grade of Recommendation: B)" (Heuser et al., 2021).

International Workshop on Chronic Lymphocytic Leukemia (iwCLL)

In the iwCLL guidelines for the diagnosis, indications for treatment, response assessment, and supportive management of CLL, the iwCLL reference the World Health Organization in describing "CLL as leukemic, lymphocytic lymphoma, being only distinguishable from small lymphocytic lymphoma (SLL) by its leukemic manifestation" and adduce that "CLL, by definition, is always a disease of neoplastic B cells, whereas the entity formerly described as T-CLL is now called T-cell prolymphocytic leukemia" (Hallek et al., 2018). The iwCLL states that "The diagnosis of CLL requires the presence of ≥5 × 109/L B lymphocytes in the peripheral blood, sustained for at least 3 months. The clonality of these B lymphocytes needs to be confirmed by demonstrating

immunoglobulin light chain restriction using flow cytometry." They also assert that "CLL or SLL might be suspected in otherwise healthy adults who have an absolute increase in clonal B lymphocytes, but who have <5 × 109/L B lymphocytes in the blood. However, in the absence of lymphadenopathy or organomegaly (as detected by physical examination or imaging studies), or of disease-related cytopenias or symptoms, the presence of <5 × 109/L B lymphocytes is defined as monoclonal B lymphocytosis (MBL)", explaining that "The presence of a cytopenia caused by a typical marrow infiltrate establishes the diagnosis of CLL regardless of the number of peripheral blood B lymphocytes or of the lymph node involvement." Meanwhile, "The definition of SLL requires the presence of lymphadenopathy and the absence of cytopenias caused by a clonal marrow infiltrate. Additionally, the number of B lymphocytes in the peripheral blood should be <5 × 109/L. In SLL, the diagnosis should be confirmed by histopathological evaluation of a lymph node biopsy or biopsy of other tissues"(Hallek et al., 2018).

They also mention that "It is important to verify that the patient has CLL and not some other lymphoproliferative disease that can masquerade as CLL, such as hairy cell leukemia or leukemic manifestations of mantle cell lymphoma, marginal zone lymphoma, splenic marginal zone lymphoma with circulating villous lymphocytes, or follicular lymphoma. To achieve this, it is necessary to evaluate the blood smear, the immunophenotype, and, in some cases, the genetic features of the circulating lymphoid cells" (Hallek et al., 2018).

British Society for Haematology (BSH)

The Haemato-oncology Task Force of BCSH (British Committee for Standards in Haematology) had produced a draft outline of guidelines for the diagnosis and management of AML in pregnancy, which was subsequently reviewed by the British Society of Haematology due to a consultation process resulting in the name change from BCSH to BSH in 2016. These guidelines state that "As for non-pregnant patients, acute myeloid leukaemia (AML) should be diagnosed using the World Health Organization (WHO) classification (Grade 1A)" and [w]here a diagnosis of leukemia is suspected, care must be taken to ensure that marrow samples are directed for immunophenotypic, cytogenetic and molecular analysis to allow accurate sub-typing and understanding of prognostic features" (Ali et al., 2015).

On the management of older patients with frailty and acute myeloid leukemia, BSH asserts that "Older adults who are suspected of having AML and are deemed to be fit to receive anti-leukaemia therapy should undergo the same diagnostic workup as any other patient (Table 1)" (Dennis et al., 2022).

At diagnosis, "Patients should have a bone marrow examination (aspirate and trephine biopsy) for blast enumeration [with a 300 (or 500 where indicated) differential cell count]" and "flow cytometry to characterise the leukaemic clone immunophenotype to confirm lineage, and because this provides a means of assessing measurable residual disease (MRD) in the absence of a molecular marker". Moreover, "For patients with a high white blood count at presentation, diagnostic workup may be performed on the peripheral blood in lieu of a bone marrow examination, and this may also be a suitable approach for frail patients, for whom best supportive care is the most appropriate treatment option. Consideration should also be given (with appropriate patient consent) to taking trial samples at the time of diagnostic marrow sampling" (Dennis et al., 2022).

The BSH notes that "Risk stratification remains informed by cytogenetic analysis, and, given that some treatments (e.g., CPX-351) are currently only licensed in the UK for therapy-related AML or AML with myelodysplasia-related changes (AML-MRC), knowledge of cytogenetic abnormalities is a prerequisite to offer the most appropriate treatment." But while "This is likely to be most efficiently achieved using a multi-target next-generation sequencing (NGS) panel approach with a maximum turnaround time of 21 days", "we [BSH] recommended rapid screening (turnaround time within 72 hours) for mutations of *NPM1*, *FLT3*, *IDH1/2* and *TP53*, given that the presence of *NPM1* and I*DH1/2* mutations may inform treatment decisions, as these mutations have been shown to confer a superior response to venetoclax/azacitidine), whilst *TP53* mutations confer resistance to chemotherapy and an overall poor outcome (Dennis et al., 2022).

The BSH also recommends that "At relapse, patients who remain fit to receive anti-leukaemia therapy, should be re-screened for actionable/targetable mutations. At the least, this should include re-screening for mutations in FLT3 (both internal tandem duplication; ITD and tyrosine kinase domain; TKD), given the availability of gilteritinib", along with "*IDH1/2* mutations (if trial recruitment is an option). It should be noted that *FLT3* mutations may be acquired or lost at relapse". As such,

- All patients should have their disease assessed by morphology, immunophenotyping, cytogenetics and molecular studies at presentation.
- At relapse, patients should be re-screened for *FLT3* mutations as a minimum.
- Peripheral blood is a suitable alternative to bone marrow for the diagnostic evaluations if there are circulating blast cells.
- All cases should be discussed at an appropriate local or regional haemato-oncology MDT (Dennis et al., 2022).

Table 1, produced by BSH and referenced earlier, is captured below:

TABLE 1. Diagnostic investigations for AML in the older adult

Diagnostic	Diagnosis	Relapse
Bone marrow aspirate	√	√
Bone marrow trephine biopsy	√	√
Flow cytometric immunophenotyping	√	√
Cytogenetic analysis (G-banding and FISH panels) (within 5 working days)	√	√[a]
Rapid *FLT3* ITD and TKD mutation screen (within 72 hours)	√	√
Rapid *NPM1* mutation screen (within 72 hours)	√	
Multi-target NGS panel including minimum of:	√	
IDH1/2 screen	√[b,c]	
TP53 screen	√[b,c]	
ASXL1 screen	√	
RUNX1 screen	√	

Abbreviations: AML, acute myeloid leukaemia; FISH, fluorescence in situ hybridisation.

[a]If no unfavourable risk cytogenetic abnormalities were found at diagnosis, or if the patient has experienced a prolonged remission (>12 months), then cytogenetics should be rechecked at relapse.

[b]Rapid mutation screening assays for *IDH1/2* and *TP53* are recommended as these mutations may impact early treatment decisions.

[c]National Genomic Test directory for Cancer (https://www.england.nhs.uk/publication/national-genomic-test-directories/) currently recommends screening for mutations in following genes at diagnosis; *NPM1*, *CEBPA*, *RUNX1*, *FLT3*, *IDH1*, *IDH2*, *KIT*, *WT1*, *ASXL1*, *SRSF2*, *STAG2*, *RAD21*, *TP53*, *KRAS*, *NRAS*, *MLL* (*KMT2A*)-PTD, *PPM1D*.

(Dennis et al., 2022).

In a paper on best practices for laboratory testing of UK patients with AML, BSH recommends that "For a suspected acute leukaemia, most multiparametric flow cytometry laboratories apply a two-stage diagnostic panelan acute leukaemia screen (such as the Euroflow A LOT combination)" in order to confirm leukaemic blasts and allow lineage assessment, "followed immediately by an AML-specific panel if appropriate". Furthermore, "extended secondary testing may be required to diagnose blastic plasmacytoid dendritic cell neoplasm (BPDCN) or ambiguous leukaemias or acute megakaryoblastic leukaemia" (Mehta et al., 2023).

European Society for Medical Oncology (ESMO)

The ESMO released guidelines for the diagnosis of acute myeloid leukemia in adult patients. They recommend molecular testing for *c-KIT, FLT3-ITD, FLT3-TKD, NPM1, CEBPA, IDH1, IDH2,* and *TP53* mutations. They state that "next-generation sequencing (NGS) of a panel of genes commonly mutated in AML provides important additional prognostic and therapeutic information" (Heuser et al., 2020).

Acute Lymphoblastic Leukemia (ALL)

The ESMO also published clinical practice guidelines for ALL in 2016 and note that "standard cytogenetics/FISH and especially RT-PCR are routinely performed to obtain a rapid diagnosis of Ph+ ALL and identify certain intermediate/high- and high-risk karyotypes or gene, mainly:

a. t(4;11)(q21;q23)/MLL-AFA4, abn11q23/MLL, t(1;19)(q23; p13)/PBX-E2A, t(8;14) or other abn14q32 in non-Burkitt ALL
b. del(6q), del(7p), del(17p), −7, +8, low hypodiploidy, i.e., with 30–39 chromosomes/near triploidy with 60–78 chromosomes
c. complex (≥5 unrelated clonal abnormalities), and
d. T-ALL lacking NOTCH1/FBXW7 mutations and/or with RAS/PTEN abnormalities

The more prognostically favorable cytogenetic/genetic subsets are t(12;21)(p13;q22)/TEL-AML1 + ALL (rare in adults) and hyperdiploid ALL, and NOTCH-1/FBXW7-mutated T-ALL" (Hoelzer et al., 2016).

Chronic Lymphocytic Leukemia

According to ESMO, "the diagnosis of CLL is established by the following criteria:

- Presence of ≥5 × 109/l monoclonal B lymphocytes in the peripheral blood. The clonality of the circulating B lymphocytes needs to be confirmed by demonstrating light chain restriction using flow cytometry.
- The leukaemia cells found in the blood smear are characteristically small, mature-appearing lymphocytes with a narrow border of cytoplasm and a dense nucleus lacking discernible nucleoli and having partially aggregated chromatin. Larger, atypical lymphocytes or prolymphocytes may be seen but must not exceed 55%" (Eichhorst et al., 2021).

Also worth noting is that "CLL cells co-express the B-cell surface antigens CD19 and CD20 together with CD5, CD23, CD43 and CD200. The levels of surface CD20, surface immunoglobulin (Ig) and CD79b are characteristically low compared with those found on normal B cells" and that "Each clone of leukaemia cells is restricted to expression of

either kappa or lambda Ig light chains, or has no apparent expression of either of the two." ESMO also states that "Other lymphoma entities to be differentiated from CLL are mantle cell lymphoma (MCL), leukaemic marginal zone lymphoma (MZL) (in particular the splenic variant) and lymphoplasmacytic lymphoma". This differentiation can be made as "tumour cells may express B-cell surface antigens and CD5, but in most cases, they do not express CD23, in particular MZL. For cases that express CD23, reverse transcription polymerase chain reaction (RT-PCR) for determination of cyclin D1 overexpression, and FISH for detecting a translocation (11;14), but also CD200 expression, are useful for establishing the diagnosis of MCL." Moreover, "SOX11 staining may be used on tumour biopsies. A diagnosis of MZL is supported by negative or low CD43 expression and high expression of CD180" (Eichhorst et al., 2021).

Ultimately, the ESMO proposes two recommendations regarding the diagnosis of CLL:

- "Diagnosis is usually possible by immunophenotyping of peripheral blood only [III, A].
- LN biopsy and/or bone marrow biopsy may be helpful if immunophenotyping is not conclusive for the diagnosis of CLL [IV, A]" (Eichhorst et al., 2021).

During diagnostic and staging work-up, the ESMO recommends also that

- "Binet and Rai staging systems with clinical symptoms are relevant for treatment indication [III, A].
- del(17p), *TP53* mutations and IGHV status are relevant for choice of therapy and should be assessed before treatment [III, A].
- Routine evaluation of del(17p), *TP53* mutation and IGHV status in early and asymptomatic stage is not recommended [V, D].
- Routine imaging during a watch-and-wait period is not recommended unless there are clinical symptoms [V, E]" (Eichhorst et al., 2021).

The ESMO also recommends that "After the first year, when all patients should be seen at 3-monthly intervals, patients can be followed every 3-12 months depending on burden and dynamics of the disease" using history and physical examinations including a careful palpation of all LN areas, spleen and liver and complete blood cell count and differential count (Eichhorst et al., 2021).

Chronic Myeloid Leukemia

The ESMO has published guidelines for diagnosis, treatment, and follow-up of CML. These guidelines include a table which is shown below:

Table 2: Recommendations for diagnostic work-up, assessment of response and monitoring (Hochhaus et al., 2017)

Test	Baseline (diagnostic work-up)	To assess the response	To monitor the response and the treatment
Blood counts and differential	Yes	Every 15 days until a CHR [complete hematological response] without significant cytopaenias has been achieved	Every 3 months
BM [bone marrow], cytology	Yes	No	No
BM, karyotype	Yes	At 3 and 6 months	Then every 6 months until CCyR [complete cytogenetic response] has been achieved
Blood, iFISH	No	No	Only if cytogenetics of BM metaphases cannot be analyzed or is normal and molecular response cannot be assessed
Blood, RT-PCR (qualitative)	Yes	No	No
Blood, qRT-PCR (quantitative, BCR–ABL %)	No	Every 3 months	Every 4–6 weeks in first year after treatment discontinuation
Mutational Analysis	Only in AP [accelerated phase] or BP [blast phase]	No	Only in the case of failure

The ESMO has also stated that a "diagnosis must be confirmed by cytogenetics showing t(9;22)(q34;q11), and by multiplex RT-PCR showing BCR–ABL1 transcripts" (Hochhaus et al., 2017). Other warning signs include "Major route cytogenetic aberrations (+8, iso(17q), +19, +22q-), chromosome 3 aberrations and BM fibrosis at diagnosis signs"; further, a quantification of BCR-ABL mRNA is required every 3 months (Hochhaus et al., 2017). Finally, the ESMO acknowledges that mutation analysis is "due" in case of failure of first-line therapy or if BCR-ABL transcript levels increase. However, ESMO recommends against baseline mutational analysis in patients with newly diagnosed CML-CP (Hochhaus et al., 2017).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

On April 28, 2017 the FDA approved the LeukoStrat® CDx *FLT3* Mutation Assay as a "PCR-based, in vitro diagnostic test designed to detect internal tandem duplication (ITD) mutations and the tyrosine kinase domain mutations D835 and I836 in the *FLT3* gene in genomic DNA extracted from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients diagnosed with acute myelogenous leukemia (AML). The LeukoStrat® CDx FLT3 Mutation Assay is used as an aid in the selection of patients with AML for whom RYDAPT® (midostaurin) treatment is being considered. The Leukostrat® CDx FLT3 Mutation Assay is to be performed only at Laboratory for Personalized Molecular Medicine (LabPMM) LLC, a single site laboratory located at 6330 Nancy Ridge Dr., San Diego, CA 92121."

On August 1, 2017 the FDA approved the Abbott RealTime *IDH2* as an "in vitro polymerase chain reaction (PCR) assay for the qualitative detection of single nucleotide variants (SNVs) coding nine *IDH2* mutations (R140Q, R140L, R140G, R140W, R172K, R172M, R172G, R172S, and R172W) in DNA extracted from human blood (EDTA) or bone marrow (EDTA). Abbott RealTime IDH2 is for use with the Abbott m2000rt System. Abbott RealTime IDH2 is indicated as an aid in identifying acute myeloid

leukemia (AML) patients with an isocitrate dehydrogenase-2 (IDH2) mutation for treatment with IDHIFA® (enasidenib)."

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81120	IDH1 (isocitrate dehydrogenase 1 [NADP+], soluble) (eg, glioma), common variants (eg, R132H, R132C)
81121	IDH2 (isocitrate dehydrogenase 2 [NADP+], mitochondrial) (eg, glioma), common variants (eg, R140W, R172M)
81170	ABL1 (ABL proto-oncogene 1, non-receptor tyrosine kinase) (eg, acquired imatinib tyrosine kinase inhibitor resistance), gene analysis, variants in the kinase domain
81175	ASXL1 (additional sex combs like 1, transcriptional regulator) (eg, myelodysplastic syndrome, myeloproliferative neoplasms, chronic myelomonocytic leukemia), gene analysis; full gene sequence
81176	ASXL1 (additional sex combs like 1, transcriptional regulator) (eg, myelodysplastic syndrome, myeloproliferative neoplasms, chronic myelomonocytic leukemia), gene analysis; targeted sequence analysis (eg, exon 12)
81206	BCR/ABL1 (t(9:22)) (eg, chronic myelogenous leukemia) translocation analysis; major breakpoint, qualitative or quantitative
81207	BCR/ABL1 (t(9:22)) (eg, chronic myelogenous leukemia) translocation analysis; minor breakpoint, qualitative or quantitative
81208	BCR/ABL1 (t(9:22)) (eg, chronic myelogenous leukemia) translocation analysis; other breakpoint, qualitative or quantitative
81218	CEBPA (CCAAT/enhancer binding protein [C/EBP], alpha) (eg, acute myeloid leukemia), gene analysis, full gene sequence
81245	FLT3 (fms-related tyrosine kinase 3) (eg, acute myeloid leukemia), gene analysis; internal tandem duplication (ITD) variants (ie, exons 14, 15)
81246	FLT3 (fms-related tyrosine kinase 3) (eg, acute myeloid leukemia), gene analysis; tyrosine kinase domain (TKD) variants (eg, D835, I836)

CPT	Code Description
81261	IGH@ (Immunoglobulin heavy chain locus) (eg, leukemias and lymphomas, B-cell), gene rearrangement analysis to detect abnormal clonal population(s); amplified methodology (eg, polymerase chain reaction)
81262	IGH@ (Immunoglobulin heavy chain locus) (eg, leukemias and lymphomas, B-cell), gene rearrangement analysis to detect abnormal clonal population(s); direct probe methodology (eg, Southern blot)
81263	IGH@ (Immunoglobulin heavy chain locus) (eg, leukemia and lymphoma, B-cell), variable region somatic mutation analysis
81272	KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) (eg, gastrointestinal stromal tumor [GIST], acute myeloid leukemia, melanoma), gene analysis, targeted sequence analysis (eg, exons 8, 11, 13, 17, 18)
81273	KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) (eg, mastocytosis), gene analysis, D816 variant(s)
81310	NPM1 (nucleophosmin) (eg, acute myeloid leukemia) gene analysis, exon 12 variants
81334	RUNX1 (runt related transcription factor 1) (eg, acute myeloid leukemia, familial platelet disorder with associated myeloid malignancy) gene analysis, targeted sequence analysis (eg, exons 3-8)
81351	TP53 (tumor protein 53) (eg, Li-Fraumeni syndrome) gene analysis; full gene sequence
83152	TP53 (tumor protein 53) (eg, Li-Fraumeni syndrome) gene analysis; targeted sequence analysis (eg, 4 oncology)
81401	Molecular pathology procedure, Level 2 (eg, 2-**10** SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)
81403	Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons) Gene: *DNMT3A*
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis) Genes: *WT1, TP53*
81450	Hematolymphoid neoplasm or disorder, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants, and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis

CPT	Code Description
81451	Hematolymphoid neoplasm or disorder, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants, and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis
81455	Solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes, genomic sequence analysis panel, interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis
81479	Unlisted molecular pathology procedure
88237	Tissue culture for neoplastic disorders; bone marrow, blood cells
88271	Molecular cytogenetics; DNA probe, each (eg, FISH)
88272	Molecular cytogenetics; chromosomal in situ hybridization, analyze 3-5 cells (eg, for derivatives and markers)
88273	Molecular cytogenetics; chromosomal in situ hybridization, analyze 10-30 cells (eg, for microdeletions)
88274	Molecular cytogenetics; interphase in situ hybridization, analyze 25-99 cells
88275	Molecular cytogenetics; interphase in situ hybridization, analyze 100-300 cells
88291	Cytogenetics and molecular cytogenetics, interpretation and report
0016U	Oncology (hematolymphoid neoplasia), RNA, BCR/ABL1 major and minor breakpoint fusion transcripts, quantitative PCR amplification, blood or bone marrow, report of fusion not detected or detected with quantitation Proprietary test: QuantideX® Lab/Manufacturer: University of Iowa, Department of Pathology, Asuragen
0023U	Oncology (acute myelogenous leukemia), DNA, genotyping of internal tandem duplication, p.D835, p.I836, using mononuclear cells, reported as detection or non-detection of FLT3 mutation and indication for or against the use of midostaurin Proprietary test: LeukoStrat® CDx *FLT3* Mutation Assay Lab/Manufacturer: LabPMM LLC/Invivoscribe Technologies, Inc.
0040U	BCR/ABL1 (t(9;22)) (eg, chronic myelogenous leukemia) translocation analysis, major breakpoint, quantitative Proprietary test: MRDx® Lab/Manufacturer: MolecularMD
0046U	FLT3 (fms-related tyrosine kinase 3) (eg, acute myeloid leukemia) internal tandem duplication (ITD) variants, quantitative Proprietary test: *FLT3* ITD MRD by NGS Lab/Manufacturer: LabPMM LLC/Invivoscribe Technologies, Inc.

CPT	Code Description
0049U	NPM1 (nucleophosmin) (eg, acute myeloid leukemia) gene analysis, quantitative Proprietary test: NPMI MRD by NGS Lab/Manufacturer: LabPMM LLC/Invivoscribe Technologies, Inc.
0413U	Oncology (hematolymphoid neoplasm), optical genome mapping for copy number alterations, aneuploidy, and balanced/complex structural rearrangements, DNA from blood or bone marrow, report of clinically significant alterations Proprietary test: DH Optical Genome Mapping/Digital Karyotyping Assay Lab/Manufacturer: Bionano Genomics

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

ACCC. (2022). *Hematologic Malignancies*. https://www.accc-cancer.org/home/learn/cancer-types/hematologic-malignancies

Ali, S., Jones, G. L., Culligan, D. J., Marsden, P. J., Russell, N., Embleton, N. D., & Craddock, C. (2015). Guidelines for the diagnosis and management of acute myeloid leukaemia in pregnancy. *Br J Haematol*, *170*(4), 487-495. https://doi.org/10.1111/bjh.13554

Alonso, C. M., Llop, M., Sargas, C., Pedrola, L., Panadero, J., Hervás, D., Cervera, J., Such, E., Ibáñez, M., Ayala, R., Martínez-López, J., Onecha, E., de Juan, I., Palanca, S., Martínez-Cuadrón, D., Rodríguez-Veiga, R., Boluda, B., Montesinos, P., Sanz, G., . . . Barragán, E. (2019). Clinical Utility of a Next-Generation Sequencing Panel for Acute Myeloid Leukemia Diagnostics. *J Mol Diagn*, *21*(2), 228-240. https://doi.org/10.1016/j.jmoldx.2018.09.009

Amin, N. A., & Malek, S. N. (2016). Gene mutations in chronic lymphocytic leukemia. *Semin Oncol*, *43*(2), 215-221. https://doi.org/10.1053/j.seminoncol.2016.02.002

Ampasavate, C., Jutapakdee, W., Phongpradist, R., Tima, S., Tantiworawit, A., Charoenkwan, P., Chinwong, D., & Anuchapreeda, S. (2019). FLT3, a prognostic biomarker for acute myeloid leukemia (AML): Quantitative monitoring with a simple anti-FLT3 interaction and flow cytometric method. *J Clin Lab Anal*, *33*(4), e22859. https://doi.org/10.1002/jcla.22859

Arber, D. A., Borowitz, M. J., Cessna, M., Etzell, J., Foucar, K., Hasserjian, R. P., Rizzo, J. D., Theil, K., Wang, S. A., Smith, A. T., Rumble, R. B., Thomas, N. E., & Vardiman, J. W. (2017). Initial Diagnostic Workup of Acute Leukemia: Guideline From the College of

American Pathologists and the American Society of Hematology. *Arch Pathol Lab Med*, *141*(10), 1342-1393. https://doi.org/10.5858/arpa.2016-0504-CP

Arber, D. A., Orazi, A., Hasserjian, R., Thiele, J., Borowitz, M. J., Le Beau, M. M., Bloomfield, C. D., Cazzola, M., & Vardiman, J. W. (2016). The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*, *127*(20), 2391-2405. https://doi.org/10.1182/blood-2016-03-643544

Bolouri, H., Farrar, J. E., Triche, T., Jr., Ries, R. E., Lim, E. L., Alonzo, T. A., Ma, Y., Moore, R., Mungall, A. J., Marra, M. A., Zhang, J., Ma, X., Liu, Y., Liu, Y., Auvil, J. M. G., Davidsen, T. M., Gesuwan, P., Hermida, L. C., Salhia, B., . . . Meshinchi, S. (2018). The molecular landscape of pediatric acute myeloid leukemia reveals recurrent structural alterations and age-specific mutational interactions. *Nat Med*, *24*(1), 103-112. https://doi.org/10.1038/nm.4439

Brown, J. T., Beldorth, I. J., Laosinchai-Wolf, W., Fahey, M. E., Jefferson, K. L., Ruskin, A. K., Roth, J. J., Cai, L., Watt, C. D., Press, R. D., Yang, F., Hedges, J. B., & Andruss, B. F. (2019). Analytical Validation of a Highly Sensitive, Multiplexed Chronic Myeloid Leukemia Monitoring System Targeting BCR-ABL1 RNA. *The Journal of molecular diagnostics : JMD*, *21*(4), 718-733. https://doi.org/10.1016%2Fj.jmoldx.2019.03.002

Castells, M. C., & Akin, C. (2021, July 15, 2021). *Mastocytosis (cutaneous and systemic) in adults: Epidemiology, pathogenesis, clinical manifestations, and diagnosis*. UpToDate. https://www.uptodate.com/contents/mastocytosis-cutaneous-and-systemic-in-adults-epidemiology-pathogenesis-clinical-manifestations-and-diagnosis

CGARN. (2013). Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med*, *368*(22), 2059-2074. https://doi.org/10.1056/NEJMoa1301689

D'Adda, M., Farina, M., Schieppati, F., Borlenghi, E., Bottelli, C., Cerqui, E., Ferrari, S., Gramegna, D., Pagani, C., Passi, A., Maifredi, A., Tucci, A., Capucci, M. A., Ruggeri, G., & Rossi, G. (2019). The e13a2 BCR-ABL transcript negatively affects sustained deep molecular response and the achievement of treatment-free remission in patients with chronic myeloid leukemia who receive tyrosine kinase inhibitors. *Cancer*, *0*(0). https://doi.org/10.1002/cncr.31977

de Haas, V., Ismaila, N., & Zhang, L. (2019). Initial Diagnostic Workup of Acute Leukemia: ASCO Clinical Practice Guideline Endorsement Summary of the CAP and ASH Guideline. *J Oncol Pract*, *15*(2), 101-105. https://doi.org/10.1200/jop.18.00613

Dennis, M., Copland, M., Kaur, H., Kell, J., Nikolousis, E., Mehta, P., Palanicawandar, R., Potter, V., Raj, K., Thomas, I., & Wilson, A. (2022). Management of older patients with frailty and acute myeloid leukaemia: A British Society for Haematology good practice paper. *Br J Haematol*, *199*(2), 205-221. https://doi.org/10.1111/bjh.18369

Devillier, R., Mansat-De Mas, V., Gelsi-Boyer, V., Demur, C., Murati, A., Corre, J., Prebet, T., Bertoli, S., Brecqueville, M., Arnoulet, C., Recher, C., Vey, N., Mozziconacci, M. J., Delabesse, E., & Birnbaum, D. (2015). Role of ASXL1 and TP53 mutations in the

molecular classification and prognosis of acute myeloid leukemias with myelodysplasia-related changes. *Oncotarget*, *6*(10), 8388-8396. https://doi.org/10.18632/oncotarget.3460

DeZern, A. E., Sung, A., Kim, S., Smith, B. D., Karp, J. E., Gore, S. D., Jones, R. J., Fuchs, E., Luznik, L., McDevitt, M., & Levis, M. (2011). Role of allogeneic transplantation for FLT3/ITD acute myeloid leukemia: outcomes from 133 consecutive newly diagnosed patients from a single institution. *Biol Blood Marrow Transplant*, *17*(9), 1404-1409. https://doi.org/10.1016/j.bbmt.2011.02.003

Döhner, H., Wei, A. H., Appelbaum, F. R., Craddock, C., DiNardo, C. D., Dombret, H., Ebert, B. L., Fenaux, P., Godley, L. A., Hasserjian, R. P., Larson, R. A., Levine, R. L., Miyazaki, Y., Niederwieser, D., Ossenkoppele, G., Röllig, C., Sierra, J., Stein, E. M., Tallman, M. S., . . . Löwenberg, B. (2022). Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood*, *140*(12), 1345-1377. https://doi.org/10.1182/blood.2022016867

Duncavage, E. J., Schroeder, M. C., O'Laughlin, M., Wilson, R., MacMillan, S., Bohannon, A., Kruchowski, S., Garza, J., Du, F., Hughes, A. E. O., Robinson, J., Hughes, E., Heath, S. E., Baty, J. D., Neidich, J., Christopher, M. J., Jacoby, M. A., Uy, G. L., Fulton, R. S., . . . Spencer, D. H. (2021). Genome Sequencing as an Alternative to Cytogenetic Analysis in Myeloid Cancers. *N Engl J Med*, *384*(10), 924-935. https://doi.org/10.1056/NEJMoa2024534

Eichhorst, B., Robak, T., Montserrat, E., Ghia, P., Niemann, C. U., Kater, A. P., Gregor, M., Cymbalista, F., Buske, C., Hillmen, P., Hallek, M., & Mey, U. (2021). Chronic lymphocytic leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, *32*(1), 23-33. https://doi.org/10.1016/j.annonc.2020.09.019

Falini, B., Mecucci, C., Tiacci, E., Alcalay, M., Rosati, R., Pasqualucci, L., La Starza, R., Diverio, D., Colombo, E., Santucci, A., Bigerna, B., Pacini, R., Pucciarini, A., Liso, A., Vignetti, M., Fazi, P., Meani, N., Pettirossi, V., Saglio, G., . . . Martelli, M. F. (2005). Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. *N Engl J Med*, *352*(3), 254-266. https://doi.org/10.1056/NEJMoa041974

FDA. (2016). *EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR QUANTIDEX QPCR BCR-ABL IS KIT DECISION SUMMARY*. U.S. Food and Drug Administration Retrieved from https://www.accessdata.fda.gov/cdrh_docs/reviews/DEN160003.pdf

FDA. (2017a). *Premarket Approval (PMA) for LeukoStrat CDx FLT3 Mutation Assay*. U.S. Food and Drug Administration Retrieved from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P160040

FDA. (2017b). *Premarket approval order for Abbott RealTime IDH2*. U.S. Food and Drug Administration Retrieved from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P170005

Frucht, H., & Lucas, A. L. (2023, December 8, 2023). *Molecular genetics of colorectal cancer*. UpToDate. https://www.uptodate.com/contents/molecular-genetics-of-colorectal-cancer

Greenfield, G., McMullan, R., Robson, N., McGimpsey, J., Catherwood, M., & McMullin, M. F. (2019). Response to Imatinib therapy is inferior for e13a2 BCR-ABL1 transcript type in comparison to e14a2 transcript type in chronic myeloid leukaemia. *BMC Hematol*, *19*, 7. https://doi.org/10.1186/s12878-019-0139-2

Hallek, M., Cheson, B. D., Catovsky, D., Caligaris-Cappio, F., Dighiero, G., Döhner, H., Hillmen, P., Keating, M., Montserrat, E., Chiorazzi, N., Stilgenbauer, S., Rai, K. R., Byrd, J. C., Eichhorst, B., O'Brien, S., Robak, T., Seymour, J. F., & Kipps, T. J. (2018). iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. *Blood*, *131*(25), 2745-2760. https://doi.org/10.1182/blood-2017-09-806398

Heiblig, M., Sujobert, P., Hayette, S., Balsat, M., Elhamri, M., Salles, G., & Thomas, X. (2019). Impact of NPM1 mutation subtypes on treatment outcome in AML: The Lyon-University Hospital experience. *Leuk Res*, *76*, 29-32. https://doi.org/10.1016/j.leukres.2018.11.016

Heuser, M., Freeman, S. D., Ossenkoppele, G. J., Buccisano, F., Hourigan, C. S., Ngai, L. L., Tettero, J. M., Bachas, C., Baer, C., Béné, M.-C., Bücklein, V., Czyz, A., Denys, B., Dillon, R., Feuring-Buske, M., Guzman, M. L., Haferlach, T., Han, L., Herzig, J. K., . . . Cloos, J. (2021). 2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party. *Blood*, *138*(26), 2753-2767. https://doi.org/10.1182/blood.2021013626

Heuser, M., Ofran, Y., Boissel, N., Brunet Mauri, S., Craddock, C., Janssen, J., Wierzbowska, A., & Buske, C. (2020). Acute myeloid leukaemia in adult patients: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, *31*(6), 697-712. https://doi.org/10.1016/j.annonc.2020.02.018

Hochhaus, A., Saussele, S., Rosti, G., Mahon, F. X., Janssen, J., Hjorth-Hansen, H., Richter, J., & Buske, C. (2017). Chronic myeloid leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, *28*(suppl_4), iv41-iv51. https://doi.org/10.1093/annonc/mdx219

Hoelzer, D., Bassan, R., Dombret, H., Fielding, J., Ribera, M., & Buske, C. (2016). *ESMO Consensus Guidelines for the Management of Patients with Metastatic Colorectal Cancer*. https://www.esmo.org/guidelines/haematological-malignancies/acute-lymphoblastic-leukaemia

Hou, H. A., Huang, T. C., Lin, L. I., Liu, C. Y., Chen, C. Y., Chou, W. C., Tang, J. L., Tseng, M. H., Huang, C. F., Chiang, Y. C., Lee, F. Y., Liu, M. C., Yao, M., Huang, S. Y., Ko, B. S., Hsu, S. C., Wu, S. J., Tsay, W., Chen, Y. C., & Tien, H. F. (2010). WT1 mutation in 470 adult patients with acute myeloid leukemia: stability during disease evolution and

implication of its incorporation into a survival scoring system. *Blood*, *115*(25), 5222-5231. https://doi.org/10.1182/blood-2009-12-259390

Iacobucci, I., & Mullighan, C. G. (2017). Genetic Basis of Acute Lymphoblastic Leukemia. *J Clin Oncol*, *35*(9), 975-983. https://doi.org/10.1200/jco.2016.70.7836

Jongen-Lavrencic, M., Grob, T., Hanekamp, D., Kavelaars, F. G., Al Hinai, A., Zeilemaker, A., Erpelinck-Verschueren, C. A. J., Gradowska, P. L., Meijer, R., Cloos, J., Biemond, B. J., Graux, C., van Marwijk Kooy, M., Manz, M. G., Pabst, T., Passweg, J. R., Havelange, V., Ossenkoppele, G. J., Sanders, M. A., . . . Valk, P. J. M. (2018). Molecular Minimal Residual Disease in Acute Myeloid Leukemia. *N Engl J Med*, *378*(13), 1189-1199. https://doi.org/10.1056/NEJMoa1716863

Khoury, J. D., Solary, E., Abla, O., Akkari, Y., Alaggio, R., Apperley, J. F., Bejar, R., Berti, E., Busque, L., Chan, J. K. C., Chen, W., Chen, X., Chng, W. J., Choi, J. K., Colmenero, I., Coupland, S. E., Cross, N. C. P., De Jong, D., Elghetany, M. T., . . . Hochhaus, A. (2022). The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. *Leukemia*, *36*(7), 1703-1719. https://doi.org/10.1038/s41375-022-01613-1

Kurzrock, R., Kantarjian, H. M., Druker, B. J., & Talpaz, M. (2003). Philadelphia chromosome-positive leukemias: from basic mechanisms to molecular therapeutics. *Ann Intern Med*, *138*(10), 819-830. http://thinkkimi.com/Work/philadelphia.pdf

Kuwatsuka, Y., Tomizawa, D., Kihara, R., Nagata, Y., Shiba, N., Iijima-Yamashita, Y., Shimada, A., Deguchi, T., Miyachi, H., Tawa, A., Taga, T., Kinoshita, A., Nakayama, H., Kiyokawa, N., Saito, A. M., Koh, K., Goto, H., Kosaka, Y., Asou, N., . . . Kiyoi, H. (2018). Prognostic value of genetic mutations in adolescent and young adults with acute myeloid leukemia. *Int J Hematol*, *107*(2), 201-210. https://doi.org/10.1007/s12185-017-2340-z

Leonard, D. G. B. (2016). *Molecular Pathology in Clinical Practice* (D. G. B. Leonard, Ed.). Springer. http://www.springer.com/us/book/9783319196732

Leoni, V., & Biondi, A. (2015). Tyrosine kinase inhibitors in BCR-ABL positive acute lymphoblastic leukemia. *Haematologica*, *100*(3), 295-299. https://doi.org/10.3324/haematol.2015.124016

Li, W. (2022). The 5(th) Edition of the World Health Organization Classification of Hematolymphoid Tumors. In W. Li (Ed.), *Leukemia*. Exon Publications. https://doi.org/10.36255/exon-publications-leukemia-who-5th-edition-hematolymphoid-tumors

Mehta, P., Telford, N., Wragg, C., Dillon, R., Freeman, S., Finnegan, D., Hamblin, A., Copland, M., & Knapper, S. (2023). Recommendations for laboratory testing of UK patients with acute myeloid leukaemia. *Br J Haematol*, *200*(2), 150-159. https://doi.org/10.1111/bjh.18516

NCBI. (2023a). *Lymphoid Neoplasm*. https://www.ncbi.nlm.nih.gov/medgen/108626

NCBI. (2023b). *Myeloid Neoplasm*. https://www.ncbi.nlm.nih.gov/medgen/445430

NCCN. (2023, December 5, 2023). *NCCN Clinical Practice Guidelines in Oncology: Chronic Myeloid Leukemia Version 2.2024 — December 5, 2023*. https://www.nccn.org/professionals/physician_gls/pdf/cml.pdf

NCCN. (2024a, March 26, 2024). *Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma Version 3.2024 — March 26, 2024*. https://www.nccn.org/professionals/physician_gls/pdf/cll.pdf

NCCN. (2024b). *NCCN Clinical Practice Guidelines in Oncology Pediatric Acute Lymphoblastic Leukemia Version 1.2024*. National Comprehensive Cancer Network. https://www.nccn.org/professionals/physician_gls/pdf/ped_all.pdf

NCCN. (2024c, February 5, 2024). *NCCN Clinical Practice Guidelines in Oncology: Acute Lymphoblastic Leukemia Version 4.2023 — February 05, 2024*. https://www.nccn.org/professionals/physician_gls/pdf/all.pdf

NCCN. (2024d, March 22, 2024). *NCCN Clinical Practice Guidelines in Oncology; Acute Myeloid Leukemia Version 2.2024 — March 22, 2024*. https://www.nccn.org/professionals/physician_gls/pdf/aml.pdf

Nowell, P., & Hungerford, D. (1960). A minute chromosome in human granulocytic leukemia. *Science*, 1497. https://doi.org/10.1126/science.132.3438.1488

Papaemmanuil, E., Gerstung, M., Bullinger, L., Gaidzik, V. I., Paschka, P., Roberts, N. D., Potter, N. E., Heuser, M., Thol, F., Bolli, N., Gundem, G., Van Loo, P., Martincorena, I., Ganly, P., Mudie, L., McLaren, S., O'Meara, S., Raine, K., Jones, D. R., . . . Campbell, P. J. (2016). Genomic Classification and Prognosis in Acute Myeloid Leukemia. *N Engl J Med*, *374*(23), 2209-2221. https://doi.org/10.1056/NEJMoa1516192

Pastore, F., Greif, P. A., Schneider, S., Ksienzyk, B., Mellert, G., Zellmeier, E., Braess, J., Sauerland, C. M., Heinecke, A., Krug, U., Berdel, W. E., Buechner, T., Woermann, B., Hiddemann, W., & Spiekermann, K. (2014). The NPM1 mutation type has no impact on survival in cytogenetically normal AML. *PLoS One*, *9*(10), e109759. https://doi.org/10.1371/journal.pone.0109759

PDQ. (2023). *Adult Acute Lymphoblastic Leukemia Treatment (PDQ®)*. https://www.ncbi.nlm.nih.gov/books/NBK65727/

Pui, C. H. (2011). Recent advances in acute lymphoblastic leukemia. *Oncology (Williston Park)*, *25*(4), 341, 346-347. https://www.cancernetwork.com/hematologic-malignancies/recent-advances-acute-lymphoblastic-leukemia

Rai, K. R., & Stilgenbauer, S. (2023, August 31, 2023). *Pathobiology of chronic lymphocytic leukemia*. UpToDate. https://www.uptodate.com/contents/pathobiology-of-chronic-lymphocytic-leukemia

Rai, K. R., Stilgenbauer, S., & Aster, J. C. (2024, January 26, 2024). *Clinical features and diagnosis of chronic lymphocytic leukemia/small lymphocytic lymphoma*. UpToDate. https://www.uptodate.com/contents/clinical-features-and-diagnosis-of-chronic-lymphocytic-leukemia-small-lymphocytic-lymphoma

Ren, R. (2005). Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukaemia. *Nat Rev Cancer*, *5*(3), 172-183. https://doi.org/10.1038/nrc1567

Sasaki, K., Kanagal-Shamanna, R., Montalban-Bravo, G., Assi, R., Jabbour, E., Ravandi, F., Kadia, T., Pierce, S., Takahashi, K., Nogueras Gonzalez, G., Patel, K., Soltysiak, K. A., Cortes, J., Kantarjian, H. M., & Garcia-Manero, G. (2020). Impact of the variant allele frequency of ASXL1, DNMT3A, JAK2, TET2, TP53, and NPM1 on the outcomes of patients with newly diagnosed acute myeloid leukemia. *Cancer*, *126*(4), 765-774. https://doi.org/10.1002/cncr.32566

Schiffer, C., & Uy, G. (2024, January 19, 2024). *Acute myeloid leukemia: Risk factors and prognosis*. https://www.uptodate.com/contents/acute-myeloid-leukemia-risk-factors-and-prognosis

Schiffer, C. A., & Gurbuxani, S. (2024, February 26, 2024). *Acute myeloid leukemia: Clinical manifestations, pathologic features, and diagnosis*. https://www.uptodate.com/contents/acute-myeloid-leukemia-clinical-manifestations-pathologic-features-and-diagnosis

Schrijver, I., & Zehnder, J. L. (2022, April 15, 2022). *Genetic abnormalities in hematologic and lymphoid malignancies*. https://www.uptodate.com/contents/genetic-abnormalities-in-hematologic-and-lymphoid-malignancies

Siegel, R. L., Miller, K. D., & Jemal, A. (2017). Cancer Statistics, 2017. *CA Cancer J Clin*, *67*(1), 7-30. https://doi.org/10.3322/caac.21387

Sperr, W. R., Zach, O., Poll, I., Herndlhofer, S., Knoebl, P., Weltermann, A., Streubel, B., Jaeger, U., Kundi, M., & Valent, P. (2016). Karyotype plus NPM1 mutation status defines a group of elderly patients with AML (>/=60 years) who benefit from intensive post-induction consolidation therapy. *Am J Hematol*, *91*(12), 1239-1245. https://doi.org/10.1002/ajh.24560

Stock, W., & Thirman, M. J. (2022, April 18, 2022). *Acute myeloid leukemia: Molecular genetics*. https://www.uptodate.com/contents/acute-myeloid-leukemia-molecular-genetics

Tachibana, T., Kondo, T., Uchida, N., Doki, N., Takada, S., Takahashi, S., Yano, S., Mori, T., Kohno, A., Kimura, T., Fukuda, T., Atsuta, Y., Nagamura-Inoue, T., & on behalf of the adult, C. w. g. o. t. J. S. f. T. a. C. T. (2022). The clinical significance of BCR-ABL1 mutations in patients with Philadelphia chromosome-positive chronic myeloid leukemia who underwent allogeneic hematopoietic cell transplantation. *Transplantation and Cellular Therapy*. https://doi.org/10.1016/j.jtct.2022.03.009

Van Etten, R. A. (2022, May 31, 2022). *Clinical manifestations and diagnosis of chronic myeloid leukemia*. https://www.uptodate.com/contents/clinical-manifestations-and-diagnosis-of-chronic-myeloid-leukemia

Van Etten, R. A. (2023, July 31, 2023). *Cellular and molecular biology of chronic myeloid leukemia*. https://www.uptodate.com/contents/cellular-and-molecular-biology-of-chronic-myeloid-leukemia

Vosberg, S., Hartmann, L., Metzeler, K. H., Konstandin, N. P., Schneider, S., Varadharajan, A., Hauser, A., Krebs, S., Blum, H., Bohlander, S. K., Hiddemann, W., Tischer, J., Spiekermann, K., & Greif, P. A. (2018). Relapse of acute myeloid leukemia after allogeneic stem cell transplantation is associated with gain of WT1 alterations and high mutation load. *Haematologica*, *103*(12), e581-e584. https://doi.org/10.3324/haematol.2018.193102

Warmuth, M., Danhauser-Riedl, S., & Hallek, M. (1999). Molecular pathogenesis of chronic myeloid leukemia: implications for new therapeutic strategies. *Ann Hematol*, *78*(2), 49-64. https://doi.org/10.1007/s002770050473

Willemze, R., Suciu, S., Meloni, G., Labar, B., Marie, J. P., Halkes, C. J., Muus, P., Mistrik, M., Amadori, S., Specchia, G., Fabbiano, F., Nobile, F., Sborgia, M., Camera, A., Selleslag, D. L., Lefrere, F., Sr., Magro, D., Sica, S., Cantore, N., . . . de Witte, T. (2014). High-dose cytarabine in induction treatment improves the outcome of adult patients younger than age 46 years with acute myeloid leukemia: results of the EORTC-GIMEMA AML-12 trial. *J Clin Oncol*, *32*(3), 219-228. https://doi.org/10.1200/jco.2013.51.8571

Xu, J., Zhang, Y., Hu, J., Ren, Y., & Wang, H. (2020). Clinical features and prognosis of normal karyotype acute myeloid leukemia pediatric patients with WT1 mutations: an analysis based on TCGA database. *Hematology*, *25*(1), 79-84. https://doi.org/10.1080/16078454.2020.1720102

Yamamoto, J. F., & Goodman, M. T. (2008). Patterns of leukemia incidence in the United States by subtype and demographic characteristics, 1997-2002. *Cancer Causes Control*, *19*(4), 379-390. https://doi.org/10.1007/s10552-007-9097-2

Yeung, C. C., Egan, D., & Radich, J. P. (2016). Molecular monitoring of chronic myeloid leukemia: present and future. *Expert Rev Mol Diagn*, *16*(10), 1083-1091. https://doi.org/10.1080/14737159.2016.1227243

Yui, S., Kurosawa, S., Yamaguchi, H., Kanamori, H., Ueki, T., Uoshima, N., Mizuno, I., Shono, K., Usuki, K., Chiba, S., Nakamura, Y., Yanada, M., Kanda, J., Tajika, K., Gomi, S., Fukunaga, K., Wakita, S., Ryotokuji, T., Fukuda, T., & Inokuchi, K. (2017). D816 mutation of the KIT gene in core binding factor acute myeloid leukemia is associated with poorer prognosis than other KIT gene mutations. *Ann Hematol*, *96*(10), 1641-1652. https://doi.org/10.1007/s00277-017-3074-y

Zhu, Y. M., Wang, P. P., Huang, J. Y., Chen, Y. S., Chen, B., Dai, Y. J., Yan, H., Hu, Y., Cheng, W. Y., Ma, T. T., Chen, S. J., & Shen, Y. (2017). Gene mutational pattern and expression level in 560 acute myeloid leukemia patients and their clinical relevance. *J Transl Med*, *15*(1), 178. https://doi.org/10.1186/s12967-017-1279-4

Liquid Biopsy

Policy Number: AHS – G2054 – Liquid Biopsy	Prior Policy Name and Number, as applicable: • AHS-G2054 Detection of Circulating Tumor Cells and Cell Free DNA in Cancer Management • Consolidated: AHS-M2140 – Liquid Biopsy
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

The National Cancer Institute (NCI) defines "liquid biopsy" as a test done on a sample of blood, urine, or other bodily fluid to look for cancer cells from a tumor or small pieces of DNA, RNA, or other molecules released by tumor cells into a person's body fluids. Liquid biopsies are non-invasive blood tests since circulating tumor cells (CTCs) and cell-free tumor DNA (cfDNA) fragments are shed into the bloodstream from existing tumors and can be detected in blood (Curigliano, 2014; Haber & Velculescu, 2014). The presence of CTCs can be indicative of metastatic disease (Alix-Panabieres & Pantel, 2013).

For guidance concerning Tumor Mutational Burden Testing (TMB) and/or Microsatellite instability (MSI) analysis please refer to AHS-M2178-Microsatellite Instability and Tumor Mutational Burden Testing policy.

II. Related Policies

Policy Number	Policy Title
AHS-G2125	Urinary Tumor Markers for Bladder Cancer
AHS-M2160	Molecular Testing of Pulmonary Specimens
AHS-M2166	Gene Expression Profiling and Protein Biomarkers for Prostate
AHS-M2168	Proteogenomic Testing of Individuals with Cancer
AHS-M2178	Microsatellite Instability and Tumor Mutational Burden Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals diagnosed with non-small cell lung cancer (NSCLC), cell-free DNA/circulating tumor DNA (cfDNA/ctDNA) testing **MEETS COVERAGE CRITERIA** in **any** of the following situations:
   a) When tissue-based testing is infeasible (i.e., quantity not sufficient for tissue-based test or invasive biopsy is medically contraindicated).
   b) In the initial diagnostic setting when there is insufficient tissue to allow testing for broad molecular analysis following pathological confirmation of NSCLC (if an oncogenic driver is not identified, follow-up tissue-based analysis should be considered).
   c) In the initial diagnostic setting when tissue-based molecular analysis does not completely assess all recommended biomarkers due to tissue quantity or testing methodologies available. Recommended biomarkers include:
      i) *ALK* rearrangements.
      ii) *BRAF* mutations.
      iii) *EGFR* mutations.
      iv) *ERBB2* (*HER2*) mutations.
      v) *KRAS* mutations.
      vi) *MET*ex14 skipping mutations.
      vii) *NTRK1/2/3* fusions.
      viii) *RET* rearrangements.
      ix) *ROS1* rearrangements.
      x) PD-L1 expression levels.
   d) To aid in biomarker evaluation for treatment selection in the initial diagnostic setting (when the feasibility of timely tissue-based testing is uncertain).
2) For individuals diagnosed with HR-positive/HER2-negative breast cancer and who are being considered for targeted therapy, cfDNA/ctDNA testing for *PIK3CA* **MEETS COVERAGE CRITERIA**.

3) For individuals diagnosed with castration-resistant prostate cancer, cfDNA/ctDNA testing of the following biomarkers **MEETS COVERAGE CRITERIA**:
   a) Androgen receptor variant 7 (AR-V2) to guide therapy selection in the post-abiraterone/enzalutamide metastatic CRPC setting.
   b) Somatic analysis of *BRCA1* and *BRCA2* to select patients for rucaparib treatment.

4) For individuals meeting the above criteria, cfDNA/ctDNA testing (annually) **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

5) For all other situations not described above, liquid biopsy testing for screening, detecting, and/or monitoring any other malignancy or tumor **DOES NOT MEET COVERAGE CRITERIA**.

6) For all situations not addressed above, analysis of PD-L1 by liquid biopsy **DOES NOT MEET COVERAGE CRITERIA**.

7) For the screening, detection, and/or diagnosis of cancer, urinary liquid biopsy (i.e., use of cell-free DNA ["UcfDNA"] or circulating tumor DNA obtained in a urine sample) (e.g., SelectMDX) **DOES NOT MEET COVERAGE CRITERIA**.

8) Liquid biopsy testing on CSF samples **DOES NOT MEET COVERAGE CRITERIA**.

9) Cell capture-enumeration assays of CTCs (e.g., CELLSEARCH® CTC) **DO NOT MEET COVERAGE CRITERIA**.

IV. Table of Terminology

Term	Definition
AACC	American Association for Clinical Chemistry
ALK	*Anaplastic lymphoma receptor tyrosine kinase*
AMP	The Association for Molecular Pathology
AR	*Androgen receptor*
AR-V7	*Androgen receptor splice variant 7*
ASCO	American Society of Clinical Oncology
BRAF	*B-Raf proto-oncogene*
BRCA1	*Breast cancer type 1 susceptibility gene*
BRCA1/2	*Breast cancer type 1/2 susceptibility gene*
BRCA2	*Breast cancer type 2 susceptibility gene*

CAM	Cell adhesion molecule
CAP	College of American Pathologists
CF	Cell-free
cfDNA	Cell-free tumour deoxyribonucleic acid
CGP	Comprehensive somatic genomic profiling
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
c-MET	*Cellular mesenchymal epithelial transition*
CNS	Central nervous system
CRC	Colorectal cancer
CRPC	Castration-resistant prostate cancer
CSCO	Chinese Society of Clinical Oncology
CSF	Cerebrospinal fluid
CSF-CTC	Circulating tumor Cells in cerebrospinal fluid
CTCs	Circulating tumor cells
ctDNA	Circulating tumor deoxyribonucleic acid
CTLA-3	Cytotoxic T-lymphocyte-associated protein 3
DLX1	Distal-less 1
DNA	Deoxyribonucleic acid
DRE	Digital rectal examination
EAU	European Association of Urology
EGFR	*Epidermal growth factor receptor*
EpCAM	Epithelial cell adhesion molecule
ER+ MBCs	Estrogen receptor-positive metastatic breast cancer
ERCC1	*Excision repair cross-complementation group 1*
ER-CTCs	Estrogen receptor-negative circulating tumor cells
ESMO	European Society for Medical Oncology
ESTRO	European Society of Urogenital Radiology
EV	Extracellular vesicle
ExoRNA	Exosome ribonucleic acid
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
GC	Gastric cancer
gDNA	Genomic deoxyribonucleic acid
HCC	Hierarchical condition category
HDL	High-density lipoprotein
hENT1	*Human equilibrative nucleoside transporter 1*
HER2	Human epidermal growth factor receptor 2
HOXC6	Homeobox C6
IASLC	International Association for the Study of Lung Cancer

InDels	Insertions/Deletions
KLK3	*Kallikreins 3*
KRAS	*Kirsten rat sarcoma viral oncogene homolog*
LAG-3	Lymphocyte-activation gene 3
LM	Leptomeningeal metastasis
MDX	Molecular diagnostics
MET	*MET Proto-Oncogene*
MRI	Myotubularin 1
mRNA	Messenger ribonucleic acid
MSI	Microsatellite instability
MSI-H	Microsatellite instability-high
NACB	National Academy of Clinical Biochemistry
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NGS	Next-generation sequencing
NIH	National Institute of Health
NK	Natural killer
NRAS	*Neuroblastoma rat sarcoma*
NSCLC	Non-small cell lung cancer
PCR	Polymerase chain reaction
PD-L1	Programmed death-ligand 1
PFS	Progression-free survival
PIK3CA	*Phosphatidylinositol 3-Kinase*
PSA	Prostate-specific antigen
RET	*Rearranged during transfection*
RGQ	Rapid gas quenching
RNA	Ribonucleic acid
RNases	Ribonucleases
RRM1	*Ribonucleotide reductase, M1 subunit*
RT-PCR	Reverse transcriptase polymerase chain reaction
SIOG	International Society of Geriatric Oncology
SNVs	Single nucleotide variants
tDNA	Tissue deoxyribonucleic acid
TEXs	Tumor-derived exosomes
TIM-3	T cell immunoglobulin and mucin-domain containing-3
TKIs	Tyrosine kinase inhibitors
TMB	Tumor mutational burden
TOP1	*DNA topoisomerase 1*
TOP2A	*DNA topoisomerase 2 alpha*

TOP2B	*DNA topoisomerase 2 beta*
TP	*Tumour protein*
TUBB3	*Tubulin beta 3 class III*
UcfDNA	Urinary cell-free deoxyribonucleic acid
utDNA	Urine-derived tumor deoxyribonucleic acid
XRCC1	*X-ray repair cross-complementing 1*

V. Scientific Background

The science of noninvasive disease monitoring has advanced greatly since circulating cell-free DNA (cfDNA) was first reported in body fluids by Mandel and Metais. Since then, the evolution of sensitive cfDNA detection technologies has enabled the development of liquid biopsies with many clinical applications. For example, in oncology, the use of liquid biopsy allows for patient stratification, screening, monitoring treatment response and detection of minimal residual disease after surgery or recurrence. Liquid biopsies have grown in importance because the genetic profile of tumors can affect how well patients respond to a certain treatment. However, this characterization is currently achieved through a biopsy despite the inherent problems in procurement of tissue samples and the limitations of tumor analyses. For example, the invasive nature of a biopsy poses a risk to patients and can have a significant cost (Brock et al., 2015).

Tumor sampling from some cancer types also remains difficult resulting in inadequate amount of tissue for genetic testing (Brock et al., 2015). In the case of advanced or metastatic non-small cell lung cancers (NSCLC), as many as 69% of cases do not have accessible tissue (Douillard et al., 2009). Even when tissue can be collected, preservation methods such as formalin fixation can cause false positive results for genetic tests (Quach et al., 2004). Finally, due to tumor heterogeneity, biopsies often suffer from sample bias (Bedard et al., 2013). Liquid biopsies are becoming more popular as they provide an opportunity to genotype in a less invasive and expensive manner. However, the low sensitivity (between 60-80%) and higher number of false negative cases compared to traditional tissue biopsy are limitations associated with liquid biopsies (Sequist & Neal, 2024).

Approaches to Liquid Biopsy Analysis

Circulating tumor cells (CTCs)

According to Brock et al. (2015), CTCs are cells shed into the vasculature from a primary tumor and may constitute seeds for subsequent growth of additional tumors (metastasis) in distant organs. CTCs generally confer the advantage of containing RNA, DNA, and protein from tumor cells including both nuclear and cytoplasmic biomarkers,

which is not attainable from ctDNA or exosomes (Yu et al., 2021). They have been detected in various metastatic carcinomas (Mavroudis, 2010) but are extremely rare in healthy subjects and patients with nonmalignant diseases (Brock et al., 2015). Clinical evidence indicates that patients with metastatic lesions are more likely to have CTCs amenable to isolation but their frequency is low, often ~1-10 CTCs per mL of whole blood (Miller et al., 2010). As one mL of blood contains ~7×10^6 white blood cells and ~5×10^9 red blood cells, technologies capable of reproducibly isolating a single CTC from the background of all other blood components are fundamental. While such levels of sensitivity are challenging, there are several novel developments in this area, including positive selection, negative selection, physical properties or even enrichment-free assays to efficiently isolate these rare CTCs (Alix-Panabieres & Pantel, 2013). However, Bettegowda et al. (2014)) stated that an advantage of ctDNA is that it can be analyzed from bio-banked biofluids, such as frozen plasma (Bettegowda et al., 2014).

Typically, CTCs are defined as cells with an intact viable nucleus, cytokeratin positive, epithelial cell adhesion molecule (EpCAM) positive and with the absence of CD45 (Brock et al., 2015). Unfortunately, EpCAM and other markers are not always expressed on CTCs (Grover et al., 2014). In addition, non-tumor epithelial cells are known to circulate in the blood of patients with prostatitis or patients undergoing surgery (Brock et al., 2015; Murray et al., 2013). The heterogeneity of CTCs is a major challenge from a technical standpoint. This has led to alternative strategies of CTC enrichment such as the CTC-iChip which does not rely on tumor antigen expression (Brock et al., 2015; Karabacak et al., 2014).

Sequencing the genetic material from CTCs has demonstrated that the majority are not cancer cells, even when the isolated cell(s) fit the phenotypic criteria of being a CTC. One study by Marchetti et al. (2014) developed a protocol to recover the CTC enriched samples from the cartridge of the Veridex platform and found that from 37 NSCLC patients, the EGFR mutation allele abundance ranged between 0.02% and 24.79% with a mean of 6.34%. Brock et al. (2015) concluded that the number of CTCs found in the blood is therefore highly dependent on how the platform defines a cell as a CTC (Brock et al., 2015; Marchetti et al., 2014). The CellSearch CTC test, a Food and Drug Administration (FDA) approved actionable CTC test, requires that samples are processed within 96 hours of collection after being drawn into the *Cellsave* preservative tube. This test does not analyze the molecular genetics of the tumor; rather *Cellsave* is a platform for CTC numeration. A positive test (more than five detected CTCs for metastatic breast and prostate cancer and more than three CTCs for metastatic colorectal cancer per 7.5 mL of blood) is associated with decreased progression-free survival and decreased overall survival in these patients (Aggarwal et al., 2013).

Overall, although CTCs have produced some promising results in evaluating prognosis of patients with varying cancers, further studies are needed to assess the clinical utility of these biomarkers (Adamczyk et al., 2015; Bidard et al., 2016; Foukakis & Bergh, 2022; Ignatiadis & Dawson, 2014).

Cell-free DNA (cfDNA)

There is currently an intensive research effort to understand the utility of cfDNA in various clinical fields, such as cancer research, non-invasive prenatal testing and transplant rejection diagnostics (Brock et al., 2015). In a systematic review and meta-analysis of 20 studies and 2012 cases covering assessment of EGFR mutational status in NSCLC, Luo et al. (2014) found a sensitivity of 0.674, a specificity of 0.935, and area under the curve of 0.93. The authors concluded that detection of EGFR mutation by cfDNA is of adequate diagnostic accuracy and cfDNA analysis could be a promising screening test for NSCLC (Luo et al., 2014).

In a study, Jiang et al. (2015)observed that most cfDNA in plasma is reportedly fragmented, around 150-180 bp in length with a higher prevalence of tumor associated mutations in the shorter fragments. Per authors, when analyzing the mutation abundance with massively parallel sequencing, a significant correlation was found between mutations and fragments less than 150 bp. Notably, the size of the majority of cfDNA fragments overlaps well with the size of histone DNA (Jiang et al., 2015).

A direct comparison of mutation detection on cfDNA *vs.* CTCs showed a higher abundance of the mutation on the cfDNA from the same patient; moreover, recent large studies comparing the effectiveness of cfDNA analysis to tissue biopsy in NSCLC showed the clinical value of the liquid biopsy approach (Douillard et al., 2014). This positive result led to an approval to use cfDNA analysis for *EGFR* mutation analysis for IRESSA in Europe (in patients where a tumor sample was not evaluable), making it the first *EGFR* tyrosine kinase inhibitor for which cfDNA testing is included in the label. Although promising, challenges remain when using cfDNA to characterize the mutation status of a tumor. In addition to the low copy number of mutant alleles, the median half-life of cfDNA in circulation ranges from 15 minutes to a few hours (Brock et al., 2015).

Brock et al. (2015), in their review, observed that the total concentration of cfDNA in the blood of cancer patients varies considerably with tumor specific mutations ranging from undetectable (less than one copy per five mL of plasma) to patients with over a hundred thousand copies of the mutation per mL of plasma. The authors note that "the challenge of how to maximize the yield of the cfDNA and pair this with a platform

sensitive enough to detect rare variants in the background of wild-type DNA remains. Optimally, the ability to detect mutations in plasma should not be limited to a subpopulation of patients with very high mutant copy numbers in circulation" (Brock et al., 2015). This has been proven to be challenging in early stage cancers (Yu et al., 2021).

While many analytical platforms report the mutation load with an allelic frequency compared to the wild-type DNA platforms relying solely on the allelic frequency without recording the number of mutations have limitations. This is because the allelic frequency of a gene is affected by the amount of wild-type DNA not related to the tumor. Therefore, it is important to consider the processes that affect the amount of wild-type DNA in circulation (Brock et al., 2015). For example, exercise increases cfDNA levels almost 10-fold (Breitbach et al., 2014). Other pre-analytical variables such as blood collection, the cellular process leading to its release, and extraction protocols affect the amount and size range of cfDNA fragments in a sample (Devonshire et al., 2014).

Exosomes

In the last few years, the exosome field has grown exponentially impacting various areas of research. Studies demonstrating that exosomes are actively released vesicles (carrying RNA, DNA, and protein) and can function as intercellular messengers. Yanez-Mo et al. (2015) highlights these developments in a review outlining the biological properties of exosomes and other extracellular vesicles (EVs). However, Gould and Raposo (2013) observed that the exosome field still lags behind as the standardization of extracellular vesicle (EV) types are not yet firmly established. The majority of exosomes range in size from 30-200 nanometers (nm) in diameter and are isolated from all bio-fluids, including serum, plasma, saliva, urine and cerebrospinal fluid (Brock et al., 2015).

Due to the size of an exosome, on average just over 100 nanometers, the entire transcriptome cannot be packaged inside every vesicle. By way of comparison, retrovirus particles with a similar size can package only around 10 kb, so it is likely that a single vesicle of that size carries only a limited number of transcripts. However, exosomes are extremely abundant (10^{11} per mL of plasma) and when isolating the vesicle fraction, most of the transcriptome can be detected (Brock et al., 2015). Per Huang et al. (2013)and Kahlert et al. (2014), exosomal RNA can be used for mutation detection as well as global profiling of most types of RNA, and the profile alone (without mutation characterization) can be utilized for diagnostics (Brock et al., 2015). In the study 'Immune modulation of T-cell and NK (natural killer) cell activities by TEXs (tumor-derived exosomes)', Whiteside (2013) observed that exosome investigations

have focused on the important physiologic and pathophysiologic functions of these vesicles in micro-metastasis, angiogenesis and immune modulation and as a means for detection of tumor specific mutations in bio-fluids (Whiteside, 2013). Consequently, in 2012, interest in this new field increased when the National Institute of Health (NIH) dedicated the large strategic Common Fund to study these new entities of extracellular RNA. The goal of this effort is to better understand how exosomes can be utilized for biomarkers and therapeutics as well as understanding this new mechanism of intercellular communication (NIH, 2024).

Mutation detection and RNA profiling

Analysis of nucleic acids present in bodily fluids can provide a better understanding of the disease, as summarized in Table below.

Analysis capability	Examples	CTCs	cfDNA	Exosomes
Mutations	Point mutations, InDels, amplifications, deletions, translocations	Yes	Yes	Yes
Epigenetic modifications	Methylation patterns	Yes	Yes	Yes
RNA transcription profiles	Levels/activity of mRNA, microRNA, long non-coding RNA, RNA splice variants	Yes	No	Yes
Phenotypic studies of cells from the tumor	Cell morphology, protein localization, *in vivo* studies	Yes	No	No
Inflammatory response, stromal and other systemic changes	Inflammatory RNA and protein markers	No	No	Yes
Analysis of RNA as well as DNA and protein profiles from tumor cells	Separate or in combination	Yes	No	Yes
Can utilize bio-banked samples	Frozen plasma, urine and other bio-fluids	No	Yes	Yes

CTCs, circulating tumor cells; cfDNA, cell-free DNA; InDels, insertions/deletions. (Brock et al., 2015)

RNA profiling from biofluids is also difficult. However, since exosomes contain RNA, it was possible to separate the fragile RNA from the large amounts of RNases and PCR inhibitors. As cell-free RNA in blood is immediately degraded, RNAs in serum and plasma were either protected inside vesicles, in protein complexes or associated with HDL particles (Brock et al., 2015). The levels of these microRNAs are tightly regulated in normal cells, and dysregulation has been implicated in several human diseases, e.g., cardiovascular (Thum & Condorelli, 2015) and neurological, and is strongly linked to cancer development and progression. However, microRNAs represent only a minor fraction of the transcriptome. By contrast, the nucleic acids in exosomes can be isolated and the entire transcriptome examined (Brock et al., 2015).

The most significant hurdle for all forms of liquid biopsy remains the relative rarity of nucleic acid derived from a tumor against the background of normal material found in most patient samples. In fact, the majority of cell, cell-free nucleic acids, microRNAs and exosomes in a liquid biopsy will have originated from normal cells with numbers fluctuating as a consequence of biological variations (Brock et al., 2015).

Furthermore, although liquid biopsy was first introduced with serum, other liquid media, such as urine and cerebrospinal fluid (CSF), have been used to evaluate other conditions. Cell-free DNA is not necessarily confined to blood, and other media have been proposed.

Urine

Urine's primary advantage over blood is that it is non-invasive, allowing for more convenient testing. Urinary cell-free DNA (UcfDNA) has been proposed as a biomarker for the detection and diagnosis of certain cancers, particularly bladder and prostate cancer (Lu & Li, 2017). An example of this is SelectMDX. SelectMDX evaluates two mRNA cancer-related biomarkers (HOXC6 and DLX1 with *KLK3* as a reference gene) to assist a clinician in deciding to continue routine screening or to order a prostate biopsy. This test is considered a "non-invasive urine test" (a liquid biopsy) and reports a binary result of "increased risk" or "very low risk" (MDx, 2023). Van Neste et al. evaluated this test at a 0.90 area under curve in a validation cohort. The authors concluded that the mRNA signature was one of the most significant components of the validation results (Van Neste et al., 2016). Shore et al. (2019) assessed the effect of SelectMDX results on clinical decision making and found that out of 253 patients SelectMDX evaluated as "negative," only 12% underwent a biopsy (Shore et al., 2019).

Xu et al. (2021) assessed the diagnostic value of urinary exosomes for urological tumors. The authors performed a systematic review and meta-analysis of 16 studies with a total of 3224 patients. Diagnostic value was calculated based on the number of

true positives, false positives, true negatives, and false negatives. The sensitivity of using urinary exosomes for the diagnosis of urological tumors was 83% and the specificity was 88%. Sensitivity and specificity results were similar regardless of urinary exosome content type and tumor type. The authors conclude that "urinary exosomes may serve as novel non-invasive biomarkers for urological cancer detection" (Xu et al., 2021).

Cerebrospinal Fluid (CSF)

Cerebrospinal Fluid is a colorless, clear liquid produced by the choroid plexus. CSF acts to control flow of molecules to the central nervous system (CNS). Due to the tight control of the CSF, it may play a significant role in assessing several conditions. CSF is traditionally used to evaluate conditions such as meningitis, but it has also been used to assess central nervous system cancers, such as leptomeningeal metastases (Demopoulos, 2022; Johnson & Sexton, 2023). In addition to widely-known measures of pathology in CSF (opening pressure, total protein, glucose, cell count with differential), circulating tumor cells in CSF have also been proposed as markers for epithelial tumors (Demopoulos, 2022).

Lin et al. (2017) evaluated the diagnostic accuracy of circulating tumor cells in CSF (CSF-CTC) in patients with leptomeningeal metastasis (LM). There were 30 of 95 total patients diagnosed with LM based on a combination of CSF cytology and MRI. CSF-CTCs were detected in 43 patients (median 19.3 CSF-CTC/mL). Based on receiver operating curve analysis, the optimal cutoff was found to be one CSF-CTC/mL, identifying patients at a rate of 93% sensitivity, 95% specificity, positive predictive value 90%, and negative predictive value 97% (Lin et al., 2017). Diaz et al. (2022)studied the clinical utility of CSF-CTC by evaluating how CSF-CTC quantification was able to predict the outcome of LM. The authors performed a single institution retrospective study of 101 LM patients with solid tumors. The CSF-CTC count significantly predicted survival continuously ($p=0.0027$). The authors conclude that "CSF-CTCs quantification predicts survival in newly diagnosed LM, and outperforms neuroimaging" and suggest CSF-CTC can be used for LM prognosis and to assess disease burden (Diaz et al., 2022).

Mathios and Phallen (2022) published a review paper noting "significant strides" towards understanding the molecular mechanisms of brain cancer. Research advances in the field include a focus on the "tumor microenvironment" and identifying molecular biomarkers with liquid-based analyses (such as CSF in liquid biopsy). While it is a rapidly advancing area of research, clinical utility is currently limited, that is, there are currently "no approved noninvasive tests that are clinically useful" for gliomas. The authors point to Cristiano et al. (2019) as an example of a study that analyzed genome-wide cfDNA fragment features (in a variety of cancers); the authors were able to

distinguish patients with cancer from non-cancer patients (as well as isolate the tissue of origin). In another glioma-specific study, Mouliere et al. (2018) detected five of 13 patients' brain tumors (38%) using a cfDNA fragmentation-based approach to analyze cfDNA fragments and copy number alterations in CSF. In conclusion, the authors note that, despite recent excitement over promising studies, liquid biopsy approaches to brain cancer are still "in their infancy" (Mathios & Phallen, 2022).

Proprietary Testing

The FDA approval of use of Roche Cobas *EGFR* Mutation Test in plasma was based on evaluation of plasma samples from the ENSURE study (Wu et al., 2015), a multicenter, open-label, randomized, Phase III study of stage IIIB/IV NSCLC patients. A total of 98.6% of the patients enrolled (214/217) had a plasma sample available for testing. The agreement between the Cobas *EGFR* Mutation Test in plasma and tissue was evaluated for detection of *EGFR* mutations. In 76.7% of tissue-positive specimens, plasma was also positive for an *EGFR* mutation. Plasma was negative for *EGFR* mutation in 98.2% (95.4%, 99.3%) of tissue-negative cases. The patients whose plasma results were positive for exon 19 deletion and/or an L858R mutations treated with erlotinib had improved progression-free survival (PFS) compared to those treated with chemotherapy (FDA, 2016).

Another commercially available, FDA-approved test is Guardant360 by Guardant Health Inc. Guardant360 is a gene panel that sequences 74 genes (including 18 amplifications and six fusions) associated with NSCLC and reports the percentage of cfDNA (Guardant, 2023). The manufacturer purports that this genetic test will allow providers to make better treatment decisions based on the mutations present in the patient (Health, 2023). The gene panel was analytically validated, with 99.8% accuracy on 1000 consecutive samples (Lanman et al., 2015).

FoundationOne has also created a proprietary FDA-approved test that examines cell-free DNA. FoundationOne's liquid CDx test evaluates 324 genes using circulating cell-free DNA and is FDA-approved to report short variants in 311 genes (FoundationOne, 2022, 2024). A prior version of this test (covering 62 genes) was evaluated based on 2666 reference samples. The assay reached >99% sensitivity of short variants of allele frequencies of >0.5%, >95% sensitivity of allele frequencies 0.25%-0.5%, and >70% sensitivity of allele frequencies 0.125%-0.25%. Out of 62 healthy volunteers, no false positives were detected (Clark et al., 2018).

Biodesix is another laboratory that offers a liquid biopsy panel. Biodesix offers two tests; one called GeneStrat, tests *EGFR, ALK, ROS1, RET, BRAF*, and *KRAS* (Biodesix, 2023). Sensitivities of 78%-100% for *EGFR, ALK,* and *KRAS* with the GeneStrat test were

shown in multiple validation studies (Mellert et al., 2017). GeneStrat also detected over 88% of *RET* or *ROS1*-positive patients (Mellert et al., 2018). Biodesix also offers GeneStrat NGS, a broad 52 gene panel also evaluated through blood-based liquid biopsy technology.

Other firms that offer liquid biopsy testing include ResolutionBio (now part of Agilent) which offers Agilent Resolution ctDx FIRST ("companion diagnostic to KRAZATI™ (adagrasib) for the detection of *KRAS* G12C in non-small cell lung cancer [NSCLC]") and Agilent Resolution ctDx LUNG, which focuses on actionable genes for lung cancer such as EGFR and ALK; Circulogene (tests *BRAF, EGFR, KRAS, ALK, ROS1,* PD-L1, and MSI), Neogenomics (InvisionFirst, 37-gene panel including 10 actionable genes), and Biocept (CNSide™). As liquid biopsy is a rapidly emerging field, it is possible that many more tests will find their way into the clinical setting (Biocept, 2023; Circulogene, 2023; Neogenomics, 2023; ResolutionBio, 2024).

In addition to panels designed to target cancer or cancer type specific mutations, tests are beginning to emerge that perform the role of a genetic screen in asymptomatic individuals. One such test includes the GRAIL-Galleri multi-cancer early detection (MCED) test, which claims to look for a signal shared by at least 50 types of cancer with a single blood test (Grail, 2024).

According to Turnbull C. (2024) the Galleri-MCED test has an overall sensitivity of only 27.5% for early-stage cancer. The test sensitivity improves to 52.8% by restricting analysis to 12 cancers that the Galleri authors specified as being of high unmet need. However, for several of these 12 cancers, including pancreatic, oesophageal, biliary, and liver, the mortality gains from population screening may be low because these cancers are mainly diagnosed in individuals older than 70 years and prognosis is poor regardless of stage (Turnbull C., 2024).

Klein et al. (2021) held a Circulating Cell-free Genome Atlas substudy including 4077 participants in an independent validation set of Galleri (cancer: n=2823; non-cancer: n=1254, non-cancer status confirmed at year-one follow-up). Specificity for cancer signal detection was 99.5%. Overall sensitivity for cancer signal detection was 51.5% with the sensitivity increasing with stage. Stage I-III sensitivity was 67.6% in the 12 pre-specified cancers and was 40.7% in all cancers. Cancer signals were detected across more than 50 cancer types. Overall accuracy of cancer signal origin prediction in true positives was 88.7%. The study concluded that "The MCED test demonstrated high specificity and accuracy of cancer signal origin prediction and detected cancer signals across a wide diversity of cancers. These results support the feasibility of this blood-based MCED test as a complement to existing single-cancer screening tests" (Klein et al., 2021).

Clinical Utility and Validity

Seeberg et al. (2015) conducted a prospective study to assess the prognostic and predictive value of CTCs in 194 patients with colorectal liver metastasis referred to surgery. A total of 153 patients underwent a resection (41 patients had an unresectable tumor), and CTCs were detected in 19.6% of patients. Patients with unresectable tumors had a 46% CTC positivity rate compared to 11.7% for resectable tumors. Patients with two or more CTCs experienced reduced time to relapse/progression. Two or more CTCs was a strong predictor of progression and mortality in all subgroups of patients. The authors concluded that "CTCs predict nonresectability and impaired survival. CTC analysis should be considered as a tool for decision-making before liver resection in these patients" (Seeberg et al., 2015).

Groot Koerkamp et al. (2013) performed systematic review and meta-analysis to investigate the prognostic value of CTCs in patients with resectable colorectal liver metastases or widespread metastatic colorectal cancer (CRC). The results of 12 studies representing 1,329 patients were suitable for pooled analysis. The overall survival and progression-free survival were worse in patients with CTCs, with hazard ratios of 2.47 for overall survival rate and 2.07 for progression-free survival. The authors concluded that "the detection of CTCs in peripheral blood of patients with resectable colorectal liver metastases or widespread metastatic CRC is associated with disease progression and poor survival" (Groot Koerkamp et al., 2013).

Zhang et al. (2012) conducted a meta-analysis of published literature on the prognostic value of CTC in breast cancer. Forty-nine eligible studies enrolling 6,825 patients were identified. The presence of CTC was significantly associated with shorter survival in the total population and the prognostic value of CTC was significant in both early and metastatic breast cancer. The authors concluded that "the detection of CTC is a stable prognosticator in patients with early-stage and metastatic breast cancer. Further studies are required to explore the clinical utility of CTC in breast cancer" (Zhang et al., 2012).

Pinzani et al. (2021) assessed that the clinical validity of CTCs has been demonstrated in cancer screening, prognosis, and monitoring treatment responses. In the original article by Cabel et al. (2017), using the Cellsearch® technique in early non-metastatic cancer has reported low CTC detection rates (5-30% depending on cancer type), with limited specificity since "some circulating epithelial cells can be found in individuals with inflammatory disease or even in some healthy individuals." However, in the preliminary report of another study, it was found that a CTC count >25 could "distinguish lung cancer from benign lesions in patients with abnormal lung imaging. CTC count was also shown to be an "independent prognostic factor in non-small cell

lung cancer and small cell lung cancer;" despite this, CTCs are rare in the non-metastatic setting, and thus cannot be completely utilized as an independent prognostic factor in the localized setting. With respect to the independent cancers, Cabel et al. (2017) summarizes the clinical validity of CTC detection in Figure 1.

On the clinical utility of CTC, Cabel et al. (2017) initially stated "the clinical utility of CTC detection (i.e. does it improve patient outcome) has yet to be demonstrated before it can be implemented in routine clinical practice." In recent time, it was seen that specific CTC features may have clinical utility in "[predicting] the sensitivity to specific immunotherapies," and in the case of ER+ MBCs, ER-CTCs can develop and reflect "acquisition of therapy resistance by the primary tumor" (Pinzani et al., 2021).

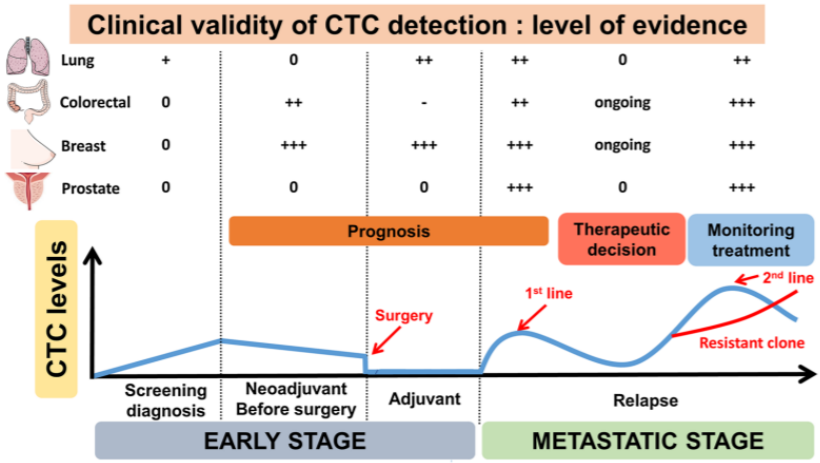


Figure 1. Clinical validity of circulating tumor cells (CTC): level of evidence according to clinical settings (Cabel et al., 2017).

Oxnard et al. (2016) found that: "Sensitivity of plasma genotyping for detection of T790M was 70%. Of 58 patients with T790M-negative tumors, T790M was detected in plasma of 18 (31%). ORR and median PFS were similar in patients with T790M-positive plasma (Objective response rate [ORR], 63%; progression-free survival [PFS], 9.7 months) or T790M-positive tumor (ORR, 62%; PFS, 9.7 months) results. Although patients with T790M-negative plasma had overall favorable outcomes (ORR, 46%; median PFS, 8.2 months), tumor genotyping distinguished a subset of patients positive for T790M who had better outcomes (ORR, 69%; PFS, 16.5 months) as well as a subset of patients negative for T790M with poor outcomes (ORR, 25%; PFS, 2.8 months)" (Oxnard et al., 2016). The authors concluded that "upon availability of validated plasma T790M assays, some patients could avoid a tumor biopsy for T790M genotyping" (Oxnard et al., 2016).

A review by Sacher et al. (2016) genotyped 180 patients with NSCLC using plasma droplet PCR (plasma ddPCR). This was done to validate the plasma droplet PCR

technique, and the study identified 115 *EGFR* mutations and 25 *KRAS* mutations. The plasma ddPCR was measured to have 82% sensitivity for the *EGFR* 19 del, 74% for L858R, 77% for T790M, and 64% for *KRAS*. The positive predictive value was 100% for every mutation apart from T790M at 79%. The authors concluded that the technique "detected *EGFR* and *KRAS* mutations rapidly with the high specificity needed to select therapy and avoid repeat biopsies". The authors also noted that this assay "may also detect *EGFR* T790M missed by tissue genotyping due to tumor heterogeneity in resistant disease" (Sacher et al., 2016).

Kim et al. (2017) evaluated the clinical utility of Guardant360. This study used the Guardant360 panel to detect mutations in patients with metastatic NSCLC and other cancers. Somatic mutations were detected in 59 patients, 25 of which had actionable mutations. Out of the 73-patient NSCLC cohort, 62 were found to have somatic mutations and 34 had actionable mutations. After these genetic findings were identified, molecularly matched therapy was provided to 10 patients with gastric cancer (GC) and 17 with NSCLC. Response rate was 67% in GC and 87% in patients with NSCLC, while disease control rate was 100% for both types (Kim et al., 2017).

Odegaard et al. (2018) validated the Guardant360 cell-free DNA sequencing test and aimed to "demonstrate its clinical feasibility". The authors found that the test could detect variants down to "0.02% to 0.04% allelic fraction/2.12 copies with ≤0.3%/2.24-2.76 copies". Clinical validation in a cohort of over 750 patients demonstrated high accuracy and specificity, with positive percent agreement (with PCR) of 92%-100% and negative percent agreement of over 99%. In terms of feasibility, the authors performed the test in 10593 patients and found the technical success rate to be over 99.6% and the clinical sensitivity to be 85.9%. The authors also noted that 16.7% of these mutations were targetable with FDA-approved treatments (with 72% with "treatment or trial recommendations") with as many as 34.5% of non-small cell cancer samples having a targetable mutation (Odegaard et al., 2018).

Aggarwal et al. (2019) evaluated the utility of plasma-based sequencing in improving mutation detection in patients with non-small cell lung cancer. The authors first performed next-generation sequencing (NGS) on tissue, then plasma-based sequencing. A total of 229 patients had concurrent sequencing, and NGS alone detected 47 targetable mutations. Addition of plasma sequencing brought that number to 82 targetable mutations. Furthermore, 36 of 42 patients that received "plasma next-generation sequencing–indicated therapy" achieved a "complete or a partial response or stable disease". The authors concluded that "adding plasma next-generation sequencing testing to the routine management of metastatic non–small cell lung cancer appears to increase targetable mutation detection and improve delivery of targeted therapy" (Aggarwal et al., 2019).

Leighl et al. (2019) evaluated the utility of "comprehensive cell-free DNA analysis" to identify genomic biomarkers in patients with newly diagnosed metastatic non-small cell lung cancer (NSCLC). A total of 282 patients were included. Tissue genotyping (current standard of care) identified a guideline-recommended biomarker in 60 patients, whereas cell-free DNA identified a relevant biomarker in 77 patients. Concordance between the two methods was 80% (48 biomarkers detected in both methods). For FDA-approved targets (*EGFR*, *ALK*, *ROS1*, *BRAF*), concordance was >98.2% with 100% positive predictive value for cell-free DNA. Cell-free DNA was also found to have a faster median turnaround time (nine days compared to 15 for tissue genotyping), and "guideline-complete" (assessment of all eight guideline-recommended biomarkers [*EGFR*, *ALK*, *ROS1*, *BRAF*, *RET*, *MET* amplification and exon 14 skipping, and HER2]), was significantly more likely (268 patients vs 51) (Leighl et al., 2019).

Dudley et al. (2019) have developed a novel high-throughput sequencing method that uses urine-derived tumor DNA (utDNA) known as utDNA CAPP-Seq (uCAPP-Seq) to detect bladder cancer. This technique was used to analyze samples from 118 patients with early-stage bladder cancer and 67 healthy adults. "We detected utDNA pretreatment in 93% of cases using a tumor mutation-informed approach and in 84% when blinded to tumor mutation status, with 96% to 100% specificity" (Dudley et al., 2019). These results show that utDNA can be used to diagnose early-stage bladder cancer with high sensitivity and specificity.

Wang et al. (2018) performed a meta-analysis to determine the diagnostic performance of cell-free DNA (both blood and urine) assays in bladder cancer. Eleven studies encompassing 802 patients were included. The authors evaluated cell-free DNA assays at the following statistics: "sensitivity 0.71, specificity 0.78 positive likelihood ratio 3.3, negative likelihood ratio 0.37, diagnostic odds ratio nine, and area under curve 0.80. No publication bias was identified. The authors concluded that "cell-free DNA has a high diagnostic value in bladder cancer" (Wang et al., 2018).

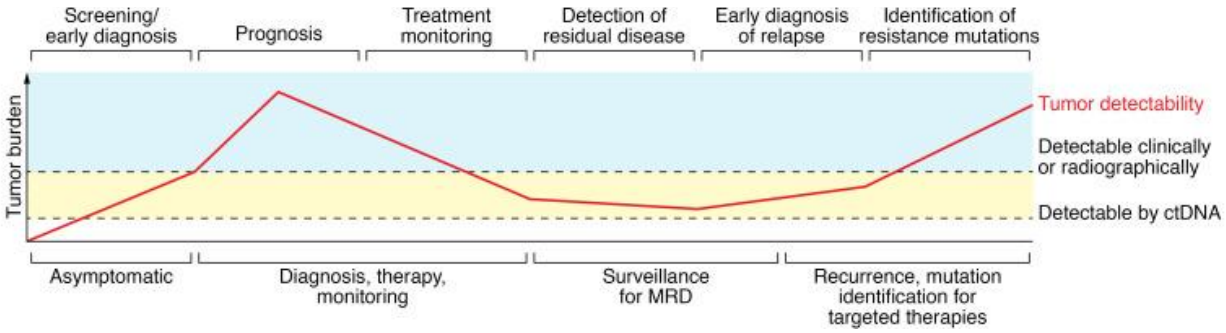
Hopefully, cfDNA can be used to indicate prognoses of personalized peptide vaccine therapy in patients with NSCLC. Waki et al. (2021) identified that cfDNA integrity "decreased after the first cycle of vaccination" and that those with "high prevaccination cfDNA integrity survived longer than those with low prevaccination integrity (median survival time (MST): 17.9 versus 9.0 months, respectively; hazard ratio (HR): 0.58, p= .0049)," showing that monitoring cfDNA levels could contribute to quantifying treatment success and predicting patient lifespans.

For exosome-based liquid biopsy, Yu et al. (2021) have proposed a synergistic alternative of combining cfDNA and exosomal RNA to "increase the sensivity of

mutation detection… the exosome component enables a combination of exosomal RNA, cfDNA, and disease specific proteins… the unique composition of the exosome compartment makes these vesicles particularly amenable for multi-analyte testing, since they carry cancer-informative DNA, RNA, proteins, lipids, oligosaccharides, and metabolites. In one study, a high sensitivity (92%) for *EGFR* mutations was found for utilizing exosomal RNA and ctDNA together and remained high in a subpopulation that's been difficult for ctDNA assays to detect (88% sensitivity). ExoRNA and ctDNA combined analyses on *BRAF, KRAS,* and *EGFR* mutations in exosomes and respective ctDNA have also better correlated the biomarkers with treatment outcomes when compared to ctDNA alone (Yu et al., 2021).

Lee et al. (2021) analyzed the clinical utility of ctDNA to reliably detect *EGFR* in ctDNA. The authors compared *EGFR* analysis results between tissueDNA (tDNA) and ctDNA from 554 NSCLC cases. ctDNA analysis detected *EGFR* mutation in 57.3% of cases. ctDNA detection correlated with metastatic stage and disease progression ($p<0.001$). The authors followed up after an average of 41.09 month and found that, "survival analysis revealed ctDNA status and M stage ($p < 0.001$) to be independent predictors of overall survival in the multivariate analysis." The authors conclude that ctDNS is clinically useful for *EGFR* analysis, but note the possibility of false negatives and recommend using tDNA to confirm ctDNA results in some situations (Lee et al., 2021). Syeda et al. (2021) evaluated the use of ctDNA as a biomarker for melanoma. The authors measured changes in ctDNA and survival following "BRAF, MEK, or BRAF plus MEK inhibitor therapy" in patients participating in two clinical trials. The $BRAF^{V600}$-mutant was measured in ctDNA before and during treatment. "Elevated baseline $BRAF^{V600}$ mutation-positive ctDNA concentration was associated with worse overall survival outcome." The authors conclude that $BRAF^{V600}$-mutation ctDNA analysis can be used as a biomarker to predict clinical outcomes (Syeda et al., 2021).

Dang and Park (2022) completed a systematic review on the use of ctDNA for liquid biopsy and the potential challenges as a primary cancer screening marker for minimal residual disease. They cite that more studies need to be performed to evaluate the positive and negative predictive values of existing tests utilizing ctDNA for this purpose. Despite this, the figure below details their understanding of positive benefits in the potential utility of ctDNA across the cancer spectrum (Dang & Park, 2022):



VI. Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

The NCCN guidelines for non-small cell lung cancer (NSCLC) strongly advise "broader molecular profiling with the goal of identifying rare driver mutations for which effective drugs may already be available, or to appropriately counsel patients regarding the availability of clinical trials... Broad molecular profiling is a key component of the improvement of care of patients with NSCLC." Furthermore, the NCCN states that "Data suggest that plasma genotyping (also known as plasma ctDNA testing or liquid biopsy) may be considered at progression instead of tissue biopsy to detect whether patients have T790M; however, if the plasma biopsy is negative, then tissue biopsy is recommended" (NCCN, 2023k).

However, the NCCN goes on to state that "cell-free/circulating tumor DNA testing should not be used in lieu of histologic tissue diagnosis." The NCCN notes that specificity is generally very high for cell-free tumor testing but is lacking in sensitivity (up to 30% false-negative rate) and that standards for analytic performance characteristics of cell-free tumor DNA have not been well established. The use of cell-free or circulating tumor DNA may be considered in specific clinical situations, such as if a patient is medically unfit for an invasive tissue sampling or if there is insufficient material for a molecular analysis following pathologic confirmation of an NSCLC diagnosis in the initial diagnostic setting (but "follow-up tissue-based analysis for all patients in which an oncogenic driver is not identified should be planned)." The NCCN notes that "data suggest that plasma ctDNA testing can be used to identify *ALK*, *BRAF, EGFR, HER2, MET* exon 14 skipping, *RET, ROS1* and other oncogenic biomarkers that would otherwise not be identified in patients with metastatic NSCLC" (NCCN, 2023k).

For NSCLC, the NCCN provides the following specific recommendations for liquid biopsy:

> "The use of cell-free/circulating tumor DNA testing can be considered in specific clinical circumstances, most notably:

- If a patient is medically unfit for invasive tissue sampling
- In the initial diagnostic setting, if following pathologic confirmation of a NSCLC diagnosis there is insufficient material for molecular analysis, cell-free/circulating tumor DNA can be used; however, follow-up tissue-based analysis for all patients in which an oncogenic driver is not identified should be planned (see NSCL-18 for oncogenic drivers with available targeted therapy options).
- In the initial diagnostic setting, if tissue-based testing does not completely assess all recommended biomarkers owing to tissue quantity or testing methodologies available, consider repeat biopsy and/or cell-free/circulating tumor DNA testing.
- In the initial diagnostic setting, if the feasibility of timely tissue-based testing is uncertain, concurrent cfDNA testing may aid in biomarker evaluation for treatment selection, provided negative results are considered per above limitations" (NCCN, 2023k).

The NCCN lists "comprehensive germline and somatic profiling to identify candidates for additional targeted therapies" as part of the workup for recurrent stage IV (M1) breast cancer." They go on to specifically note that "tissue or plasma-based circulating tumor DNA (ctDNA) assays may be used. Tissue-based assays have greater sensitivity, but ctDNA may reflect tumor heterogeneity more accurately." The NCCN also states that assessment of the PIK3CA mutation may be performed through liquid biopsy if the tumor is HR-positive, HER2 negative, and if therapy with alpelisib plus fulvestrant is being considered. Finally, for the management of breast cancer with liquid biopsy techniques, the NCCN states that "the clinical use of Circulating Tumor Cells (CTC) or circulating DNA (ctDNA) in metastatic breast cancer is not yet included in the NCCN Guidelines for Breast Cancer for disease assessment and monitoring", though the sentence that follows would indicate that this statement refers to a count of CTCs, not their use for genotyping: "Patients with persistently increased CTC after three weeks of first-line chemotherapy have a poor PFS and OS" (NCCN, 2023e).

The NCCN states that AR-V7 testing in CTCs "can be considered to help guide selection of therapy in the post-abiraterone/enzalutamide metastatic CRPC [castration-resistant prostate cancer] setting." The NCCN does not comment on any particular liquid medium over another (e.g., urine, CSF, serum). However, the NCCN does specify the use of circulating DNA for rucaparib treatment, stating that "the preferred method of selecting patients for rucaparib treatment is somatic analysis of *BRCA1* and *BRCA2* using a circulating tumor DNA sample" (NCCN, 2023a). SelectMDx is also acknowledged by the NCCN; "the panel believes that SelectMDx score is potentially informative in patients who have never undergone biopsy, and it can therefore be considered in such individuals" (NCCN, 2023m).

With regards to circulating tumor DNA (ctDNA) in colon cancer, the NCCN "panel believes that there are insufficient data to recommend the use of multigene assays, Immunoscore, or post-surgical ctDNA to estimate risk of recurrence or determine adjuvant therapy" (NCCN, 2023g).

For neuroendocrine tumors, NCCN notes that CTCs have been studied as prognostic markers, but state that more research is required. There is no single biomarker available that is satisfactory as a diagnostic, prognostic, or predictive marker (NCCN, 2023j).

For a primary CNS lymphoma, the NCCN remarks that cerebrospinal fluid analysis may "possibly" include gene rearrangement evaluation. For leptomeningeal metastases, the NCCN notes that assessment of ctDNA in CSF "increases sensitivity of tumor cell detection and assessment of response to treatment" (NCCN, 2023f).

For pancreatic adenocarcinomas, the NCCN acknowledges that circulating cell-free DNA is being investigated as a biomarker for screening. The NCCN also notes that if tumor tissue is not available, cell-free DNA testing may be considered (NCCN, 2023l).

For esophageal, esophagogastric junction cancers, and gastric cancers, the NCCN states "testing using a validated NGS-based [next generation sequencing] genomic profiling assay performed in a CLIA-approved laboratory may be considered. A negative result should be interpreted with caution, as this does not exclude the presence of tumor mutations or amplifications" (NCCN, 2023h).

For acute myeloid leukemia, the NCCN notes that "morphologically detectable," circulating leukemic blasts from peripheral blood may be used to detect molecular abnormalities (NCCN, 2023b).

For bladder cancer, the NCCN mentions RT-PCR testing for *FGFR2/3* gene alterations but does not specify whether this can be done through a liquid biopsy or cell-free DNA. The only comment made is that the laboratory should be CLIA-approved (NCCN, 2023d).

The NCCN guidelines for small cell lung cancer and hepatocellular carcinoma do not address use of CTCs, ctDNA, or liquid biopsy for patient management (NCCN, 2023i, 2023n). In biliary tract cancers, the NCCN states that "a cell-free DNA (cfDNA) test may also be considered for identifying gene mutations. This technique may not reliably identify gene fusions or rearrangements depending on the panel used and the specific partner gene" (NCCN, 2023c).

American Society of Clinical Oncology (ASCO)

In 2016, ASCO published updated recommendations for the use of tumor markers in treatment of metastatic breast cancer. ASCO found that although CTCs may be prognostic, they are not predictive for clinical benefit when used to guide or influence decisions on systemic therapy for metastatic breast cancer. ASCO recommends clinicians to not use these markers as adjunctive assessments (Van Poznak et al., 2015). Similarly, ASCO recommended against use of CTCs to guide decisions about adjuvant systemic therapy for individual's with early stage invasive breast cancer (Andre et al., 2019).

In 2019, ASCO stated that clinicians "should not use circulating biomarkers as a surveillance strategy for detection of recurrence in patients who have undergone curative-intent treatment of stage I-III NSCLC or SCLC". ASCO states that further data is required to validate this approach (Schneider et al., 2019).

In 2018, ASCO and the College of American Pathologists (CAP) released a joint review on "circulating tumor DNA analysis in patients with cancer." In it, they note that apart from the assays that have received "regulatory appeal", most assays have "insufficient evidence" for both clinical validity and clinical utility. They note discordant results between circulating DNA assays and tissue genotyping. Furthermore, they remark on the lack of evidence for use in monitoring therapy effectiveness, diagnosing early-stage cancer, or cancer screening.

However, they point to evidence that well-validated assays may support initiation of targeted therapy (Merker et al., 2018).

National Academy of Clinical Biochemistry (NACB), now known as the American Association for Clinical Chemistry (AACC)

In 2010, the NACB issued practice guidelines for the use of tumor markers in liver, bladder, cervical, and gastric cancers. It found that CTCs had "questionable" clinical utility in the assessment of liver cancer and did not recommend their use (Sturgeon et al., 2010).

The NACB published an updated guideline in 2020. For liver cancer, they note circulating cell-free serum DNA as "undergoing evaluation" for "predictive marker for distant metastasis of hepatitis C virus–related HCC." The plasma proteasome is also undergoing evaluation for "assessment of early HCC in patients with chronic viral chronic hepatitis; assessment of metastatic potential of HCC." Finally, circulating methylated DNA is undergoing evaluation for HCC screening, detection, and

prognosis. No other circulating tumor markers for bladder, cervical, and gastric cancers were mentioned (Sturgeon et al., 2020).

College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP)

An expert panel was convened to review and update the CAP-IASLC-AMP Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors. This panel consists of practicing pathologists, oncologists, and a methodologist.

The panel states there is "insufficient evidence to support the use of circulating cell-free plasma DNA (cfDNA) molecular methods for the diagnosis of primary lung adenocarcinoma". According to the panel, there is also "insufficient evidence to support the use of circulating tumor cell (CTC) molecular analysis for the diagnosis of primary lung adenocarcinoma, the identification of EGFR or other mutations, or the identification of EGFR T790M mutations at the time of EGFR TKI-resistance" (Lindeman et al., 2018).

However, the panel acknowledges that "In some clinical settings in which tissue is limited and/or insufficient for molecular testing, physicians may use a cell-free plasma DNA (cfDNA) assay to identify EGFR mutations" (Lindeman et al., 2018).

In 2021, the IASLC published an updated consensus statement on liquid biopsy testing. They note that liquid biopsy "includes a variety of methodologies for circulating analytes. From a clinical point of view, plasma circulating tumor DNA is the most extensively studied and widely adopted alternative to tissue tumor genotyping in solid tumors, including NSCLC" (Rolfo et al., 2021).

The following recommendations were presented in a consensus statement:

1. In clinical practice, ctDNA collection, sample handling, and automated processing should be performed using standardized and clinically validated procedures to reduce operator variability and false-negative results.
2. Because of the growing number of guideline-recommended oncogene targets to be assessed in advanced NSCLC, testing of plasma ctDNA should be performed by a clinically validated NGS platform rather than single-gene, PCR-based approaches, both in treatment-naive patients and those associated with multiple mechanisms of acquired resistance (MOR) to targeted agents. Where plasma NGS is not available owing to technical and economic constraints, single-gene or low multiplex-based approaches may represent appropriate alternatives. Use of limited PCR analysis for

EGFR mutations as the initial step in molecular assessment, for example, remains highly relevant in areas of the world where the EGFR mutation rate is high. Nevertheless, single-gene testing should not be considered complete, and if negative, serial testing for additional actionable biomarkers must be pursued.
3. The benefit of tissue and plasma NGS is now established in several clinical practice settings. It is anticipated, owing to broad-based coverage of requisite oncogenes, decreased turnaround times, and emerging data on cost effectiveness, that in the near future, NGS will become increasingly available worldwide. Implementation of a multidisciplinary MTB to assist clinicians in treatment decision-making is advisable, as described previously.
4. In patients with oncogene-addicted NSCLC, liquid biopsy is emerging as not only complementary to tissue-based analysis but also acceptable as the initial approach ("plasma first") for biomarker evaluation at the time of diagnosis and for monitoring the efficacy of targeted therapies. Finally, a plasma-first approach is appropriate for identification of MOR to targeted therapies in many clinical settings.
5. Indications for liquid biopsy in patients with nononcogene-addicted NSCLC are less well defined at this time, although there are several promising areas of investigation. As noted previously, bTMB is an emerging biomarker, pending completion of ongoing prospective randomized trials and refinement of methodology.

American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology

These joint guidelines from these societies were published regarding molecular biomarkers for colorectal cancer. Despite the potential of liquid biopsy for assessment of tumor recurrence and treatment resistance, the technique "awaits robust validation and further studies to determine their clinical utility" (Sepulveda et al., 2017).

European Society for Medical Oncology (ESMO) and Chinese Society of Clinical Oncology (CSCO)

These guidelines state that liquid biopsy can be used as "the initial test for the detection of a T790M mutation [for EGFR in NSCLC], and if tests are negative, a re-biopsy should be attempted if feasible" (Wu et al., 2018).

European Association of Urology (EAU), European Society for Radiotherapy and Oncology (ESTRO), European Society of Urogenital Radiology (ESUR), International Society of Geriatric Oncology (SIOG)

The joint guidelines on prostate cancer state that "In asymptomatic [individuals] with a prostate-specific antigen level between three to ten ng/mL and a normal [digital rectal examination], use one of the following tools for biopsy indication:

- risk-calculator - provided it is correctly calibrated to the population prevalence; magnetic resonance imaging of the prostate - STRONG
- an additional serum, urine or tissue-based test – WEAK."

These joint guidelines acknowledged SelectMDX as a test to isolate urine biomarkers, but the guidelines noted "the clinically added value of SelectMDX in the era of upfront MRI and targeted biopsies remains unclear" (Mottet et al., 2023).

American Society of Colon and Rectal Surgeons (ASCRS)

The ASCRS released clinical practice guidelines for the management of colon cancer. The guidelines state that "the use of multigene assays, CDX2 expression analysis, and ctDNA may be used to complement multidisciplinary decision-making for patients with stage II or III colon cancer" (Vogel et al., 2022).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/overview-and-quick-search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

There are four FDA-approved liquid biopsy tests as of January 10, 2023. The cobas EGFR Mutation Test v2 from Roche Diagnostics is an assay purported to detect epidermal growth factor receptor (EGFR) gene mutations in NSCLC patients. The test is intended as a companion diagnostic test for the cancer drug Tarceva (FDA, 2016), and a similar test for the T790M mutation has been produced by the same company. A second test is the Cell Search® Circulating Tumor Cell (CTC) Test, which is used to predict and analyze outcomes for individuals with metastatic breast, prostate, or colon cancer (CellSearch, 2024). A third test is Guardant360® CDx, which detects ctDNA and other common genetic errors in order to help in the choice of a therapeutic or treatment (Health, 2023). Lastly, FoundationOne® Liquid CDx is an FDA-approved

liquid biopsy test that detects ctDNA and may be able to assist a provider in determining the type of treatment that will be most effective (FoundationOne, 2022).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81162	BRCA1 (BRCA1, DNA repair associated), BRCA2 (BRCA2, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; full sequence analysis and full duplication/deletion analysis (ie, detection of large gene rearrangements)
81163	BRCA1 (BRCA1, DNA repair associated), BRCA2 (BRCA2, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; full sequence analysis
81164	BRCA1 (BRCA1, DNA repair associated), BRCA2 (BRCA2, DNA repair associated) (eg, hereditary breast and ovarian cancer) gene analysis; full duplication/deletion analysis (ie, detection of large gene rearrangements)
81194	NTRK (neurotrophic receptor tyrosine kinase 1, 2, and 3) (eg, solid tumors) translocation analysis
81210	BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer, melanoma), gene analysis, V600 variant(s)
81235	EGFR (epidermal growth factor receptor) (eg, non-small cell lung cancer) gene analysis, common variants (eg, exon 19 LREA deletion, L858R, T790M, G719A, G719S, L861Q)
81275	KRAS (Kirsten rat sarcoma viral oncogene homolog) (eg, carcinoma) gene analysis; variants in exon 2 (eg, codons 12 and 13)
81276	KRAS (Kirsten rat sarcoma viral oncogene homolog) (eg, carcinoma) gene analysis; additional variant(s) (eg, codon 61, codon 146)
81309	PIK3CA (phosphatidylinositol-4, 5-biphosphate 3-kinase, catalytic subunit alpha) (eg, colorectal and breast cancer) gene analysis, targeted sequence analysis (eg, exons 7, 9, 20)
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)

CPT	Code Description
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons)
81462	Solid organ neoplasm, genomic sequence analysis panel, cell-free nucleic acid (eg, plasma), interrogation for sequence variants; DNA analysis or combined DNA and RNA analysis, copy number variants and rearrangements
81479	Unlisted molecular pathology procedure
86152	Cell enumeration using immunologic selection and identification in fluid specimen (eg, circulating tumor cells in blood)
86153	Cell enumeration using immunologic selection and identification in fluid specimen (eg, circulating tumor cells in blood); physician interpretation and report, when required
0011M	Oncology, prostate cancer, mRNA expression assay of 12 genes (10 content and 2 housekeeping), RT-PCR test utilizing blood plasma and urine, algorithms to predict high-grade prostate cancer risk Proprietary test: NeoLAB™ Prostate Liquid Biopsy Lab/Manufacturer: NeoGenomics Laboratories
0091U	Oncology (colorectal) screening, cell enumeration of circulating tumor cells, utilizing whole blood, algorithm, for the presence of adenoma or cancer, reported as a positive or negative result Proprietary test: FirstSightCRC Lab/Manufacturer: CellMax Life
0155U	Oncology (breast cancer), DNA, PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha) (eg, breast cancer) gene analysis (ie, p.C420R, p.E542K, p.E545A, p.E545D [g.1635G>T only], p.E545G, p.E545K, p.Q546E, p.Q546R, p.H1047L, p.H1047R, p.H1047Y), utilizing formalin-fixed paraffin-embedded breast tumor tissue, reported as PIK3CA gene mutation status Proprietary test: therascreen® PIK3CA RGQ PCR Kit Lab/Manufacturer: QIAGEN
0177U	Oncology (breast cancer), DNA, PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) gene analysis of 11 gene variants utilizing plasma, reported as PIK3CA gene mutation status Proprietary test: therascreen® PIK3CA RGQ PCR Kit Lab/Manufacturer: QIAGEN
0179U	Oncology (non-small cell lung cancer), cell-free DNA, targeted sequence analysis of 23 genes (single nucleotide variations, insertions and deletions,

CPT	Code Description
	fusions without prior knowledge of partner/breakpoint, copy number variations), with report of significant mutation(s) Proprietary test: Resolution ctDx Lung™ Lab/Manufacturer: Resolution Bioscience
0229U	BCAT1 (Branched chain amino acid transaminase 1) and IKZF1 (IKAROS family zinc finger 1) (eg, colorectal cancer) promoter methylation analysis Proprietary test: Colvera® Lab/Manufacturer: Clinical Genomics Pathology Inc
0317U	Oncology (lung cancer), four-probe FISH (3q29, 3p22.1, 10q22.3, 10cen) assay, whole blood, predictive algorithm-generated evaluation reported as decreased or increased risk for lung cancer Proprietary test: LungLB® Lab/Manufacturer: LungLife AI®
0332U	Oncology (pan-tumor), genetic profiling of 8 DNA-regulatory (epigenetic) markers by quantitative polymerase chain reaction (qPCR), whole blood, reported as a high or low probability of responding to immune checkpoint–inhibitor therapy Protietary test: EpiSwitch® CiRT (Checkpoint-inhibitor Response Test) Lab/Manufacturer: Next Bio-Research Services, LLC
0333U	Oncology (liver), surveillance for hepatocellular carcinoma (HCC) in high-risk patients, analysis of methylation patterns on circulating cell-free DNA (cfDNA) plus measurement of serum of AFP/AFP-L3 and oncoprotein des-gamma-carboxy-prothrombin (DCP), algorithm reported as normal or abnormal result Protietary test: HelioLiver™ Test Lab/Manufacturer: Fulgent Genetics, LLC
0337U	Oncology (plasma cell disorders and myeloma), circulating plasma cell immunologic selection, identification, morphological characterization, and enumeration of plasma cells based on differential CD138, CD38, CD19, and CD45 protein biomarker expression, peripheral blood Protietary test: CELLSEARCH® Circulating Multiple Myeloma Cell (CMMC) Test Lab/Manufacturer: Menarini Silicon Biosystems, Inc
0338U	Oncology (solid tumor), circulating tumor cell selection, identification, morphological characterization, detection and enumeration based on differential EpCAM, cytokeratins 8, 18, and 19, and CD45 protein biomarkers, and quantification of HER2 protein biomarker–expressing cells, peripheral blood

CPT	Code Description
	Protietary test: CELLSEARCH® HER2 Circulating Tumor Cell (CTC-HER2) Test Lab/Manufacturer: Menarini Silicon Biosystems, Inc
0343U	Oncology (prostate), exosome-based analysis of 442 small noncoding RNAs (sncRNAs) by quantitative reverse transcription polymerase chain reaction (RT-qPCR), urine, reported as molecular evidence of no-, low-, intermediate- or high-risk of prostate cancer Protietary test: miR Sentinel™ Prostate Cancer Test Lab/Manufacturer: miR Scientific, LLC
0356U	Oncology (oropharyngeal or anal), evaluation of 17 DNA biomarkers using droplet digital PCR (ddPCR), cell-free DNA, algorithm reported as a prognostic risk score for cancer recurrence. Proprietary test: NavDx® Lab/Manufacturer: Naveris, Inc
0368U	Oncology (colorectal cancer), evaluation for mutations of APC, BRAF, CTNNB1, KRAS, NRAS, PIK3CA, SMAD4, and TP53, and methylation markers (MYO1G, KCNQ5, C9ORF50, FLI1, CLIP4, ZNF132 and TWIST1), multiplex quantitative polymerase chain reaction (qPCR), circulating cell-free DNA (cfDNA), plasma, report of risk score for advanced adenoma or colorectal cancer Proprietary test: ColoScape™ Colorectal Cancer Detection Lab/Manufacturer: DiaCarta Clinical Lab
0388U	Oncology (non-small cell lung cancer), next-generation sequencing with identification of single nucleotide variants, copy number variants, insertions and deletions, and structural variants in 37 cancer-related genes, plasma, with report for alteration detection Proprietary test: InVisionFirst®-Lung Liquid Biopsy Lab/Manufacturer: Inivata, Inc
0395U	Oncology (lung), multi-omics (microbial DNA by shotgun next-generation sequencing and carcinoembryonic antigen and osteopontin by immunoassay), plasma, algorithm reported as malignancy risk for lung nodules in early-stage disease Proprietary test: OncobiotaLUNG Lab/Manufacturer: Micronoma™
0410U	Oncology (pancreatic), DNA, whole genome sequencing with 5-hydroxymethylcytosine enrichment, whole blood or plasma, algorithm reported as cancer detected or not detected Proprietary test: Avantect™ Pancreatic Cancer Test Lab/Manufacturer: ClearNote™ Health

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Adamczyk, L. A., Williams, H., Frankow, A., Ellis, H. P., Haynes, H. R., Perks, C., Holly, J. M., & Kurian, K. M. (2015). Current Understanding of Circulating Tumor Cells - Potential Value in Malignancies of the Central Nervous System. *Front Neurol*, *6*, 174. https://doi.org/10.3389/fneur.2015.00174

Aggarwal, C., Meropol, N. J., Punt, C. J., Iannotti, N., Saidman, B. H., Sabbath, K. D., Gabrail, N. Y., Picus, J., Morse, M. A., Mitchell, E., Miller, M. C., & Cohen, S. J. (2013). Relationship among circulating tumor cells, CEA and overall survival in patients with metastatic colorectal cancer. *Ann Oncol*, *24*(2), 420-428. https://doi.org/10.1093/annonc/mds336

Aggarwal, C., Thompson, J. C., Black, T. A., Katz, S. I., Fan, R., Yee, S. S., Chien, A. L., Evans, T. L., Bauml, J. M., Alley, E. W., Ciunci, C. A., Berman, A. T., Cohen, R. B., Lieberman, D. B., Majmundar, K. S., Savitch, S. L., Morrissette, J. J. D., Hwang, W.-T., Elenitoba-Johnson, K. S. J., . . . Carpenter, E. L. (2019). Clinical Implications of Plasma-Based Genotyping With the Delivery of Personalized Therapy in Metastatic Non–Small Cell Lung Cancer. *JAMA Oncol*, *5*(2), 173-180. https://doi.org/10.1001/jamaoncol.2018.4305

Alix-Panabieres, C., & Pantel, K. (2013). Circulating tumor cells: liquid biopsy of cancer. *Clin Chem*, *59*(1), 110-118. https://doi.org/10.1373/clinchem.2012.194258

Andre, F., Ismaila, N., Henry, N. L., Somerfield, M. R., Bast, R. C., Barlow, W., Collyar, D. E., Hammond, M. E., Kuderer, N. M., Liu, M. C., Van Poznak, C., Wolff, A. C., & Stearns, V. (2019). Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer: ASCO Clinical Practice Guideline Update—Integration of Results From TAILORx. *Journal of Clinical Oncology*, *37*(22), 1956-1964. https://doi.org/10.1200/JCO.19.00945

Bedard, P. L., Hansen, A. R., Ratain, M. J., & Siu, L. L. (2013). Tumour heterogeneity in the clinic. *Nature*, *501*(7467), 355-364. https://doi.org/10.1038/nature12627

Bettegowda, C., Sausen, M., Leary, R. J., Kinde, I., Wang, Y., Agrawal, N., Bartlett, B. R., Wang, H., Luber, B., Alani, R. M., Antonarakis, E. S., Azad, N. S., Bardelli, A., Brem, H., Cameron, J. L., Lee, C. C., Fecher, L. A., Gallia, G. L., Gibbs, P., . . . Diaz, L. A., Jr. (2014). Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med*, *6*(224), 224ra224. https://doi.org/10.1126/scitranslmed.3007094

Bidard, F. C., Proudhon, C., & Pierga, J. Y. (2016). Circulating tumor cells in breast cancer. *Mol Oncol*, *10*(3), 418-430. https://doi.org/10.1016/j.molonc.2016.01.001
Biocept. (2023). *CNSide™ Biomarkers*. https://biocept.com/biomarkers-test/
Biodesix. (2023). GENESTRAT® GENOMIC TEST. https://www.biodesix.com/products/lung-cancer/lung-reflex/genestrat
Breitbach, S., Sterzing, B., Magallanes, C., Tug, S., & Simon, P. (2014). Direct measurement of cell-free DNA from serially collected capillary plasma during incremental exercise. *J Appl Physiol (1985)*, *117*(2), 119-130. https://doi.org/10.1152/japplphysiol.00002.2014
Brock, G., Castellanos-Rizaldos, E., Hu, L., Coticchia, C., & Skog, J. (2015). Liquid biopsy for cancer screening, patient stratification and monitoring [Review Article]. *Transl Cancer Res*, *4*. https://api.semanticscholar.org/CorpusID:31810404
Cabel, L., Proudhon, C., Gortais, H., Loirat, D., Coussy, F., Pierga, J.-Y., & Bidard, F.-C. (2017). Circulating tumor cells: clinical validity and utility. *International Journal of Clinical Oncology*, *22*(3), 421-430. https://doi.org/10.1007/s10147-017-1105-2
CellSearch. (2024). *What is a CellSearch CTC Test?* https://www.cellsearchctc.com/about-cellsearch/what-is-cellsearch-ctc-test
Circulogene. (2023). *How It Works*. https://circulogene.com/patients/how-it-works/
Clark, T. A., Chung, J. H., Kennedy, M., Hughes, J. D., Chennagiri, N., Lieber, D. S., Fendler, B., Young, L., Zhao, M., Coyne, M., Breese, V., Young, G., Donahue, A., Pavlick, D., Tsiros, A., Brennan, T., Zhong, S., Mughal, T., Bailey, M., . . . Lipson, D. (2018). Analytical Validation of a Hybrid Capture-Based Next-Generation Sequencing Clinical Assay for Genomic Profiling of Cell-Free Circulating Tumor DNA. *J Mol Diagn*, *20*(5), 686-702. https://doi.org/10.1016/j.jmoldx.2018.05.004
Cristiano, S., Leal, A., Phallen, J., Fiksel, J., Adleff, V., Bruhm, D. C., Jensen, S., Medina, J. E., Hruban, C., White, J. R., Palsgrove, D. N., Niknafs, N., Anagnostou, V., Forde, P., Naidoo, J., Marrone, K., Brahmer, J., Woodward, B. D., Husain, H., . . . Velculescu, V. E. (2019). Genome-wide cell-free DNA fragmentation in patients with cancer. *Nature*, *570*(7761), 385-389. https://doi.org/10.1038/s41586-019-1272-6
Curigliano, G. (2014). Liquid biopsies: Tumour diagnosis and treatment monitoring in a blood test | ESMO. ESMO 2014,
Dang, D. K., & Park, B. H. (2022). Circulating tumor DNA: current challenges for clinical utility. *J Clin Invest*, *132*(12). https://doi.org/10.1172/jci154941
Demopoulos, A. (2022, May 24). *Clinical features and diagnosis of leptomeningeal metastases from solid tumors*. https://www.uptodate.com/contents/clinical-features-and-diagnosis-of-leptomeningeal-metastases-from-solid-tumors
Devonshire, A. S., Whale, A. S., Gutteridge, A., Jones, G., Cowen, S., Foy, C. A., & Huggett, J. F. (2014). Towards standardisation of cell-free DNA measurement in plasma: controls for extraction efficiency, fragment size bias and quantification. *Anal Bioanal Chem*, *406*(26), 6499-6512. https://doi.org/10.1007/s00216-014-7835-3

Diaz, M., Singh, P., Kotchetkov, I., Skakodub, A., Meng, A., Tamer, C., Young, R., Reiner, A., Panageas, K., & Ramanathan, L. (2022). Quantitative Assessment of Circulating Tumor Cells in Cerebrospinal Fluid as a Clinical Tool to Predict Survival in Leptomeningeal Metastases. https://doi.org/10.1007/s11060-022-03949-1

Douillard, J.-Y., Shepherd, F. A., Hirsh, V., Mok, T., Socinski, M. A., Gervais, R., Liao, M.-L., Bischoff, H., Reck, M., Sellers, M. V., Watkins, C. L., Speake, G., Armour, A. A., & Kim, E. S. (2009). Molecular Predictors of Outcome With Gefitinib and Docetaxel in Previously Treated Non–Small-Cell Lung Cancer: Data From the Randomized Phase III INTEREST Trial. *Journal of Clinical Oncology*, *28*(5), 744-752. https://doi.org/10.1200/JCO.2009.24.3030

Douillard, J. Y., Ostoros, G., Cobo, M., Ciuleanu, T., Cole, R., McWalter, G., Walker, J., Dearden, S., Webster, A., Milenkova, T., & McCormack, R. (2014). Gefitinib treatment in EGFR mutated caucasian NSCLC: circulating-free tumor DNA as a surrogate for determination of EGFR status. *J Thorac Oncol*, *9*(9), 1345-1353. https://doi.org/10.1097/jto.0000000000000263

Dudley, J. C., Schroers-Martin, J., Lazzareschi, D. V., Shi, W. Y., Chen, S. B., Esfahani, M. S., Trivedi, D., Chabon, J. J., Chaudhuri, A. A., Stehr, H., Liu, C. L., Lim, H., Costa, H. A., Nabet, B. Y., Sin, M. L. Y., Liao, J. C., Alizadeh, A. A., & Diehn, M. (2019). Detection and Surveillance of Bladder Cancer Using Urine Tumor DNA. *Cancer Discov*, *9*(4), 500-509. https://doi.org/10.1158/2159-8290.Cd-18-0825

FDA. (2016). *Approved Drugs - cobas EGFR Mutation Test v2* [WebContent]. https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm504540.htm

Foukakis, T., & Bergh, J. (2022, November 9). *Prognostic and predictive factors in early, nonmetastatic breast cancer*. https://www.uptodate.com/contents/prognostic-and-predictive-factors-in-early-nonmetastatic-breast-cancer

FoundationOne. (2022). *Technical Specifications*. https://assets.ctfassets.net/w98cd481qyp0/wVEm7VtICYR0sT5C1VbU7/fd055e0476183a6acd4eae6b583e3a00/F1LCDx_Technical_Specs_072021.pdf

FoundationOne. (2024). *Liquid CDx*. https://www.foundationmedicine.com/test/foundationone-liquid-cdx

Gould, S. J., & Raposo, G. (2013). As we wait: coping with an imperfect nomenclature for extracellular vesicles. *J Extracell Vesicles*, *2*. https://doi.org/10.3402/jev.v2i0.20389

Grail. (2024). *ABOUT GALLERI*. https://www.galleri.com/what-is-galleri

Groot Koerkamp, B., Rahbari, N. N., Buchler, M. W., Koch, M., & Weitz, J. (2013). Circulating tumor cells and prognosis of patients with resectable colorectal liver metastases or widespread metastatic colorectal cancer: a meta-analysis. *Ann Surg Oncol*, *20*(7), 2156-2165. https://doi.org/10.1245/s10434-013-2907-8

Grover, P. K., Cummins, A. G., Price, T. J., Roberts-Thomson, I. C., & Hardingham, J. E. (2014). Circulating tumour cells: the evolving concept and the inadequacy of their

enrichment by EpCAM-based methodology for basic and clinical cancer research. *Ann Oncol*, *25*(8), 1506-1516. https://doi.org/10.1093/annonc/mdu018

Guardant. (2023). Retrieved 2/10/21 from https://guardant360cdx.com/genelist/

Haber, D. A., & Velculescu, V. E. (2014). Blood-based analyses of cancer: circulating tumor cells and circulating tumor DNA. *Cancer Discov*, *4*(6), 650-661. https://doi.org/10.1158/2159-8290.Cd-13-1014

Health, G. (2023). *Guardant360*. http://www.guardant360.com/

Huang, X., Yuan, T., Tschannen, M., Sun, Z., Jacob, H., Du, M., Liang, M., Dittmar, R. L., Liu, Y., Kohli, M., Thibodeau, S. N., Boardman, L., & Wang, L. (2013). Characterization of human plasma-derived exosomal RNAs by deep sequencing. *BMC Genomics*, *14*, 319. https://doi.org/10.1186/1471-2164-14-319

Ignatiadis, M., & Dawson, S. J. (2014). Circulating tumor cells and circulating tumor DNA for precision medicine: dream or reality? *Ann Oncol*, *25*(12), 2304-2313. https://doi.org/10.1093/annonc/mdu480

Jiang, P., Chan, C. W., Chan, K. C., Cheng, S. H., Wong, J., Wong, V. W., Wong, G. L., Chan, S. L., Mok, T. S., Chan, H. L., Lai, P. B., Chiu, R. W., & Lo, Y. M. (2015). Lengthening and shortening of plasma DNA in hepatocellular carcinoma patients. *Proc Natl Acad Sci U S A*, *112*(11), E1317-1325. https://doi.org/10.1073/pnas.1500076112

Johnson, K. S., & Sexton, D. J. (2023, August 16). *Cerebrospinal fluid: Physiology and utility of an examination in disease states*. https://www.uptodate.com/contents/cerebrospinal-fluid-physiology-and-utility-of-an-examination-in-disease-states

Kahlert, C., Melo, S. A., Protopopov, A., Tang, J., Seth, S., Koch, M., Zhang, J., Weitz, J., Chin, L., Futreal, A., & Kalluri, R. (2014). Identification of double-stranded genomic DNA spanning all chromosomes with mutated KRAS and p53 DNA in the serum exosomes of patients with pancreatic cancer. *J Biol Chem*, *289*(7), 3869-3875. https://doi.org/10.1074/jbc.C113.532267

Karabacak, N. M., Spuhler, P. S., Fachin, F., Lim, E. J., Pai, V., Ozkumur, E., Martel, J. M., Kojic, N., Smith, K., Chen, P. I., Yang, J., Hwang, H., Morgan, B., Trautwein, J., Barber, T. A., Stott, S. L., Maheswaran, S., Kapur, R., Haber, D. A., & Toner, M. (2014). Microfluidic, marker-free isolation of circulating tumor cells from blood samples. *Nat Protoc*, *9*(3), 694-710. https://doi.org/10.1038/nprot.2014.044

Kim, S. T., Banks, K. C., Lee, S.-H., Kim, K., Park, J. O., Park, S. H., Park, Y. S., Lim, H. Y., Kang, W. K., Lanman, R. B., Talasaz, A., Park, K., & Lee, J. (2017). Prospective Feasibility Study for Using Cell-Free Circulating Tumor DNA–Guided Therapy in Refractory Metastatic Solid Cancers: An Interim Analysis. *JCO Precision Oncology*, *1*(1), 1-15. https://doi.org/10.1200/PO.16.00059

Klein, E. A., Richards, D., Cohn, A., Tummala, M., Lapham, R., Cosgrove, D., Chung, G., Clement, J., Gao, J., Hunkapiller, N., Jamshidi, A., Kurtzman, K. N., Seiden, M. V., Swanton, C., & Liu, M. C. (2021). Clinical validation of a targeted methylation-based

multi-cancer early detection test using an independent validation set. *Ann Oncol*, *32*(9), 1167-1177. https://doi.org/10.1016/j.annonc.2021.05.806

Lanman, R. B., Mortimer, S. A., Zill, O. A., Sebisanovic, D., Lopez, R., Blau, S., Collisson, E. A., Divers, S. G., Hoon, D. S. B., Kopetz, E. S., Lee, J., Nikolinakos, P. G., Baca, A. M., Kermani, B. G., Eltoukhy, H., & Talasaz, A. (2015). Analytical and Clinical Validation of a Digital Sequencing Panel for Quantitative, Highly Accurate Evaluation of Cell-Free Circulating Tumor DNA. *PLoS One*, *10*(10), e0140712. https://doi.org/10.1371/journal.pone.0140712

Lee, H., Han, J., & Choi, Y.-L. (2021). Real-World Analysis of the EGFR Mutation Test in Tissue and Plasma Samples from Non-Small Cell Lung Cancer. *Diagnostics*, *11*(9), 1695. https://doi.org/10.3390/diagnostics11091695

Leighl, N. B., Page, R. D., Raymond, V. M., Daniel, D. B., Divers, S. G., Reckamp, K. L., Villalona-Calero, M. A., Dix, D., Odegaard, J. I., Lanman, R. B., & Papadimitrakopoulou, V. A. (2019). Clinical Utility of Comprehensive Cell-Free DNA Analysis to Identify Genomic Biomarkers in Patients with Newly Diagnosed Metastatic Non-Small Cell Lung Cancer. *Clinical Cancer Research*, clincanres.0624.2019. https://doi.org/10.1158/1078-0432.CCR-19-0624

Lin, X., Fleisher, M., Rosenblum, M., Lin, O., Boire, A., Briggs, S., Bensman, Y., Hurtado, B., Shagabayeva, L., DeAngelis, L. M., Panageas, K. S., Omuro, A., & Pentsova, E. I. (2017). Cerebrospinal fluid circulating tumor cells: a novel tool to diagnose leptomeningeal metastases from epithelial tumors. *Neuro Oncol*, *19*(9), 1248-1254. https://doi.org/10.1093/neuonc/nox066

Lindeman, N. I., Cagle, P. T., Aisner, D. L., Arcila, M. E., Beasley, M. B., Bernicker, E. H., Colasacco, C., Dacic, S., Hirsch, F. R., Kerr, K., Kwiatkowski, D. J., Ladanyi, M., Nowak, J. A., Sholl, L., Temple-Smolkin, R., Solomon, B., Souter, L. H., Thunnissen, E., Tsao, M. S., . . . Yatabe, Y. (2018). Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors: Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. *J Mol Diagn*, *20*(2), 129-159. https://doi.org/10.1016/j.jmoldx.2017.11.004

Lu, T., & Li, J. (2017). Clinical applications of urinary cell-free DNA in cancer: current insights and promising future. *Am J Cancer Res*, *7*(11), 2318-2332. https://pubmed.ncbi.nlm.nih.gov/29218253/

Luo, J., Shen, L., & Zheng, D. (2014). Diagnostic value of circulating free DNA for the detection of EGFR mutation status in NSCLC: a systematic review and meta-analysis. *Sci Rep*, *4*, 6269. https://doi.org/10.1038/srep06269

Marchetti, A., Del Grammastro, M., Felicioni, L., Malatesta, S., Filice, G., Centi, I., De Pas, T., Santoro, A., Chella, A., Brandes, A. A., Venturino, P., Cuccurullo, F., Crino, L., & Buttitta, F. (2014). Assessment of EGFR mutations in circulating tumor cell preparations from

NSCLC patients by next generation sequencing: toward a real-time liquid biopsy for treatment. *PLoS One*, *9*(8), e103883. https://doi.org/10.1371/journal.pone.0103883
Mathios, D., & Phallen, J. (2022). Advances in molecular biomarkers and liquid biopsy in gliomas. *Neuro-Oncology Advances*, *4*(Supplement_2), ii15-ii21. https://doi.org/10.1093/noajnl/vdac151
Mavroudis, D. (2010). Circulating cancer cells. *Ann Oncol*, *21 Suppl 7*, vii95-100. https://doi.org/10.1093/annonc/mdq378
MDx. (2023). SelectMDx for Prostate Cancer. https://mdxhealth.com/select-mdx-for-patients/
Mellert, H., Foreman, T., Jackson, L., Maar, D., Thurston, S., Koch, K., Weaver, A., Cooper, S., Dupuis, N., Sathyanarayana, U. G., Greer, J., Hahn, W., Shelton, D., Stonemetz, P., & Pestano, G. A. (2017). Development and Clinical Utility of a Blood-Based Test Service for the Rapid Identification of Actionable Mutations in Non-Small Cell Lung Carcinoma. *J Mol Diagn*, *19*(3), 404-416. https://doi.org/10.1016/j.jmoldx.2016.11.004
Mellert, H. S., Alexander, K. E., Jackson, L. P., & Pestano, G. A. (2018). A Blood-based Test for the Detection of ROS1 and RET Fusion Transcripts from Circulating Ribonucleic Acid Using Digital Polymerase Chain Reaction. *J Vis Exp*(134). https://doi.org/10.3791/57079
Merker, J. D., Oxnard, G. R., Compton, C., Diehn, M., Hurley, P., Lazar, A. J., Lindeman, N., Lockwood, C. M., Rai, A. J., Schilsky, R. L., Tsimberidou, A. M., Vasalos, P., Billman, B. L., Oliver, T. K., Bruinooge, S. S., Hayes, D. F., & Turner, N. C. (2018). Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. *Journal of Clinical Oncology*, *36*(16), 1631-1641. https://doi.org/10.1200/JCO.2017.76.8671
Miller, M. C., Doyle, G. V., & Terstappen, L. W. (2010). Significance of Circulating Tumor Cells Detected by the CellSearch System in Patients with Metastatic Breast Colorectal and Prostate Cancer. *J Oncol*, *2010*, 617421. https://doi.org/10.1155/2010/617421
Mottet, N., Cornford, P., van den Bergh, R. C. N., Briers, E., Eberli, D., De Meerleer, G., De Santis, M., Gillessen, S., Grummet, J., Henry, A. M., van Leenders, G. J. L. H., Mason, M. D., O'Hanlon, S., van Oort, I. M., Oprea-Lager, D. E., Ploussard, G., Rouvière, O., I.G., S., Stranne, J., . . . Wiegel, T. (2023). *EAU-EANM-ESTRO-ESUR-ISUP-SIOG Guidelines on Prostate Cancer Edn. presented at the EAU Annual Congress Milan 2023*. EAU Guidelines Office. https://uroweb.org/guidelines/prostate-cancer
Mouliere, F., Mair, R., Chandrananda, D., Marass, F., Smith, C. G., Su, J., Morris, J., Watts, C., Brindle, K. M., & Rosenfeld, N. (2018). Detection of cell-free DNA fragmentation and copy number alterations in cerebrospinal fluid from glioma patients. *EMBO Mol Med*, *10*(12). https://doi.org/10.15252/emmm.201809323
Murray, N. P., Reyes, E., Badinez, L., Orellana, N., Fuentealba, C., Olivares, R., Porcell, J., & Duenas, R. (2013). Circulating Prostate Cells Found in Men with Benign Prostate

Disease Are P504S Negative: Clinical Implications. *J Oncol*, *2013*, 165014. https://doi.org/10.1155/2013/165014
NCCN. (2023a, September 7). *NCCN Clinical Practice Guidelines for Prostate Cancer version 4.2023*. https://www.nccn.org/professionals/physician_gls/pdf/prostate.pdf
NCCN. (2023b, October 24). *NCCN Clinical Practice Guidelines in Oncology for Acute Myeloid Leukemia version 6.2023*. https://www.nccn.org/professionals/physician_gls/pdf/aml.pdf
NCCN. (2023c, November 8). *NCCN Clinical Practice Guidelines in Oncology for Biliary Tract Cancers version 3.2023*. https://www.nccn.org/professionals/physician_gls/pdf/btc.pdf
NCCN. (2023d, May 25). *NCCN Clinical Practice Guidelines in Oncology for Bladder Cancer version 3.2023*. https://www.nccn.org/professionals/physician_gls/pdf/bladder.pdf
NCCN. (2023e, December 5). *NCCN Clinical Practice Guidelines in Oncology for Breast Cancer version 5.2023*. https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf
NCCN. (2023f, March 24). *NCCN Clinical Practice Guidelines in Oncology for Central Nervous System Cancers version 1.2023*. https://www.nccn.org/professionals/physician_gls/pdf/cns.pdf
NCCN. (2023g, November 16). *NCCN Clinical Practice Guidelines in Oncology for Colon Cancer version 4.2023*. https://www.nccn.org/professionals/physician_gls/pdf/colon.pdf
NCCN. (2023h, August 29). *NCCN Clinical Practice Guidelines in Oncology for Esophageal and Esophagogastric Junction Cancers version 3.2023*. https://www.nccn.org/professionals/physician_gls/pdf/esophageal.pdf
NCCN. (2023i, September 14). *NCCN Clinical Practice Guidelines in Oncology for Hepatocellular Carcinoma version 2.2023*. https://www.nccn.org/professionals/physician_gls/pdf/hcc.pdf
NCCN. (2023j, August 2). *NCCN Clinical Practice Guidelines in Oncology for Neuroendocrine and Adrenal Tumors version 1.2023*. https://www.nccn.org/professionals/physician_gls/pdf/neuroendocrine.pdf
NCCN. (2023k, November 8). *NCCN Clinical Practice Guidelines in Oncology for Non-Small Cell Lung Cancer version 5.2023*. https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf
NCCN. (2023l, December 13). *NCCN Clinical Practice Guidelines in Oncology for Pancreatic Adenocarcinoma version 1.2024*. https://www.nccn.org/professionals/physician_gls/pdf/pancreatic.pdf
NCCN. (2023m, September 26). *NCCN Clinical Practice Guidelines in Oncology for Prostate Cancer Early Detection version 2.2023*. https://www.nccn.org/professionals/physician_gls/pdf/prostate_detection.pdf

NCCN. (2023n, November 21). *NCCN Clinical Practice Guidelines in Oncology for Small Cell Lung Cancer version 2.2024*. https://www.nccn.org/professionals/physician_gls/pdf/sclc.pdf

Neogenomics. (2023). *InvisionFirst-Lung (Liquid Biopsy)*. https://neogenomics.com/patients/invisionfirstr-lung-liquid-biopsy

NIH. (2024). Extracellular RNA Communication - Home | NIH Common Fund. https://commonfund.nih.gov/exrna/faq

Odegaard, J. I., Vincent, J. J., Mortimer, S., Vowles, J. V., Ulrich, B. C., Banks, K. C., Fairclough, S. R., Zill, O. A., Sikora, M., Mokhtari, R., Abdueva, D., Nagy, R. J., Lee, C. E., Kiedrowski, L. A., Paweletz, C. P., Eltoukhy, H., Lanman, R. B., Chudova, D. I., & Talasaz, A. (2018). Validation of a Plasma-Based Comprehensive Cancer Genotyping Assay Utilizing Orthogonal Tissue- and Plasma-Based Methodologies. *Clin Cancer Res*, *24*(15), 3539-3549. https://doi.org/10.1158/1078-0432.Ccr-17-3831

Oxnard, G. R., Thress, K. S., Alden, R. S., Lawrance, R., Paweletz, C. P., Cantarini, M., Yang, J. C., Barrett, J. C., & Janne, P. A. (2016). Association Between Plasma Genotyping and Outcomes of Treatment With Osimertinib (AZD9291) in Advanced Non-Small-Cell Lung Cancer. *J Clin Oncol*, *34*(28), 3375-3382. https://doi.org/10.1200/jco.2016.66.7162

Pinzani, P., D'Argenio, V., Del Re, M., Pellegrini, C., Cucchiara, F., Salvianti, F., & Galbiati, S. (2021). Updates on liquid biopsy: current trends and future perspectives for clinical application in solid tumors. *Clin Chem Lab Med*. https://doi.org/10.1515/cclm-2020-1685

Quach, N., Goodman, M. F., & Shibata, D. (2004). In vitro mutation artifacts after formalin fixation and error prone translesion synthesis during PCR. *BMC Clin Pathol*, *4*(1), 1. https://doi.org/10.1186/1472-6890-4-1

ResolutionBio. (2024). Resolution ctDx Lung™. https://www.resolutionbio.com/assays/Resolution-ctDx-Lung.html

Rolfo, C., Mack, P., Scagliotti, G. V., Aggarwal, C., Arcila, M. E., Barlesi, F., Bivona, T., Diehn, M., Dive, C., Dziadziuszko, R., Leighl, N., Malapelle, U., Mok, T., Peled, N., Raez, L. E., Sequist, L., Sholl, L., Swanton, C., Abbosh, C., . . . Gandara, D. (2021). Liquid Biopsy for Advanced NSCLC: A Consensus Statement From the International Association for the Study of Lung Cancer. *Journal of Thoracic Oncology*, *16*(10), 1647-1662. https://doi.org/10.1016/j.jtho.2021.06.017

Sacher, A. G., Paweletz, C., Dahlberg, S. E., Alden, R. S., O'Connell, A., Feeney, N., Mach, S. L., Janne, P. A., & Oxnard, G. R. (2016). Prospective Validation of Rapid Plasma Genotyping for the Detection of EGFR and KRAS Mutations in Advanced Lung Cancer. *JAMA Oncol*, *2*(8), 1014-1022. https://doi.org/10.1001/jamaoncol.2016.0173

Schneider, B. J., Ismaila, N., Aerts, J., Chiles, C., Daly, M. E., Detterbeck, F. C., Hearn, J. W. D., Katz, S. I., Leighl, N. B., Levy, B., Meyers, B., Murgu, S., Nekhlyudov, L., Santos, E. S., Singh, N., Tashbar, J., Yankelevitz, D., & Altorki, N. (2019). Lung Cancer Surveillance

After Definitive Curative-Intent Therapy: ASCO Guideline. *Journal of Clinical Oncology*, JCO.19.02748. https://doi.org/10.1200/JCO.19.02748

Seeberg, L. T., Waage, A., Brunborg, C., Hugenschmidt, H., Renolen, A., Stav, I., Bjornbeth, B. A., Brudvik, K. W., Borgen, E. F., Naume, B., & Wiedswang, G. (2015). Circulating tumor cells in patients with colorectal liver metastasis predict impaired survival. *Ann Surg*, *261*(1), 164-171. https://doi.org/10.1097/sla.0000000000000580

Sepulveda, A. R., Hamilton, S. R., Allegra, C. J., Grody, W., Cushman-Vokoun, A. M., Funkhouser, W. K., Kopetz, S. E., Lieu, C., Lindor, N. M., Minsky, B. D., Monzon, F. A., Sargent, D. J., Singh, V. M., Willis, J., Clark, J., Colasacco, C., Rumble, R. B., Temple-Smolkin, R., Ventura, C. B., & Nowak, J. A. (2017). Molecular Biomarkers for the Evaluation of Colorectal Cancer: Guideline From the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology. *J Mol Diagn*, *19*(2), 187-225. https://doi.org/10.1016/j.jmoldx.2016.11.001

Sequist, L. V., & Neal, J. W. (2024, February 1). *Personalized, genotype-directed therapy for advanced non-small cell lung cancer*. https://www.uptodate.com/contents/personalized-genotype-directed-therapy-for-advanced-non-small-cell-lung-cancer

Shore, N., Hafron, J., Langford, T., Stein, M., DeHart, J., Brawer, M., Hessels, D., Schalken, J., Criekinge, W. V., Groskopf, J., & Wojno, K. (2019). Urinary Molecular Biomarker Test Impacts Prostate Biopsy Decision Making in Clinical Practice. *Urology Practice*, *6*(4), 256-261. https://doi.org/doi:10.1016/j.urpr.2018.09.002

Sturgeon, C. M., Duffy, M. J., Hofmann, B. R., Lamerz, R., Fritsche, H. A., Gaarenstroom, K., Bonfrer, J., Ecke, T. H., Grossman, H. B., Hayes, P., Hoffmann, R.-T., Lerner, S. P., Löhe, F., Louhimo, J., Sawczuk, I., Taketa, K., & Diamandis, E. P. (2020). National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for Use of Tumor Markers in Liver, Bladder, Cervical, and Gastric Cancers. *Clin Chem*, *56*(6), e1-e48. https://doi.org/10.1373/clinchem.2009.133124

Sturgeon, C. M., Duffy, M. J., Hofmann, B. R., Lamerz, R., Fritsche, H. A., Gaarenstroom, K., Bonfrer, J., Ecke, T. H., Grossman, H. B., Hayes, P., Hoffmann, R. T., Lerner, S. P., Lohe, F., Louhimo, J., Sawczuk, I., Taketa, K., & Diamandis, E. P. (2010). National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for use of tumor markers in liver, bladder, cervical, and gastric cancers. *Clin Chem*, *56*(6), e1-48. https://doi.org/10.1373/clinchem.2009.133124

Syeda, M. M., Wiggins, J. M., Corless, B. C., Long, G. V., Flaherty, K. T., Schadendorf, D., Nathan, P. D., Robert, C., Ribas, A., Davies, M. A., Grob, J. J., Gasal, E., Squires, M., Marker, M., Garrett, J., Brase, J. C., & Polsky, D. (2021). Circulating tumour DNA in patients with advanced melanoma treated with dabrafenib or dabrafenib plus trametinib: a clinical validation study. *Lancet Oncol*, *22*(3), 370-380. https://doi.org/10.1016/s1470-2045(20)30726-9

Thum, T., & Condorelli, G. (2015). Long noncoding RNAs and microRNAs in cardiovascular pathophysiology. *Circ Res*, *116*(4), 751-762. https://doi.org/10.1161/circresaha.116.303549

Turnbull C., W. N., Sullivan R., Pharoah P., Houlston R., Aggarwal A., Hogarth S., McCartney M. (2024). GRAIL-Galleri: why the special treatment? *The Lancet*, *403*(10425), 431-432. https://doi.org/10.1016/S0140-6736(23)02830-1

Van Neste, L., Hendriks, R. J., Dijkstra, S., Trooskens, G., Cornel, E. B., Jannink, S. A., de Jong, H., Hessels, D., Smit, F. P., Melchers, W. J., Leyten, G. H., de Reijke, T. M., Vergunst, H., Kil, P., Knipscheer, B. C., Hulsbergen-van de Kaa, C. A., Mulders, P. F., van Oort, I. M., Van Criekinge, W., & Schalken, J. A. (2016). Detection of High-grade Prostate Cancer Using a Urinary Molecular Biomarker-Based Risk Score. *Eur Urol*, *70*(5), 740-748. https://doi.org/10.1016/j.eururo.2016.04.012

Van Poznak, C., Somerfield, M. R., Bast, R. C., Cristofanilli, M., Goetz, M. P., Gonzalez-Angulo, A. M., Hicks, D. G., Hill, E. G., Liu, M. C., Lucas, W., Mayer, I. A., Mennel, R. G., Symmans, W. F., Hayes, D. F., & Harris, L. N. (2015). Use of Biomarkers to Guide Decisions on Systemic Therapy for Women With Metastatic Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol*, *33*(24), 2695-2704. https://doi.org/10.1200/JCO.2015.61.1459

Vogel, J. D., Felder, S. I., Bhama, A. R., Hawkins, A. T., Langenfeld, S. J., Shaffer, V. O., Thorsen, A. J., Weiser, M. R., Chang, G. J., Lightner, A. L., Feingold, D. L., & Paquette, I. M. (2022). The American Society of Colon and Rectal Surgeons Clinical Practice Guidelines for the Management of Colon Cancer. *Dis Colon Rectum*, *65*(2), 148-177. https://doi.org/10.1097/dcr.0000000000002323

Waki, K., Yokomizo, K., Yoshiyama, K., Takamori, S., Komatsu, N., & Yamada, A. (2021). Integrity of circulating cell-free DNA as a prognostic biomarker for vaccine therapy in patients with nonsmall cell lung cancer. *Immunopharmacol Immunotoxicol*, 1-14. https://doi.org/10.1080/08923973.2021.1872619

Wang, X. S., Zhao, M. Q., Zhang, L., Kong, D. J., Ding, X. Z., Hu, X. C., Yang, J. Q., & Gao, S. G. (2018). Cell-free DNA in blood and urine as a diagnostic tool for bladder cancer: a meta-analysis. *Am J Transl Res*, *10*(7), 1935-1948. https://pubmed.ncbi.nlm.nih.gov/30093933/

Whiteside, T. L. (2013). Immune modulation of T-cell and NK (natural killer) cell activities by TEXs (tumour-derived exosomes). *Biochem Soc Trans*, *41*(1), 245-251. https://doi.org/10.1042/bst20120265

Wu, Y. L., Planchard, D., Lu, S., Sun, H., Yamamoto, N., Kim, D. W., Tan, D. S. W., Yang, J. C. H., Azrif, M., Mitsudomi, T., Park, K., Soo, R. A., Chang, J. W. C., Alip, A., Peters, S., & Douillard, J. Y. (2018). Pan-Asian adapted Clinical Practice Guidelines for the management of patients with metastatic non-small-cell lung cancer: a CSCO–ESMO initiative endorsed by JSMO, KSMO, MOS, SSO and TOS. *Annals of Oncology*, *30*(2), 171-210. https://doi.org/10.1093/annonc/mdy554

Wu, Y. L., Zhou, C., Liam, C. K., Wu, G., Liu, X., Zhong, Z., Lu, S., Cheng, Y., Han, B., Chen, L., Huang, C., Qin, S., Zhu, Y., Pan, H., Liang, H., Li, E., Jiang, G., How, S. H., Fernando, M. C., . . . Zuo, Y. (2015). First-line erlotinib versus gemcitabine/cisplatin in patients with advanced EGFR mutation-positive non-small-cell lung cancer: analyses from the phase III, randomized, open-label, ENSURE study. *Ann Oncol*, *26*(9), 1883-1889. https://doi.org/10.1093/annonc/mdv270

Xu, Y., Lou, J., Yu, M., Jiang, Y., Xu, H., Huang, Y., Gao, Y., Wang, H., Li, G., Wang, Z., & Zhao, A. (2021). Urinary Exosomes Diagnosis of Urological Tumors: A Systematic Review and Meta-Analysis. *Front Oncol*, *11*, 734587. https://doi.org/10.3389/fonc.2021.734587

Yanez-Mo, M., Siljander, P. R., Andreu, Z., Zavec, A. B., Borras, F. E., Buzas, E. I., Buzas, K., Casal, E., Cappello, F., Carvalho, J., Colas, E., Cordeiro-da Silva, A., Fais, S., Falcon-Perez, J. M., Ghobrial, I. M., Giebel, B., Gimona, M., Graner, M., Gursel, I., . . . De Wever, O. (2015). Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles*, *4*, 27066. https://doi.org/10.3402/jev.v4.27066

Yu, W., Hurley, J., Roberts, D., Chakrabortty, S., Enderle, D., Noerholm, M., Breakefield, X. O., & Skog, J. (2021). Exosome-based Liquid Biopsies in Cancer: Opportunities and Challenges. *Ann Oncol*. https://doi.org/10.1016/j.annonc.2021.01.074

Zhang, L., Riethdorf, S., Wu, G., Wang, T., Yang, K., Peng, G., Liu, J., & Pantel, K. (2012). Meta-analysis of the prognostic value of circulating tumor cells in breast cancer. *Clin Cancer Res*, *18*(20), 5701-5710. https://doi.org/10.1158/1078-0432.ccr-12-1587

Lynch Syndrome

Policy Number: AHS – M2004 – Lynch Syndrome	Prior Policy Name and Number, as applicable:
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Lynch syndrome (LS) (also known as hereditary non-polyposis colorectal cancer; HNPCC) is the most common form of hereditary colorectal (CRC) and endometrial cancers (EMC), resulting from an autosomal dominant inactivation of any of four mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) leading to microsatellite instability (MSI) (Rumilla et al., 2011) and associated with an increased risk of colorectal, endometrial, stomach, small bowel, and ovarian cancers (Hunter et al., 2015; Lynch et al., 2009; Moreira et al., 2012).

For guidance concerning Tumor Mutational Burden Testing (TMB) and/or Microsatellite instability (MSI) analysis please refer to the AHS-M2178-Microsatellite Instability and Tumor Mutational Burden Testing policy.

II. Related Policies

Policy Number	Policy Title
AHS-M2003	Genetic Testing for Breast, Ovarian, Pancreatic, and Prostate
AHS-M2024	Genetic Testing for Polyposis Syndromes
AHS-M2026	Testing for Colorectal Cancer Management
AHS-M2178	Microsatellite Instability and Tumor Mutational Burden Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

Consideration of both maternal and paternal family histories is necessary in the evaluation of individuals for risk of carrying a Lynch syndrome gene mutation; each lineage must be considered separately.

1) For asymptomatic individuals in a family with a pathogenic familial Lynch Syndrome (LS) gene mutation who are at least 18 years of age and who have received genetic counseling, the following testing **MEETS COVERAGE CRITERIA**:
    a) Testing restricted to the known familial mutation.
    b) Comprehensive genetic testing, including muti-gene panel testing, when the specific familial mutation is unknown.

2) For individuals with a diagnosis of any LS-related cancer (see Note 1) who have received genetic counseling, multi-gene panel testing (see Note 2, Note 3) **MEETS COVERAGE CRITERIA** when one of the following conditions is met:
    a) When the individual has a personal history of a tumor with MMR deficiency, determined by PCR, NGS, or IHC.
    b) When the individual was diagnosed before 50 years of age.
    c) When the individual has at any age had one or more additional LS-related cancers.
    d) When the individual has at least one first- or second-degree relative diagnosed before 50 years of age with LS-related cancer.
    e) When the individual has at least two first- or second-degree relatives diagnosed at any age with LS-related cancers.
    f) The individual has a gene mutation associated with LS-related cancers that was detected by tumor genomic profiling in the absence of germline mutation testing.

3) For individuals with a known family history (see Note 4) of LS related cancer (see Note 1) who have received genetic counseling and are at least 18 years of age, multi-gene panel testing (see Note 2, Note 3) **MEETS COVERAGE CRITERIA** only if the family mutation is unknown (i.e., family member is unavailable for testing or testing results are unavailable) and one of the following conditions is met:
    a) The individual has at least one first-degree relative diagnosed before 50 years of age with LS-related cancer diagnosed.
    b) The individual has at least one first-degree relative diagnosed with LS-related cancer and another synchronous or metachronous LS-related cancer.

c) The individual has at least two first- or second-degree relatives diagnosed with LS-related cancer, with at least one of the relatives diagnosed by 50 years of age.

d) The individual has at least three first- or second-degree relatives diagnosed with LS-related cancers, regardless of their age at diagnosis.

e) The individual has at least a 5% risk of having a pathogenic MMR gene variant based on predictive models (PREMM5, MMRpro, MMRpredict).

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

4) For all other purposes, including, but not limited to, testing of the general population, genetic testing for susceptibility to LS related cancer **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: According to the NCCN, "LS-related cancers include colorectal, endometrial, gastric, ovarian, pancreas, urothelial [renal pelvis, ureter, and/or bladder], brain . . . , biliary tract, and small intestinal cancers, as well as sebaceous adenomas, sebaceous carcinomas, and keratoacanthomas as seen in Muir-Torre syndrome" (NCCN, 2023).

Note 2: When germline multigene panel testing is performed in individuals with LS-related cancer, the panel should include "at minimum the following CRC risk-associated genes: *APC, MUTYH, MLH1, MSH2, MSH6, PMS2, EPCAM, BMPR1A, SMAD4, PTEN, STK11,* and *TP53*" (NCCN, 2023).

Note 3: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

Note 4: Close blood relatives include 1st-degree relatives (e.g., parents, siblings, and children), 2nd-degree relatives (e.g., grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings), and 3rd-degree relatives (great-grandparents, great-aunts, great-uncles, great-grandchildren, and first cousins), all of whom are on the same side of the family.

IV. Table of Terminology

Term	Definition
ACG	American College of Gastroenterology

AMP	Association for Molecular Pathology
APC	*Adenomatous polyposis coli*
ASCO	American Society of Clinical Oncology
ASCP	American Society for Clinical Pathology
AUC	Area under the curve
CAP	College of American Pathologists
CGA-IGC	Collaborative Group of the Americas on Inherited Gastrointestinal Cancer
CLIA	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid Services
CRC	Colorectal cancer
DFCI	Dana-Farber Cancer Institute (Harvard)
EGAPP	Evaluation of Genomic Applications in Practice and Prevention
EMC	Endometrial cancers
EPCAM	*Epithelial cellular adhesion molecule*
ESMO	European Society for Medical Oncology
GCU	Genetic counselling unit
GEMCAD	Grupo Español Multidisciplinar de Cáncer Digestivo
GREM1	*Gremlin 1*
HNPCC	Hereditary non-polyposis colorectal cancer
ICG-HNPCC	The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer
IHC	Immunohistochemistry
LDTs	Laboratory-developed tests
LS	Lynch syndrome
MGPT	Multigene panel test
MLH1	*MutL homolog 1*
MLH6	*MutL homolog 6*
MMR	Mismatch repair
MMR-D	Mismach repair protein deficiency
MSH2	*MutS homolog 2*
MSH6	*MutS homolog 6*
MSI	Microsatellite instability
MUTYH	*MutY DNA glycosylase*
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NGS	Next-generation sequencing

NICE	National Institute for Health and Care Excellence
NSGC	National Society of Genetic Counsellors
NTHL1	*Nth like deoxyribonucleic acid glycosylase 1*
O/E	Observed-to-expected ratio
PCR	Polymerase chain reaction
PMS2	*Post meiotic segregation increased 2 (S. cerevisiae)/PMS1 homolog 2*
POLD1	*Deoxyribonucleic acid polymerase delta 1*
POLE	*Deoxyribonucleic acid polymerase epsilon, catalytic subunit*
PREMM5	Prediction model for gene mutations 5
SEOM	Spanish Society of Medical Oncology
TP53	*Tumor protein 53*
TTD	Grupo Español de Tumores Digestivos
UGI	Upper gastrointestinal
USMSTF	United States Multi-Society Task Force

V. Scientific Background

Lynch syndrome (LS) is recognized by a hereditary predisposition to colorectal, endometrial, and other cancers due to inactivation by germline mutations or epigenetic silencing in any of four DNA mismatch repair genes—*MLH1, MSH2, MSH6,* and *PMS2*. Mutations in *MLH1* and *MSH2* are most common (90%) followed by *MSH6* (10%) and *PMS2* (6%) (Jansen et al., 2014). Mutations of the upstream *EPCAM* gene which result in silencing of the *MSH2* gene produce a phenotype very similar to LS (Ligtenberg et al., 2009). LS accounts for approximately 3% to 5% of all colorectal cancers (Yilmaz et al., 2020) and 2% to 5% of endometrial cancers (Hampel et al., 2005). In addition to colorectal and endometrial cancers, patients may present with ovarian, urinary tract, stomach, small bowel, hepatobiliary, sebaceous gland and central nervous system neoplasms (Barrow et al., 2013).

The lifetime risk of colorectal cancer (CRC) is greatly increased in LS patients but varies significantly from 10-74% dependent on which MMR gene is inactivated (Brosens et al., 2015). The average age at CRC diagnosis in LS patients is 44 to 61 years with tumors primarily arising proximal to the splenic flexure (Giardiello et al., 2014). There is also a high rate of metachronous CRC (16% at 10 years; 41% at 20 years) in LS patients (Win et al., 2013). The histopathology of LS colorectal cancer is often poorly differentiated with signet cell histology, abundant extracellular mucin, tumor infiltrating lymphocytes, and a lymphoid host response to tumor (Peltomäki PT, 2010). LS patients have improved survival rates compared to similar stage spontaneous CRC (Brosens et al., 2015). Lifetime risk of endometrial cancer is significantly increased to 15 – 71% of

individuals with mutation specific variability (Giardiello et al., 2014). Increased lifetime risks have also been observed in urinary, ovarian, stomach, hepatobiliary, small bowel, brain, pancreatic and prostate cancers (Brosens et al., 2015).

Cancer Risks in Individuals with Lynch Syndrome Age ≤70 Years Compared to the General Population (Brosens et al., 2015).

Cancer Type	General Population Risk	Lynch Syndrome (*MLH1* and *MSH2* heterozygotes)	
		Risk	Mean Age of Onset
Colon	4.8%	52%-82%	44-61 years
Endometrium	2.7%	25%-60%	48-62 years
Stomach	<1%	6%-13%	56 years
Ovary	1.4%	4%-12%	42.5 years
Hepatobiliary tract	<1%	1.4%-4%	Not reported
Urinary tract	<1%	1%-4%	~55 years
Small bowel	<1%	3%-6%	49 years
Brain/central nervous	<1%	1%-3%	~50 years
Sebaceous neoplasms	<1%	1%-9%	Not reported

Several sets of clinical criteria have been developed to identify patients with LS. In 1990, the International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer (HNPCC) established criteria (Amsterdam I Criteria) for HNPCC (Vasen et al., 1991), which were updated to be more sensitive in 1999 (Vasen et al., 1999). The Revised Bethesda Guidelines are a third set of clinicopathologic criteria developed in 2004 to improve identification of individuals who deserve investigation for LS; however, they state, "The goal of the Bethesda Guidelines is to identify HNPCC patients, not to identify MSI-H tumors from patients in sporadic populations that may have better prognoses or different therapeutic implications" (Umar et al., 2004).

Analytical Validity

Currently, there exist two main approaches to diagnosing Lynch syndrome. One approach leverages molecular screening of colorectal and endometrial tumor specimens for evidence of defective MMR function (MMR-D) or high-level MSI (MSI-H) to identify patients with cancer who should undergo germline testing for pathogenic MMR gene variants. The other focuses on using direct germline testing performed on patients whose family histories of cancer are suspicious for Lynch syndrome. In recent years, molecular testing has gained traction for identification of individuals with Lynch syndrome due to its robust sensitivity and specificity, testing of which can be

generalized into one of four categories: polymerase chain reaction (PCR)-based MSI testing, immunohistochemical staining (or immunohistochemistry [IHC]) for the MMR proteins, *MLH1* promoter methylation analysis (or somatic *BRAF V600E* mutation analysis), and next-generation somatic (and/or germline) sequencing assays (Yurgelun & Hampel, 2018).

The specificity and sensitivity of these methods can be polemical, and thus engender questions of what tests to even employ. Stinton et al. (2021) conducted a systematic review of literature published up to August 2019 to assess the immunohistochemistry and microsatellite instability-based testing (with or without *MLH1* promoter methylation testing) for Lynch syndrome in individuals with endometrial cancer. Thirteen studies consisting of approximately 3500 people were examined, and the researchers determined that, after adjusting for studies with highly selective inclusion criteria, sensitivity ranged from 60.9%-83.3% for immunohistochemistry, 69.2-89.9% for microsatellite instability-based testing, and 72.4-92.3% for studies combining immunohistochemistry, microsatellite instability-based testing, and *MLH1* promoter methylation testing. According to the authors, they "found no statistically significant differences in test accuracy estimates (sensitivity, specificity) in head-to-head studies of immunohistochemistry versus microsatellite instability-based testing" and thus concluded that "sensitivity of the index tests were generally high, though most studies had much lower specificity." However, though the authors "found no evidence that test accuracy differed between IHC and MSI based strategies", they acknowledged that the evidence base is still quite small and at risk of bias (Stinton et al., 2021).

The complexity of Lynch syndrome likewise evokes the use of complex diagnostic algorithms, oftentimes involving multiple subsequent germline and somatic tests. The utility and efficacy of these algorithms are also points of contention, given the novelty of said algorithms. Through retrospectively reviewing a consecutive series of 702 patients with colorectal cancer and endometrial cancer undergoing paired tumor/germline analysis of the LS genes at a clinical diagnostic laboratory, Salvador et al. (2019) asserted that "Paired testing identified a cause for MMRd tumors in 76% and 61% of patients without and with prior LS germline testing, respectively," leading the researchers to support inclusion of tumor sequencing as well as comprehensive LS germline testing in the LS testing algorithm.

Statistical models to predict risk of MMR mutations include PREMM5, MMRpredict, and MMRpro. The PREMM5 clinical prediction algorithm, available at http://premm.dfci.harvard.edu/, "estimates the cumulative probability of an individual carrying a germline mutation in the *MLH1, MSH2, MSH6, PMS2,* or *EPCAM* genes" using an individual's personal and family history of colorectal cancer, endometrial cancer, or

other LS-related cancers with the results given as a percentage of overall predicted probability of mutation in one of the four LS-related genes (DFCI, 2020). A study using the clinical and germline data from more than 18,000 individuals published in 2017 validated the use of the PREMM5 model. The report shows that for the four LS-related genes, PREMM5 can distinguish "carriers from noncarriers with an area under the curve (AUC) of 0.81 (95% CI, 0.79 to 0.82), and performance was similar in the validation cohort (AUC, 0.83; 95% CI, 0.75 to 0.92). Prediction was more difficult for *PMS2* mutations (AUC, 0.64; 95% CI, 0.60 to 0.68) than for other genes." The authors conclude, " $PREMM_5$ provides comprehensive risk estimation of all five LS genes and supports LS genetic testing for individuals with scores ≥ 2.5%" (Kastrinos et al., 2017). Kastrinos et al. (2018) published another article the following year stating that a threshold of ≥ 2.5% is now recommended to improve the identification of *PMS2* carriers by enhancing the model's sensitivity (a threshold of ≥ 5% was previously recommended).

MMRpro, statistical model and software using family history of colorectal and endometrial cancers, is available for free download at http://www4.utsouthwestern.edu/breasthealth/cagene/. "The results give useful information about an individual's colon cancer risk before he or she decides to undergo invasive screenings or expensive genetic testing" (Harvard, 2019). A study released in 2015 concluded that MMRpro was comparable to the PREMM1,2,6 model in discriminating both clinic- and population-based cohorts (Kastrinos et al., 2016). Another study in 2017 investigated the use of MMRpro in predicting *MLH1* mutations since, unlike the other LS-related genes, immunohistochemistry is less sensitive as a prescreening test for these mutations. By limiting the scope of the study to *MLH1* mutations, MMRpro outperforms the PREMM1,2,6 algorithm (AUC 0.83 versus 0.68, respectively). The authors state, "Considering a threshold of 5%, MMRpro would eliminate unnecessary germline mutation analysis in a significant proportion of cases while keeping very high sensitivity. We conclude that MMRpro is useful to correctly predict who should be screened for a germline *MLH1* gene mutation and propose an algorithm to improve the cost-effectiveness of LS diagnosis" (Cabreira et al., 2017).

Likewise, the MMRpredict algorithm, available at http://hnpccpredict.hgu.mrc.ac.uk/, is jointly operated by the Colon Cancer Genetics Group at the University of Edinburgh and MRC Human Genetics Unit of Edinburgh. This algorithm predicts the probability of a mutation carrier of an affected individual using criteria consisting of the age at time of diagnosis, gender, tumor location, synchronicity of tumor, and family history (MRC, 2023). A 2018 study shows that MMRpredict performs better than the PREMM5 model in identifying *PMS2* mutation carriers (AUCs 0.72 and 0.51, respectively), and the efficacy of the PREMM5 model is more dependent on the location of the tumor. Both

algorithms were comparable in predicting *MLH1* and *MSH2* mutation carriers (Goverde et al., 2018). These data apparently contradict earlier findings where a previous version of the PREMM model, PREMM1,2,6, performed better than MMRpredict in predicting carriers of *MLH1, MSH2*, or *MSH6* gene mutations. "For clinic- and population-based cohorts, O/E [observed-to-expected ratio] deviated from 1 for MMRPredict (0.38 and 0.31, respectively) and MMRPro (0.62 and 0.36) but were more satisfactory for PREMM1,2,6 (1.0 and 0.70). MMRPro or PREMM1,2,6 predictions were clinically useful at thresholds of 5% or greater and in particular at greater than 15%" (Kastrinos et al., 2016).

Mercado et al. (2012) published a study to assess the sensitivity and specificity of PREMM1,2,6, MMRpredict, and MMRpro in 692 endometrial cancer cases (563 population-based and 129 clinic-based cases). Pathogenic mutations were identified in 3% of the population-based participants and in 62% of the clinic-based participants. "PREMM(1,2,6), MMRpredict, and MMRpro were able to distinguish mutation carriers from noncarriers (AUC of 0.77, 0.76, and 0.77, respectively), among population-based cases. All three models had lower discrimination for the clinic-based cohort, with AUCs of 0.67, 0.64, and 0.54, respectively" (Mercado et al., 2012). For PREMM1,2,6, a sensitivity of 93% and a specificity of 5% was identified in population-based participants and a sensitivity of 99% and specificity of 2% was identified in clinic-based cases. For MMRpredict, a sensitivity of 71% and a specificity of 64% was identified in population-based participants and a sensitivity of 90% and specificity of 0% was identified in clinic-based cases. For MMRpro, a sensitivity of 57% and a specificity of 85% was identified in population-based participants and a sensitivity of 95% and specificity of 10% was identified in clinic-based cases (Mercado et al., 2012). These authors state that the PREMM1,2,6, MMRpredict, and MMRpro seem to have limited utility in the determining which endometrial cancer patients would benefit from Lynch syndrome testing.

Clinical Utility and Validity

As use of clinical criteria and modeling to identify patients with LS has less than optimal sensitivity and can vary in efficacy between different ethnic populations (Lee et al., 2016), universal screening for LS has been recommended (Cohen et al., 2016; Kidambi et al., 2015). Analysis by immunohistochemical testing for the MLH1/MSH2/MSH6/PMS2 proteins and/or MSI testing are commonly used to screen for LS phenotypes (Syngal et al., 2015). Tumors with loss of *MLH1* should undergo analysis to exclude *BRAF* mutation or *MLH1* promoter hypermethylation according to the USPSTF (Giardiello et al., 2014). Moreover, patients with evidence of LS should be

referred for genetic evaluation (EGAPP, 2009; Robson et al., 2015; Sepulveda et al., 2017).

Adar et al. (2018) completed a study to determine the value of screening both CRC and endometrial cancer (EMC) tumors in the same population. An immunohistochemistry (IHC) screening program evaluated all patients at two centers newly diagnosed with CRC and/or EMCs. "Genetic testing was recommended for those who had tumors with absent mutS homolog 2 (*MSH2*), *MSH6*, or postmeiotoic segregation increased 2 (*PMS2*) expression and for those who had tumors with absent mutL homolog 1 (*MLH1*) expression and no v-Raf murine sarcoma viral oncogene homolog B (*BRAF*) mutation or *MLH1* promoter methylation" (Adar et al., 2018). Scores from the PREMM1,2,6 and PREMM5 prediction models were also obtained, along with traditional Amsterdam II criteria and revised Bethesda criteria. Of the 1774 total patients screened for LS (1290 with CRC and 484 with EMC), genetic testing was recommended for 169 patients. LS was diagnosed in 16 patients with CRC and 8 patients with EMC based on traditional detection methods (Amsterdam II criteria, revised Bethesda criteria, PREMM1,2,6 and PREMM5 prediction models). Of the patients genetically tested, the LS diagnosis rate was higher. Specifically, "The Amsterdam II criteria, revised Bethesda criteria, and both PREMM calculators would have missed 62.5%, 50.0%, and 12.5% of the identified patients with LS, respectively" (Adar et al., 2018). The results of this study show that risk assessment tools are likely to miss a percentage of LS diagnoses.

Laish et al. (2021) conducted a retrospective cohort study on young patients with colorectal adenomatous polyps that aimed to "evaluate the yield of germline mutational analysis in diagnosis of LS." All patients were 45 years or younger, with at least one adenoma removal, and underwent genetic testing by a multigene panel or LS-Jewish founder mutation panel. They found that from the 92 patients that underwent both panels, "18 patients were identified with pathogenic mutations in actionable genes, including LS-associated genes in 6 (6.5%), *BRCA2* in 2 (2.5%), *GREM1* in 1 (1.2%), and low-penetrance genes – *APC* I1307K and *CHECK2*- in 9 (11.4%) patients." Generally, routine screening for establishing LS in young patients with adenomas is not recommended due to low yield, but the researchers proposed that due to these findings, genetic screening should be offered when they fulfill the clinical guidelines for LS.

VI. Guidelines and Recommendations

Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group

In 2009, the EGAPP Working Group recommended (EGAPP, 2009):

1. Offering genetic testing for Lynch Syndrome to individuals with newly diagnosed colorectal cancer to reduce morbidity and mortality in relatives. However, they do not recommend a specific testing protocol.
2. That individuals with newly diagnosed CRC should be routinely offered counseling and educational materials aimed at informing them and their relatives of the potential benefits and harms associated with genetic testing to identify Lynch Syndrome.
3. "Microsatellite instability (MSI) testing or immunohistochemical (IHC) testing (with or without *BRAF* mutation testing) of the tumor tissue are examples of preliminary testing strategies that could be used to select patients for subsequent diagnostic testing. Diagnostic testing involves MMR gene mutation (and deletion/duplication) testing of the proband, usually using a blood sample. Lynch syndrome is most commonly caused by mutations in the two MMR genes MLH1 and MSH2; less commonly by mutations in MSH6 and PMS2."

The EGAPP was launched by the CDC Office of Public Health Genomics in 2004. EGAPP's website, which includes the 2009 Lynch Syndrome guidelines, states that the page is archived and is no longer being updated (EGAPP, 2016).

National Comprehensive Cancer Network (NCCN)

In their *Genetic/Familial High-Risk Assessment: Colorectal*, the NCCN lists the following criteria for the evaluation of Lynch Syndrome:

- "Known LS pathogenic variant in the family
- An individual with a LS-related cancer and any of the following:
  - Diagnosed <50 y
  - A synchronous or metachronous LS-related cancer regardless of age
  - 1 first-degree or second-degree relative with LS-related cancer diagnosed <50 y
  - ≥2 first-degree or second-degree relatives with LS-related cancers regardless of age
- Family history of any of the following:
  - ≥1 first-degree relative with colorectal or endometrial cancer diagnosed <50 y
  - ≥1 first-degree relative with colorectal or endometrial cancer and a synchronous or metachronous LS-related cancer regardless of age
  - ≥2 first-degree or second-degree relatives with LS-related cancer including ≥1 diagnosed <50 y
  - ≥3 first-degree or second-degree relatives with LS-related cancers regardless of age

- Increased model-predicted risk for LS
  - An individual with a ≥5% risk of having an MMR gene pathogenic variant based on predictive models (ie, PREMM5, MMRpro, MMRpredict)
    - Individuals with a personal history of colorectal and/or endometrial cancer with a PREMM5 score of ≥2.5% should be considered for multi-gene panel testing.
    - For individuals without a personal history of colorectal cancer and/or endometrial cancer, some data have suggested using a PREMM5 score threshold of ≥2.5% rather than ≥5% to select individuals for MMR genetic testing. Based on these data, it is reasonable for testing to be done based on the ≥2.5% score result and clinical judgment. Of note, with the lower threshold, there is an increase in sensitivity, but a decrease in specificity.
- Personal history of a tumor with MMR deficiency determined by PCR, NGS, or IHC diagnosed at any age" (NCCN, 2023)

The NCCN considers LS-related cancers to "include colorectal, endometrial, gastric, ovarian, pancreas, urothelial, brain (usually glioblastoma), biliary tract, and small intestinal cancers, as well as sebaceous adenomas, sebaceous carcinomas, and keratoacanthomas as seen in Muir-Torre syndrome." When there is no known familial mutation, the NCCN recommends germline multigene panel test (MGPT) evaluation for LS and other hereditary cancer syndromes (NCCN, 2023).

The NCCN also "recommends tumor screening for MMR deficiency for all CRC and endometrial cancers regardless of age at diagnosis. Tumor screening for CRCs for MMR deficiency for purposes of screening for LS is not required if MGPT is chosen as the strategy for screening for LS, but may still be required for CRC therapy selection. Consider tumor screening for MMR deficiency for sebaceous neoplasms as well as the following adenocarcinomas: small bowel, ovarian, gastric, pancreatic, biliary tract, brain, bladder/urothelial, and adrenocortical cancers regardless of age at diagnosis." Moreover, the panel states that "Direct referral for germline testing to rule out LS may be preferred in patients with a strong family history or if diagnosed age <50y, …MSI-H, or loss of MMR protein expression" (NCCN, 2023).

Regarding "Strategies for Evaluating for Lynch syndrome in Individuals Meeting Criteria for the Evaluation of Lynch Syndrome", it is recommended that when a deleterious Lynch syndrome pathogenic variant in a family is known, "the individual should be tested for the familial pathogenic variant." Moreover, the guidelines recommend that genetic testing should also be offered to at-risk family members.

When no Lynch syndrome pathogenic variant is present in proband or in family, individuals should first refer to the Amsterdam and Bethesda criteria. However, overall, "for individuals without a previously known Lynch syndrome-associated pathogenic variant, the panel recommends additional evaluation for Lynch syndrome based on clinical criteria, including for individuals with no known Lynch syndrome pathogenic variant who meet the Amsterdam II criteria or Bethesda Guidelines, have a CRC diagnosis < 50 years of age, or have predicted risk for Lynch syndrome greater than 5% on one of the following prediction models: MMRpro, PREMM5, or MMRpredict." However, due to issues of suboptimal sensitivity of clinical criteria when it comes to identifying individuals with Lynch syndrome, "the panel recommends universal screening of all CRCs, and endometrial cancers to maximize sensitivity for Lynch syndrome detection and simplify care processes." When germline MGPT is performed, the panel should include "at minimum the following CRC risk-associated genes: *APC, MUTYH, MLH1, MSH2, MSH6, PMS2, EPCAM, BMPR1A, SMAD4, PTEN, STK11,* and *TP53*. Selection of a panel that includes additional genes beyond this minimal set should be based on personal and family history of cancer, as well as patient and provider preference" (NCCN, 2023).

In terms of initial tumor testing methodologies, "the panel recommends using only one test [either MSI or IHC testing] initially" and only "If normal results are found and Lynch syndrome is strongly suspected" that the other test be employed. Furthermore, "Where genetic testing is recommended, the panel recommends consultation with an individual with expertise in genetics, and germline testing to exclude presence of Lynch-associated P/LP variants."

NCCN does not recommend multi-gene testing when

1) "there is an individual from a family with a known P/LP variant and there is no other reason for multi-gene testing;
2) the patient's family history is strongly suggestive of a known hereditary syndrome" (NCCN, 2023).

In these scenarios, syndrome-specific panels may be considered. For patients whose personal history is not suspicious for a polyposis syndrome and who were diagnosed with CRC ≥50 years with no known MMR deficiency in the tumor, multigene testing may be considered (category 2B). Otherwise, tumor and family history-based criteria for evaluation of Lynch syndrome is recommended for these patients" (NCCN, 2023). While tumor testing can identify pathogenic/likely pathogenic variants and germline origin can sometimes be inferred with a high degree of confidence, "confirmatory germline testing is indicated for pathogenic/likely pathogenic variants with a

reasonable clinical suspicion of being of germline origin (based on patient/family history or clinical characteristics, presence of founder mutation, and in some cases variant allele frequency). Somatic pathogenic/likely pathogenic variants in several genes with germline implications are common (eg, *TP53, STK11, PTEN, APC),* and will rarely be indicative of a need for germline testing unless clinical/family history features suggest the possibility of a germline pathogenic/likely pathogenic variant. It should be noted that the absence of reported pathogenic/likely pathogenic variants in a particular gene based on tumor testing does not rule out the possibility of a germline pathogenic/likely pathogenic variant in that gene. Clinically indicated germline testing is still appropriate for patients meeting testing guidelines regardless of tumor profiling results" (NCCN, 2023).

The NCCN states also that "In children <18 years, genetic testing is generally not recommended unless results would impact medical management, such as initiation of early colonoscopy surveillance", though "Clear exceptions include when FAP, JPS, PJS, or constitutional MMR deficiency (CMMRD) syndrome are suspected or known to be present in a family, in which case testing prior to age 18 is recommended to guide medical management" (NCCN, 2023).

National Institute for Health and Care Excellence (NICE)

NICE, in 2017, released their guidelines concerning molecular testing for LS in people with CRC. The recommend the following (NICE, 2017):

- "Offer testing to all people with colorectal cancer, when first diagnosed, using immunohistochemistry for mismatch repair proteins or microsatellite instability testing to identify tumors with deficient DNA mismatch repair, and to guide further sequential testing for Lynch syndrome... Do not wait for the results before starting treatment.
- "If using immunohistochemistry, follow the steps in table 1."

Table 1: Steps in the immunohistochemistry testing strategy (NICE, 2017)		
Step 1	Do an immunohistochemistry 4-panel test for *MLH1, MSH2, MSH6* and *PMS2*.	
Step 2	If the *MLH1* immunohistochemistry result is abnormal, use sequential *BRAF* V600E and *MLH1* promoter hypermethylation testing to differentiate sporadic and Lynch syndrome-associated colorectal cancers. First do a *BRAF* V600E test.	If the *MSH2*, *MSH6* or *PMS2* immunohistochemistry results are abnormal, confirm Lynch

Step 3	If the *BRAF* V600E test is negative, do an *MLH1* promoter hypermethylation test.	syndrome by genetic testing of germline DNA.
Step 4	If the *MLH1* promoter hypermethylation test is negative, confirm Lynch syndrome by genetic testing of germline DNA.	

- "If using microsatellite instability testing, follow the steps in table 2."

Table 2: Steps in the microsatellite instability testing strategy (NICE, 2017)	
Step 1	Do a microsatellite instability test.
Step 2	If the microsatellite instability test result is positive, use sequential *BRAF* V600E and *MLH1* promoter hypermethylation testing to differentiate sporadic and Lynch syndrome-associated colorectal cancers. First do a *BRAF* V600E test.
Step 3	If the *BRAF* V600E test is negative, do an *MLH1* promoter hypermethylation test
Step 4	If the *MLH1* promoter hypermethylation test is negative, confirm Lynch syndrome by genetic testing of germline DNA.

- "Healthcare professionals should ensure that people are informed of the possible implications of test results for both themselves and their relatives, and ensure that relevant support and information is available. Discussion of genetic testing should be done by a healthcare professional with appropriate training" (NICE, 2017).

The NICE published new recommendations dealing with testing strategies for Lynch syndrome in people with endometrial cancer in 2020 (NICE, 2020). Said recommendations are provided below.

"1.1 Offer testing for Lynch syndrome to people who are diagnosed with endometrial cancer. Use immunohistochemistry (IHC) to identify tumours with mismatch repair (MMR) deficiency:

- If IHC is abnormal with loss of MLH1, or loss of both MLH1 and PMS2 protein expression, do MLH1 promoter hypermethylation testing of tumour DNA. If MLH1 promoter hypermethylation is not detected, offer germline genetic testing to confirm Lynch syndrome.
- If IHC is abnormal with loss of MSH2, MSH6 or isolated PMS2 protein expression, offer germline genetic testing to confirm Lynch syndrome.

1.2 Healthcare professionals should inform people about the possible implications of test results for both themselves and their relatives, and give support and information. Discussion of genetic testing and obtaining consent should be done by a healthcare professional with appropriate training.

1.3 Laboratories doing IHC for MMR proteins, MLH1 promoter hypermethylation testing or germline genetic testing should take part in a recognised external quality assurance programme."

In February 2022, NICE updated one of their quality statements to recommend that adults with a new diagnosis of colorectal cancer be tested for Lynch syndrome. NICE recommends offering of cascade testing to family members in addition to testing individuals newly diagnosed with colorectal cancer. NICE suggests the following laboratory testing: "IHC for mismatch repair proteins or microsatellite instability testing, *BRAF V600E* testing and *MLH1* promoter hypermethylation testing…" According to their statement, these tests should be a part of the "standard pathology report requested by oncology," and lab providers should "ensure that laboratory protocols are in place to provide genetic testing of germline DNA for Lynch syndrome in adults with a new diagnosis of colorectal cancer and in whom test results are suggestive of Lynch syndrome" (NICE, 2022).

American Society of Clinical Oncology (ASCO)

The American Society of Clinical Oncology (ASCO) recommends that "genetic testing only be conducted in the setting of pre- and post-test counseling" (Robson et al., 2010). In 2015, ASCO stated that "identifying inherited mutations in genes such as *BRCA1*, *BRCA2*, and the genes associated with Lynch syndrome allows for interventions that can significantly reduce the development of cancer and improve survival. Targeted capture assays employing NGS [next generation sequencing] technology allow for testing many genes simultaneously, including genes that would not necessarily have been tested using the phenotype-directed approach, as well as genes of less clearly established clinical utility" (Robson et al., 2015). According to ASCO, multi-gene panel testing is particularly useful in situations where there are multiple high-penetrance genes associated with a specific cancer, and "one example of such a situation is Lynch syndrome, when the results of immunohistochemical analysis are not available to direct testing" (Robson et al., 2015).

In 2023, ASCO endorsed the College of American Pathologists (CAP) Guidelines on testing for mismatch repair (MMR) and microsatellite instability (MSI) for patients considered for immune checkpoint inhibitor therapy: "For cancer patients being considered for immune checkpoint inhibitor therapy, if a MMR deficiency consistent

with Lynch syndrome is identified in the tumor, pathologists should communicate this finding to the treating physician. (Strong recommendation)" (Vikas et al., 2023).

U.S. Multi-Society Task Force on Colorectal Cancer (USMSTF)

The 2014 U.S. Multi-Society Task Force guidelines on colorectal cancer are captured below.

"Testing for MMR deficiency of newly diagnosed CRC should be performed. This can be done for all CRCs, or CRC diagnosed at age 70 years or younger, and in individuals older than 70 years who have a family history concerning for LS. Analysis can be done by IHC testing for the MLH1/MSH2/MSH6/PMS2 proteins and/or testing for MSI. Tumors that demonstrate loss of MLH1 should undergo BRAF testing or analysis of MLH1 promoter hypermethylation." Also, "Individuals who have a personal history of a tumor showing evidence of MMR deficiency (without evidence of ***MLH1*** promoter methylation); uterine cancer diagnosed at younger than age 50 years; a known family MMR gene mutation; fulfill Amsterdam criteria or revised Bethesda guidelines; and/or have a personal risk of ≥5% chance of LS based on prediction models should undergo genetic evaluation for LS" (Giardiello et al., 2014)

Updated 2017 guidelines from the U.S. Multi-Society Task Force give the following guideline for colorectal cancer screening and LS (Rex et al., 2017):

- "colonoscopy is recommended at 10-year intervals in average-risk persons and at 1- to 2-year intervals in those with Lynch syndrome."

However, for specific LS related screening techniques and recommendations, the updated 2017 article states that the Giardiello et al. (2014) guidelines are still the most current.

In 2022, the U.S. Multi-Society Task Force published updated guidelines on colorectal cancer (Patel et al., 2022). One of the recommendations was that "average-risk CRC screening" start at age 45 on a qualified basis because of the increasing incidence and mortality from colorectal cancer. However, this task force update did not mention Lynch syndrome specifically.

American Society for Clinical Pathology (ASCP), College of American Pathologists (CAP), Association for Molecular Pathology (AMP), and American Society of Clinical Oncology (ASCO)

The ASCP, CAP, AMP, and ASCO issued guidelines in 2017 stating "*BRAF* p.V600 mutational analysis should be performed in deficient MMR tumors with loss of *MLH1*

to evaluate for Lynch Syndrome risk. Presence of a *BRAF* mutation strongly favors a sporadic pathogenesis. The absence of *BRAF* mutation does not exclude risk of Lynch syndrome." In addition, they have added the following recommendation for clinicians: "clinicians should order mismatch repair status testing in patients with colorectal cancers for the identification of patients at high risk for Lynch syndrome and/or prognostic stratification" (Sepulveda et al., 2017).

American College of Gastroenterology (ACG)

In 2015, ACG issued the following practice guidelines for the management of patients with hereditary gastrointestinal cancer syndromes. The relevant guidelines are reported below.

- "All newly diagnosed colorectal cancers should be evaluated for mismatch repair deficiency.
- Analysis may be done by immunohistochemical (IHC) testing for the *MLH1/MSH2/MSH6/PMS2* proteins and/or testing for microsatellite instability; tumors that demonstrate loss of *MLH1* should undergo *BRAF* testing or analysis for *MLH1* promoter hypermethylation.
- Individuals who have a personal history of a tumor showing evidence of mismatch repair deficiency (and no demonstrated *BRAF* mutation or hypermethylation of *MLH1*), a known family mutation associated with LS, or a risk of ≥5% chance of LS based on risk prediction models should undergo genetic evaluation for LS.
- Genetic testing of patients with suspected LS should include germline mutation genetic testing for the *MLH1, MSH2, MSH6, PMS2*, and/or *EPCAM* genes or the altered gene(s) indicated by IHC testing" (Syngal et al., 2015).

American Society of Colon and Rectal Surgeons (ASCRS)

The American Society of Colon and Rectal Surgeons published guidelines based on 2014 U.S. Multi-Society Task Force on Colorectal Cancer. There, they recommend the following:

"Universal testing (tumor testing):

- Testing for MMR deficiency of newly diagnosed CRC should be performed
- This can be done for all CRCs or CRC diagnosed at age ≤70 y and in individuals >70 y who have a family history concerning for LS
- Analysis can be done by IHC testing for the MLH1/MSH2/MSH6/PMS2 proteins and/or testing for MSI

- Tumors that demonstrate loss of MLH1 should undergo BRAF testing or analysis of MLH1 promoter hypermethylation
- To facilitate surgical planning, tumor testing on suspected CRC should be performed on preoperative biopsy specimens, if possible

Traditional testing (germline testing):

- Individuals who have a personal history of a Lynch syndrome–related tumor showing evidence of MMR deficiency (without evidence of MLH1 promoter methylation)
- Personal history of uterine cancer diagnosed at age <50 y
- A known family MMR gene mutation
- Fulfill Amsterdam criteria or revised Bethesda guidelines
- Have a personal risk of ≥5% chance of LS based on prediction models" (Herzig et al., 2017).

Spanish Society of Medical Oncology (SEOM)

The SEOM published guidelines on hereditary colorectal cancer. These guidelines include the following recommendations:

- "Different screening strategies for LS of all newly diagnosed CRC and EC [endometrial cancers] can be considered including tumor tests for defective MMR function and/or high-level MSI and/or NGS tumor sequencing including *BRAF*.
- In case of lack of expression of *MLH1* and *PMS2* by immunohistochemistry, BRAFV600E mutation and/or *MLH1* promoter hypermethylation should be carried out to rule out sporadic cases.
- Patients with molecular profiles compatible with LS should be referred to GCU for appropriate counseling and NGS germline genetic testing.
- In families with fulfillment of rBC or a ≥ 2.5% likelihood of LS on the PREMM5 prediction model, prevalent and/or previous CRC and/or EC should follow the same screening procedure before considering referral to GCU (evidence level B, strength 1).
- Multigene panel testing for hereditary CRC and polyposis should include the genes:
  - *APC, BMPR1A, EPCAM, MLH1, MSH2, MSH6, MUTYH, PMS2, PTEN, SMAD4* and *STK11* (evidence level A, strength 1).
  - *AXIN2, BLM, GREM1, NTHL1, POLD1, POLE* and *TP53* (evidence level B, strength 2).
- Criteria for referral to a GCU and *APC/MUTYH* or multigene panel testing (evidence level B, strength 1):

- 1. Patients with > 10 synchronous adenomatous colonic polyps histologically confirmed.
- 2. Family history of adenomatous colonic polyps (> 10 in > 1 relative), at young age and extracolonic manifestations.
- 3. Gastric polyps (> 100), in body and fundus, preponderantly fundic glands polyps. Proton pump inhibitor use must be excluded.
- 4. Consider in: hepatoblastoma, desmoid tumor, cribriform-morular variant of papillary thyroid carcinoma, multifocal or bilateral congenital hypertrophy of retinal pigmented epithelium.
- 5. Known familial mutation in at-risk relatives" (Guillen-Ponce et al., 2020).

Spanish Society of Medical Oncology (SEOM), Multidisciplinary Spanish Group of Digestive Cancer (GEMCAD), Spanish Group of Digestive Tumors (TTD)

These guidelines were the result of the consensus of ten oncologist experts in treatment from two Spanish digestive cooperative groups (Grupo Español Multidisciplinar de Cáncer Digestivo, GEMCAD, and Grupo Español de Tumores Digestivos, TTD), the Spanish Society of Medical Oncology (SEOM), and an external review panel comprising two experts designated by SEOM. Using the Infectious Diseases Society of America–US Public Health Service Grading System for Ranking Recommendations in Clinical Guidelines, the collaborators assigned levels of evidence and grades of recommendation.

The authors recommend the following upon suspicion of CRC based on suggestive symptoms or screening tests:

- "A complete colonoscopy with biopsy to confirm the diagnosis is mandatory. Virtual colonoscopy is an alternative to detect potential synchronous colorectal lesions if a full colonoscopy is not feasible [I, A].
- CT scan of the chest, abdomen, and pelvis is the best technique to assess distant metastases [IV, A].
- MRI and PET-CT may be considered in selected cases [IV, B].
- Patients with mCRC should be evaluated by a multidisciplinary team to define patient management: resectable, potentially resectable, and un-resectable disease [III, A].
- The recommended staging system is that of the 8th edition of the AJCC [I, A]" (Fernández Montes et al., 2023).

The value of molecular profiling and identification of specific biomarkers in metastatic colon cancer are also acknowledged as predictive and prognostic indicators of disease and response to targeted therapies. As such, they note that

- "KRAS, NRAS exons 2, 3, and 4, and BRAF V600E mutations should be tested at the time of mCRC diagnosis [I, A].
- Assessment of mismatch repair deficiency (IHC or MSI-H) is recommended to assist genetic counseling for Lynch syndrome [II, B] and for its predictive value of benefit from ICI [I, A].
- Identification of HER 2 amplification or overexpression [III, C] and NTRK fusions are recommended in subsequent lines for access to clinical trials with targeted therapies [III, A].
- Liquid biopsy might be considered to monitor emergent mutations of resistance to targeted therapy, especially prior to re-challenge with anti-epidermal growth factor receptor (anti-EGFR) treatment, though this is not supported by our national authorities [II, B].
- Testing for DPYD deficiency is strongly recommended prior to initiating 5-fluorouracil-based chemotherapy [III, A]" (Fernández Montes et al., 2023).

Collaborative Group of the Americas on Inherited Gastrointestinal Cancer (CGA-IGC)

The Collaborative Group of the Americas on Inherited Gastrointestinal Cancer published a position statement on multigene panel testing for patients with colorectal cancer and/or polyposis, in which they addressed several key questions. The questions and results relevant to this policy are summarized below.

"Question 1: Which minimal set of genes should be included on a multigene panel for evaluation of hereditary CRC or polyposis?"

The CGA-IGC notes that "All commercially available panels do not include the same genes, and some panels might fail to include genes relevant to the evaluation of hereditary CRC and/or polyposis". However, they have defined 11 genes in which PV have been associated with hereditary or familial CRC or polyposis, and "considers this list of genes to be the minimal set of genes that should clinically be tested in all patients suspected of hereditary CRC or polyposis, and recommends this testing be conducted by multigene panel" (Heald et al., 2020). It should also be noted that the authors state that "Multigene panel testing is recommended over targeted gene testing due to overlapping clinical phenotypes, inconsistent definitions for oligopolyposis, challenges with accurately classifying polyp histology (particularly with hamartomatous polyps), and variable modes of inheritance (both dominant and recessive inheritance)" (Heald et al., 2020).

Their table of aforementioned list of 11 genes is pulled in below.

Minimal and additional genes to include on multigene panel test for evaluation of colorectal cancer and/or polyposis

Genes	Cumulative CRC risk by age 70	Prevalence	Inheritance	Cancer/polyposis phenotype
Genes associated with Lynch syndrome				
MLH1	4–79% [83–85]	1:1946 [16]	AD	CRC, EC, OV,GC, SBC, PC, HTC, UTC, PC, SC, BT
MSH2	35–77% [83–85]	1:2841 [16]		
MSH6	12–50% [83–85]	1:758 [16]		
PMS2[a]	10–19% [85, 86]	1:714 [16]		
EPCAM[b]	75% [85]	Unknown		
Genes associated with polyposis		syndromes		
APC	69–100% [3]	2.29:100,000 to 3.2:100,000	AD	Adenomatous polyposis, CRC, SBC, GC, TC, HB, desmoids
BMPR1A	38%[c] [87, 88]	1:16,000 to 1:100,000	AD	Juvenile polyposis, CRC, GC, SBC
MUTYH	71.7–75.4% [33]	1:8073 [16] (biallelic)1:45 [16] (monoallelic)	AR	Adenomatous polyposis, CRC, SBC, TC
PTEN	9–16% [89, 90]	1:200,000 [91]	AD	Mixed polyposis (hamartomas, ganglioneuromas, serrated, adenomatous), BC, TC, EC, CRC, KC, melanoma
STK11	39% [23, 92]	1:25,000 to 1:280,000 [93]	AD	Peutz-Jeghers polyps, CRC, BC, PC, GC, SBC, OC, EC, SCT, LC
SMAD4	38%[c] [87, 88]	1:16,000 to 1:100,000	AD	Juvenile polyposis, CRC, GC, SBC

AD autosomal dominant, *AR* autosomal recessive, *BC* breast cancer, *BT* brain tumor, *CRC* colorectal cancer, *EC* endometrial cancer, *GC* gastric cancer, *HB* hepatoblastoma, *HTC* hepatobiliary tract cacner, *KC* kidney cancer, *LC* lung cancer, *PC* pancreatic cancer, *SBC* small bowel cancer, *SC* sebaceous carcinoma, *SCT* sex cord tumor, *TC* thyroid cancer, *UTC* urinary tract cancer

[a]Laboratory analysis should be able to distinguish exons 12–15 in *PMS2* from the transcribed pseudocopy of *PMS2* (*PMS2CL*)
[b]Large rearrangement analysis only
[c]Estimated cumulative lifetime colorectal cancer risk

(*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*, *APC*, *BMPR1A*, *MUTYH*, *PTEN*, *STK11*, *SMAD4*) (Heald et al., 2020)

"Question 2: Which additional set of genes should be considered on a multigene panel for evaluation of hereditary CRC or polyposis?"

According to the authors, "Beyond the minimal list of genes recommended for multigene panel testing for patients with suspected hereditary CRC, CGA-IGC recommends that 16 additional genes be considered." Their table of 16 genes is captured below.

Additional genes with (a) low to moderately increased risk of colorectal cancer (CRC), (b) preliminary but limited data on CRC risk, and (c) genes with pathogenic variants found in CRC patients that are actionable in regard to other cancers but CRC causation not proven. Testing for these genes can be considered in specific circumstances

Genes	Odds ratio	Prevalence	Inheritance	Cancer/polyposis phenotype
(a) Genes associated with low to moderately increased risk of CRC				
ATM	2.81–2.97 [27]	1:100 [94]	AD	BC, PC
CHEK2	1.56–1.88[a] [95]	1:100 to 1:200 [28]	AD	BC, CRC
TP53	Not defined, but skewed towards younger age at diagnosis [96, 97]	1:5000 to 1:20,000	AD	ACC, BC, BT, CRC, LC, leukemia, sarcoma
(b) Genes with preliminary but limited data				
SCG5/GREM1[b]	Not defined, presumed to be high	Unknown	AD	Mixed (serrated, juvenile, adenomatous) polyposis, CRC
POLD1	Not defined	Unknown	AD	Adenomatous polyposis, BT, CRC, EC
POLE	Not defined	Unknown	AD	Adenomatous polyposis, CRC
AXIN2	Not defined	Unknown	AD	Adenomatous polyposis, CRC
NTHL1	Not defined	Unknown	AR	Adenomatous polyposis, CRC
MSH3	Not defined	Unknown	AR	Adenomatous polyposis, CRC
GALNT12	Not defined	Unknown	AD	CRC
RPS20	Not defined	Unknown	AD	CRC
(c) Genes with PV found in CRC patients that are actionable but CRC causation not proven				
BRCA1	Not defined	1:400 to 1:500 [98] 1:40 (Ashkenazi Jewish) [99]	AD	BC, OC, PrC, melanoma
BRCA2	Not defined	1:400 to 1:500 [98] 1:40 (Ashkenazi Jewish) [99]	AD	BC, OC, PrC, melanoma
CDKN2A	Not defined	Unknown	AD	PC, melanoma
PALB2	3.42–4.91 [27]	Unknown	AD	BC, PC

(*ATM*, *CHEK2*, *TP53*, *POLD1*, *POLE*, *AXIN2*, *NTHL1*, *MSH3*, *GALNT12*, *BSP20*, *BRCA1*, *BRCA2*, *CDKN2A*, *PALB2*)

(Heald et al., 2020)

"Question 3: Who should undergo multigene panel testing for hereditary CRC syndromes?"

The authors state that though conventional wisdom believes that "inherited cancer syndromes only occur in those with striking personal and/or family histories of cancer", multigene panel testing is endorsed for epithelial ovarian cancer, metastatic prostate cancer, triple negative breast cancer, and pancreatic cancer due to "the absence of typical "red flags" (family history of cancer and/or young age of onset)". Because "traditional criteria fail to identify individuals at risk for hereditary CRC", the CGA-IGC proposes indications for panel testing in the table referenced below.

Table 5

CGA-IGC recommendations for individuals who should be considered for multigene panel testing for evaluation of hereditary colorectal cancer and/or polyposis

Individuals who should undergo multigene panel testing[a]
Colorectal cancer diagnosed age < 50 years[b]
Multiple Lynch syndrome primary tumors[b,c]
Colorectal cancer[b] and at least one first degree relative with colorectal or endometrial cancer
$PREMM_5$ score ≥ 2.5% or MMRpro or MMRpredict score ≥ 5%
Mismatch repair-deficient colorectal cancer, not attributed to *MLH1* promoter methylation
Patients meeting any other genetic testing criteria [4, 6]
≥ 10 cumulative colorectal adenomas
≥ 3 cumulative gastrointestinal hamartomatous polyps[d]

Open in a separate window

[a]In some instances, the individual affected with colorectal cancer/polyposis is unavailable for testing. In this case, genetic testing should be offered to any first-degree relative (FDR) with a family history meeting these criteria. Consideration can be given to testing second-degree relatives with a family history meeting these criteria when the affected family member and FDRs are unavailable to be tested

[b]Irrespecitve of microsatellite instability (MSI)/immunohistochemistry (IHC) status

[c]Colorectal cancer, endometrial cancer, ovarian cancer, urothelial cancer, small bowel cancer, gastric cancer, hepatobiliary cancer, pancreatic cancer, sebaceous carcinoma, glioblastoma

[d]Including hamartomas, ganglioneuromas, Peutz-Jeghers polyps, and/or juvenile polyps

(Heald et al., 2020)

"Question 4: How should a patient with a dMMR CRC be evaluated using both germline and somatic testing?"

In the ideal situation, "the CGA-IGC endorses a simultaneous paired germline-somatic panel. However, considerations about whether to order sequential or simultaneous germline and tumor sequencing include a number of factors. If the patient's personal and/or family history are consistent with LS, it is more likely that they will have LS, so it may be efficient to order germline testing alone given that follow-up tumor testing will probably not be necessary. If the patient has an insurance that might only cover one genetic test, it might be best to order paired tumor and normal testing since it may not be possible to reflex to tumor testing if no PV is found on germline testing. In some cases (e.g. older patients with no family history with loss of MLH1 and PMS2 without *MLH1* methylation), it may actually be more likely that the patient has biallelic somatic

MMR gene PV rather than a germline MMR gene PV, and paired germline and somatic testing may be a reasonable first approach to testing". Finally, they also recommend that "patient preferences should be taken into account as some may prefer to get a complete answer quickly rather than drawing out the testing process by ordering the tests sequentially" (Heald et al., 2020).

National Society of Genetic Counselors (NSGC) and the Collaborative Group of the Americas on Inherited Colorectal Cancer (CGA-IGC)

The following guidelines were provided by the NSGC and the Collaborative Group of the Americas on Inherited Colorectal Cancer:

- "Microsatellite instability (MSI) and immunohistochemistry (IHC) tumor analyses should be performed on CRC or endometrial cancers as the first-line testing strategy for any patient being evaluated for LS (this includes individuals with CRC or endometrial cancer who meet Amsterdam I or II criteria or Bethesda guidelines).
- *MLH1* promoter methylation and *BRAF* V600E mutation testing may help to reduce the number of germline genetic tests needed when IHC reveals absence of *MLH1* and *PMS2*. However, NSGC and the CGA-ICC did not find enough data to recommend one test over the other or both concomitantly.
- IHC may occasionally yield atypical results. If IHC reveals absent *MLH1* or *MSH2* only, consider genetic testing of those genes individually. If IHC reveals loss of more than two MMR proteins, consider repeating the IHC analysis. If the results persist or if repeat testing was not performed, consider following the algorithm based on the most likely true results (i.e., if *MSH2, MSH6* and *MLH1* or *PMS2* are all absent, follow the loss of *MSH2/MSH6* pathway; if *MLH1, PMS2* and *MSH6* or *MSH2* are all absent, follow the *MLH1* and *PMS2* pathway). Further, it is worth noting that there is a mononucleotide microsatellite in *MSH6* that may cause loss of *MSH6* with another MMR germline mutation leading to aberrant IHC staining patterns
- When MSI testing is stable, but IHC shows absence of one or more MMR proteins, clinical judgment should be used to determine whether tumor studies should be repeated or germline genetic testing should be pursued
- MSI testing should include, at a minimum, the five markers included in the NCI panel
- While we recognize that some third party payers may not cover MSI and/or IHC analyses on the tumor of a patient's family member(s) (e.g., the family member is deceased), in our expert opinion, we deem testing the family member(s)' tumor is justified because: 1) LS is one of a few hereditary cancer syndromes that has a

validated screening test to determine if germline genetic testing is warranted; 2) if an affected family member is living, it is likely that MSI and IHC will be covered by that relative's insurance; 3) a negative germline genetic test for all four MMR genes in an unaffected patient is uninformative; 4) the cost of direct germline genetic testing for each MMR gene ranges from $1000 to $1500, whereas the cost of MSI and IHC together is ~$1000; 5) if IHC is abnormal, additional tumor tests (*BRAF* and *MLH1* promoter methylation) may help determine if germline genetic testing is necessary and if it is warranted, testing can be targeted to one or two genes limiting overall costs; and 6) normal MSI and IHC results on an affected individual would significantly lower the likelihood that LS is the explanation for the cancer in the family and germline genetic testing would most likely not be needed.

- Direct germline genetic testing (refers to both DNA sequencing and a technology that detects large rearrangements, insertions, deletions and duplications) may be considered on an affected or unaffected patient being evaluated for LS when MSI and IHC testing are not feasible.
    - In the event that a tumor block is not available, a family member(s) is not willing or able to participate in testing, there are financial concerns or there is insufficient tissue to do either MSI or IHC testing, when indicated (e.g., high familial risk is present such as Amsterdam criteria), direct germline genetic testing may be considered. It should be noted, however, that negative germline testing in an affected individual who has not had MMR IHC can also be uninformative because there are some individuals with unidentifiable MMR gene mutations that would be followed as having LS based on abnormal IHC" (Weissman et al., 2012).

European Society for Medical Oncology (ESMO)

The ESMO published guidelines in 2015 for familial risk-colorectal cancer. The ASCO has endorsed these guidelines, with minor modifications.

The ASCO endorsement panel has "determined that the recommendations of the ESMO guideline are clear, thorough, and based on the most relevant scientific evidence". The ASCO endorsed the ESMO guidelines (below) with a few minor qualifying statements (in bold):

- "Tumor **testing for DNA mismatch repair (MMR) deficiency** with immunohistochemistry for MMR proteins and/or MSI should be **assessed** in all CRC patients. As an alternate strategy, tumor testing should be carried out in

individuals with CRC younger than 70 years, or those older than 70 years who fulfill any of the revised Bethesda guidelines.

- If loss of MLH1/PMS2 protein expression is observed in the tumor, analysis of *BRAF* V600E mutation or analysis of methylation of the *MLH1* promoter should be carried out first to rule out a sporadic case. **If tumor is MMR deficient and somatic *BRAF* mutation is not detected or MLH1 promoter methylation is not identified, testing for germline mutations is indicated.**
- If loss of any of the other proteins (MSH2, MSH6, PMS2) is observed, germline genetic testing should be carried out **for the genes corresponding to the absent proteins (e.g., MSH2, MSH6, EPCAM, PMS2, or MLH1).**
- Full germline genetic testing **for Lynch syndrome** should include DNA sequencing and large rearrangement analysis.
- Full germline genetic testing of *APC* should include DNA sequencing and large rearrangement analysis.
- Germline testing of *MUTYH* can be initiated by screening for the most common mutations (*G396D, Y179C*) in the white population followed by analysis of the entire gene in heterozygotes. Founder mutations among ethnic groups should be taken into account. **For nonwhite individuals, full sequencing of *MUTYH* should be considered"** (Stoffel et al., 2015).

In 2019, the ESMO updated their clinical practice guidelines for hereditary gastrointestional cancers, including those for Lynch syndrome. In this set of recommendations, the ESMO maintains that tumor testing with IHC for MMR proteins and/or MSI is recommended in individuals with CRC and that if loss of *MLH1* is observed in the tumour, analysis of *BRAF V600E* mutation or analysis of the methylation of the *MLH1* promoter should be carried out first to rule out a sporadic case. They also maintained that full germline genetic testing should include DNA sequencing and large rearrangement analysis, as in the previous guidelines, but also proposed that for those with Lynch syndrome,

- "Somatic MMR gene testing for patients with unexplained abnormal tumour screening is suggested [III, B]
- Clinical risk can be assessed using Amsterdam criteria II or the revised Bethesda guidelines
- MMR IHC and/or MSI screening, with MLH1 promotor hypermethylation analysis in cases of MLH1 expression loss, is recommended for [individuals] with endometrial cancer [III, B]
- Follow-up recommendations in mutation carriers include colonoscopy every 1–2 years [III, A], and gynaecological examination (with TV US, CA 125 and endometrial biopsy) on a yearly basis from age 30 to 35 years [IV, C]. In all cases,

age of onset in the youngest member of the family is to be considered and surveillance be started 5 years earlier [V, B]. High-quality colonoscopy carried out in dedicated centres is advised [IV, C]. UGI endoscopy surveillance (every 1–3 years, from age 30–35 years) may be considered in patients at high risk. Prophylactic gynaecological surgery might be an option for. . .carriers who have completed childbearing or are postmenopausal [IV, C]" (Stjepanovic et al., 2019).

In 2023, ESMO published guidelines for Metastatic colorectal cancer diagnosis, treatment, and follow-up. The guidelines stated that "deficient mismatch repair (dMMR)/microsatellite instability (MSI) testing in mCRC can assist clinicians with genetic counselling, including for identification of Lynch syndrome, and should be done to select patients for immune checkpoint inhibition (ICI) as part of the initial molecular work-up" (Cervantes, 2023).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

On October 27, 2017, the FDA approved VENTANA MMR IHC Panel for patients diagnosed with colorectal cancer (CRC) to detect mismatch repair (MMR) proteins deficiency as an aid in the identification of probable Lynch syndrome and to detect BRAFV600E protein as an aid to differentiate between sporadic CRC and probable Lynch syndrome.

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81288	MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; promoter methylation analysis
81292	MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis
81293	MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; known familial variants
81294	MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; duplication/deletion variants
81295	MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis
81296	MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; known familial variants
81297	MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; duplication/deletion variants
81298	MSH6 (mutS homolog 6 [E. coli]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis
81299	MSH6 (mutS homolog 6 [E. coli]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; known familial variants
81300	MSH6 (mutS homolog 6 [E. coli]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; duplication/deletion variants
81301	Microsatellite instability analysis (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) of markers for mismatch repair deficiency (eg, BAT25, BAT26), includes comparison of neoplastic and normal tissue, if performed
81317	PMS2 (postmeiotic segregation increased 2 [S. cerevisiae]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis

CPT	Code Description
81318	PMS2 (postmeiotic segregation increased 2 [S. cerevisiae]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; known familial variants
81319	PMS2 (postmeiotic segregation increased 2 [S. cerevisiae]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; duplication/deletion variants
81403	Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)
81435	Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis); genomic sequence analysis panel, must include sequencing of at least 10 genes, including APC, BMPR1A, CDH1, MLH1, MSH2, MSH6, MUTYH, PTEN, SMAD4, and STK11
81436	Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis); duplication/deletion analysis panel, must include analysis of at least 5 genes, including MLH1, MSH2, EPCAM, SMAD4, and STK11
81479	Unlisted molecular pathology procedure
0101U	Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with MRNA analytics to resolve variants of unknown significance when indicated (15 genes [sequencing and deletion/duplication], EPCAM and GREM1 [deletion/duplication only]) Proprietary test: ColoNext® Lab/Manufacturer: Ambry Genetics®
0238U	Oncology (Lynch syndrome), genomic DNA sequence analysis of MLH1, MSH2, MSH6, PMS2, and EPCAM, including small sequence changes in exonic and intronic regions, deletions, duplications, mobile element insertions, and variants in non-uniquely mappable regions Proprietary test: Genomic Unity® Lynch Syndrome Analysis Lab/Manufacturer: Variantyx Inc

CPT	Code Description
0474U	Hereditary pan-cancer (eg, hereditary sarcomas, hereditary endocrine tumors, hereditary neuroendocrine tumors, hereditary cutaneous melanoma), genomic sequence analysis panel of 88 genes with 20 duplications/deletions using nextgeneration sequencing (NGS), Sanger sequencing, blood or saliva, reported as positive or negative for germline variants, each gene Proprietary test: GeneticsNow® Comprehensive Germline Panel Lab/Manufacturer: GoPath Diagnostics, Inc
0475U	Hereditary prostate cancerrelated disorders, genomic sequence analysis panel using next-generation sequencing (NGS), Sanger sequencing, multiplex ligation-dependent probe amplification (MLPA), and array comparative genomic hybridization (CGH), evaluation of 23 genes and duplications/deletions when indicated, pathologic mutations reported with a genetic risk score for prostate cancer Proprietary test: ProstateNowTM Prostate Germline Panel Lab/Manufacturer: GoPath Diagnostics, Inc

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Adar, T., Rodgers, L. H., Shannon, K. M., Yoshida, M., Ma, T., Mattia, A., Lauwers, G. Y., Iafrate, A. J., Hartford, N. M., Oliva, E., & Chung, D. C. (2018). Universal screening of both endometrial and colon cancers increases the detection of Lynch syndrome. *Cancer*, *124*(15), 3145-3153. https://doi.org/10.1002/cncr.31534

Barrow, P., Khan, M., Lalloo, F., Evans, D. G., & Hill, J. (2013). Systematic review of the impact of registration and screening on colorectal cancer incidence and mortality in familial adenomatous polyposis and Lynch syndrome. *Br J Surg*, *100*(13), 1719-1731. https://doi.org/10.1002/bjs.9316

Brosens, L. A., Offerhaus, G. J. A., & Giardiello, F. M. (2015). Hereditary Colorectal Cancer: Genetics and Screening. *Surg Clin North Am*, *95*(5), 1067-1080. https://doi.org/10.1016/j.suc.2015.05.004

Cabreira, V., Pinto, C., Pinheiro, M., Lopes, P., Peixoto, A., Santos, C., Veiga, I., Rocha, P., Pinto, P., Henrique, R., & Teixeira, M. R. (2017). Performance of Lynch syndrome predictive models in quantifying the likelihood of germline mutations in patients with abnormal MLH1 immunoexpression. *Fam Cancer*, *16*(1), 73-81. https://doi.org/10.1007/s10689-016-9926-0

Cervantes, A., Adam, R., Rosello, S., Yoshino, T., Martinelli, E. (2023). Metastatic colorectal cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol*, *34*(1). https://doi.org/10.1016/j.annonc.2022.10.003

Cohen, S. A., Laurino, M., Bowen, D. J., Upton, M. P., Pritchard, C., Hisama, F., Jarvik, G., Fichera, A., Sjoding, B., Bennett, R. L., Naylor, L., Jacobson, A., Burke, W., & Grady, W. M. (2016). Initiation of universal tumor screening for Lynch syndrome in colorectal cancer patients as a model for the implementation of genetic information into clinical oncology practice. *Cancer*, *122*(3), 393-401. https://doi.org/10.1002/cncr.29758

DFCI. (2020). *PREMM5 Model: Lynch Syndrome*. Dana-Farber Cancer Institute. http://premm.dfci.harvard.edu/

EGAPP. (2009). Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives. *Genet Med*, *11*(1), 35-41. https://doi.org/10.1097/GIM.0b013e31818fa2ff

EGAPP. (2016). *Genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives*. https://archive.cdc.gov/#/details?url=https://www.cdc.gov/egappreviews/recommendations/lynch.html

Fernández Montes, A., Alonso, V., Aranda, E., Élez, E., García Alfonso, P., Grávalos, C., Maurel, J., Vera, R., Vidal, R., & Aparicio, J. (2023). SEOM-GEMCAD-TTD clinical guidelines for the systemic treatment of metastatic colorectal cancer (2022). *Clin Transl Oncol*, *25*(9), 2718-2731. https://doi.org/10.1007/s12094-023-03199-1

Giardiello, F. M., Allen, J. I., Axilbund, J. E., Boland, C. R., Burke, C. A., Burt, R. W., Church, J. M., Dominitz, J. A., Johnson, D. A., Kaltenbach, T., Levin, T. R., Lieberman, D. A., Robertson, D. J., Syngal, S., & Rex, D. K. (2014). Guidelines on genetic evaluation and management of Lynch syndrome: a consensus statement by the US Multi-Society Task Force on colorectal cancer. *Gastroenterology*, *147*(2), 502-526. https://doi.org/10.1053/j.gastro.2014.04.001

Goverde, A., Spaander, M. C. W., Nieboer, D., van den Ouweland, A. M. W., Dinjens, W. N. M., Dubbink, H. J., Tops, C. J., Ten Broeke, S. W., Bruno, M. J., Hofstra, R. M. W., Steyerberg, E. W., & Wagner, A. (2018). Evaluation of current prediction models for Lynch syndrome: updating the PREMM5 model to identify PMS2 mutation carriers. *Fam Cancer*, *17*(3), 361-370. https://doi.org/10.1007/s10689-017-0039-1

Guillen-Ponce, C., Lastra, E., Lorenzo-Lorenzo, I., Martin Gomez, T., Morales Chamorro, R., Sanchez-Heras, A. B., Serrano, R., Soriano Rodriguez, M. C., Soto, J. L., & Robles, L. (2020). SEOM clinical guideline on hereditary colorectal cancer (2019). *Clin Transl Oncol*. https://doi.org/10.1007/s12094-019-02272-y

Hampel, H., Frankel, W. L., Martin, E., Arnold, M., Khanduja, K., Kuebler, P., Nakagawa, H., Sotamaa, K., Prior, T. W., Westman, J., Panescu, J., Fix, D., Lockman, J., Comeras, I., & de la Chapelle, A. (2005). Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). *N Engl J Med*, *352*(18), 1851-1860. https://doi.org/10.1056/NEJMoa043146

Harvard. (2019). *MMRpro*. The President and Fellows of Harvard College. Retrieved 02/07/2019 from https://projects.iq.harvard.edu/bayesmendel/mmrpro

Heald, B., Hampel, H., Church, J., Dudley, B., Hall, M. J., Mork, M. E., Singh, A., Stoffel, E., Stoll, J., You, Y. N., Yurgelun, M. B., & Kupfer, S. S. (2020). Collaborative Group of the Americas on Inherited Gastrointestinal Cancer Position statement on multigene panel testing for patients with colorectal cancer and/or polyposis. *Fam Cancer*, *19*(3), 223-239. https://doi.org/10.1007/s10689-020-00170-9

Herzig, D. O., Buie, W. D., Weiser, M. R., You, Y. N., Rafferty, J. F., Feingold, D., & Steele, S. R. (2017). Clinical Practice Guidelines for the Surgical Treatment of Patients With Lynch Syndrome. *Dis Colon Rectum*, *60*(2), 137-143. https://doi.org/10.1097/dcr.0000000000000785

Hunter, J. E., Zepp, J. M., Gilmore, M. J., Davis, J. V., Esterberg, E. J., Muessig, K. R., Peterson, S. K., Syngal, S., Acheson, L. S., Wiesner, G. L., Reiss, J. A., & Goddard, K. A. (2015). Universal tumor screening for Lynch syndrome: Assessment of the perspectives of patients with colorectal cancer regarding benefits and barriers. *Cancer*, *121*(18), 3281-3289. https://doi.org/10.1002/cncr.29470

Jansen, M., Menko, F. H., Brosens, L. A., Giardiello, F. M., & Offerhaus, G. J. (2014). Establishing a clinical and molecular diagnosis for hereditary colorectal cancer syndromes: Present tense, future perfect? *Gastrointest Endosc*, *80*(6), 1145-1155. https://doi.org/10.1016/j.gie.2014.07.049

Kastrinos, F., Ojha, R. P., Leenen, C., Alvero, C., Mercado, R. C., Balmana, J., Valenzuela, I., Balaguer, F., Green, R., Lindor, N. M., Thibodeau, S. N., Newcomb, P., Win, A. K., Jenkins, M., Buchanan, D. D., Bertario, L., Sala, P., Hampel, H., Syngal, S., & Steyerberg, E. W. (2016). Comparison of Prediction Models for Lynch Syndrome Among Individuals With Colorectal Cancer. *J Natl Cancer Inst*, *108*(2). https://doi.org/10.1093/jnci/djv308

Kastrinos, F., Uno, H., & Syngal, S. (2018). Commentary: PREMM5 threshold of 2.5% is recommended to improve identification of PMS2 carriers. *Fam Cancer*, *17*(4), 567. https://doi.org/10.1007/s10689-018-0074-6

Kastrinos, F., Uno, H., Ukaegbu, C., Alvero, C., McFarland, A., Yurgelun, M. B., Kulke, M. H., Schrag, D., Meyerhardt, J. A., Fuchs, C. S., Mayer, R. J., Ng, K., Steyerberg, E. W., & Syngal, S. (2017). Development and Validation of the PREMM5 Model for Comprehensive Risk Assessment of Lynch Syndrome. *Journal of Clinical Oncology*, *35*(19), 2165-2172. https://doi.org/10.1200/JCO.2016.69.6120

Kidambi, T. D., Blanco, A., Myers, M., Conrad, P., Loranger, K., & Terdiman, J. P. (2015). Selective Versus Universal Screening for Lynch Syndrome: A Six-Year Clinical Experience. *Dig Dis Sci*, *60*(8), 2463-2469. https://doi.org/10.1007/s10620-014-3234-z

Laish, I., Goldberg, Y., Friedman, E., Kedar, I., Katz, L., Levi, Z., Gingold-Belfer, R., Kopylov, U., Feldman, D., Levi-Reznick, G., & Half, E. (2021). Genetic testing for assessment of lynch syndrome in young patients with polyps. *Dig Liver Dis*, *53*(12), 1640-1646. https://doi.org/10.1016/j.dld.2021.05.031

Lee, S. Y., Kim, D. W., Shin, Y. K., Ihn, M. H., Lee, S. M., Oh, H. K., Ku, J. L., Jeong, S. Y., Lee, J. B., Ahn, S., Won, S., & Kang, S. B. (2016). Validation of Prediction Models for Mismatch Repair Gene Mutations in Koreans. *Cancer Res Treat*, *48*(2), 668-675. https://doi.org/10.4143/crt.2014.288

Ligtenberg, M. J., Kuiper, R. P., Chan, T. L., Goossens, M., Hebeda, K. M., Voorendt, M., Lee, T. Y., Bodmer, D., Hoenselaar, E., Hendriks-Cornelissen, S. J., Tsui, W. Y., Kong, C. K., Brunner, H. G., van Kessel, A. G., Yuen, S. T., van Krieken, J. H., Leung, S. Y., & Hoogerbrugge, N. (2009). Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3' exons of TACSTD1. *Nat Genet*, *41*(1), 112-117. https://doi.org/10.1038/ng.283

Lynch, H. T., Lynch, P. M., Lanspa, S. J., Snyder, C. L., Lynch, J. F., & Boland, C. R. (2009). Review of the Lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications. *Clin Genet*, *76*(1), 1-18. https://doi.org/10.1111/j.1399-0004.2009.01230.x

Mercado, R. C., Hampel, H., Kastrinos, F., Steyerberg, E., Balmana, J., Stoffel, E., Cohn, D. E., Backes, F. J., Hopper, J. L., Jenkins, M. A., Lindor, N. M., Casey, G., Haile, R., Madhavan, S., de la Chapelle, A., & Syngal, S. (2012). Performance of PREMM(1,2,6), MMRpredict, and MMRpro in detecting Lynch syndrome among endometrial cancer cases. *Genet Med*, *14*(7), 670-680. https://doi.org/10.1038/gim.2012.18

Moreira, L., Balaguer, F., Lindor, N., de la Chapelle, A., Hampel, H., Aaltonen, L. A., Hopper, J. L., Le Marchand, L., Gallinger, S., Newcomb, P. A., Haile, R., Thibodeau, S. N., Gunawardena, S., Jenkins, M. A., Buchanan, D. D., Potter, J. D., Baron, J. A., Ahnen, D. J., Moreno, V., . . . Castells, A. (2012). Identification of Lynch syndrome among patients with colorectal cancer. *Jama*, *308*(15), 1555-1565. https://doi.org/10.1001/jama.2012.13088

MRC. (2023, 03/03/2023). *Prediction of DNA mismatch repair gene mutation status in incident colorectal cancer cases*. https://webapps.igc.ed.ac.uk/world/research/hnpccpredict/

NCCN. (2023, October 30, 2023). *Genetic/Familial High-Risk Assessment: Colorectal Version 2.2023 — October 30, 2023*. NCCN. https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf

NICE. (2017, 02/22/2017). *Molecular testing strategies for Lynch syndrome in people with colorectal cancer*. Retrieved 02/07/2019 from https://www.nice.org.uk/guidance/dg27/resources/molecular-testing-strategies-for-lynch-syndrome-in-people-with-colorectal-cancer-pdf-1053695294917

NICE. (2020, 10/28/2020). *Testing strategies for Lynch syndrome in people with endometrial cancer*. NICE. Retrieved 2/1/2021 from https://www.nice.org.uk/guidance/dg42/chapter/1-Recommendations

NICE. (2022). *Quality statement 1: Testing for Lynch syndrome*. https://www.nice.org.uk/guidance/qs20/chapter/Quality-statement-1-Testing-for-Lynch-syndrome

Patel, S. G., May, F. P., Anderson, J. C., Burke, C. A., Dominitz, J. A., Gross, S. A., Jacobson, B. C., Shaukat, A., & Robertson, D. J. (2022). Updates on Age to Start and Stop Colorectal Cancer Screening: Recommendations From the U.S. Multi-Society Task Force on Colorectal Cancer. *Gastroenterology*, *162*(1), 285-299. https://doi.org/10.1053/j.gastro.2021.10.007

Peltomäki PT, O. G., Vasen HFA. (2010). Lynch syndrome. In C. F. Bosman FT, Hruban RH, Theise ND (Ed.), *WHO Classification of Tumours of the Digestive System* (4th ed., Vol. 3). IARC Press.

Rex, D. K., Boland, C. R., Dominitz, J. A., Giardiello, F. M., Johnson, D. A., Kaltenbach, T., Levin, T. R., Lieberman, D., & Robertson, D. J. (2017). Colorectal Cancer Screening: Recommendations for Physicians and Patients from the U.S. Multi-Society Task Force on Colorectal Cancer. *Am J Gastroenterol*, *112*(7), 1016-1030. https://doi.org/10.1038/ajg.2017.174

Robson, M. E., Bradbury, A. R., Arun, B., Domchek, S. M., Ford, J. M., Hampel, H. L., Lipkin, S. M., Syngal, S., Wollins, D. S., & Lindor, N. M. (2015). American Society of Clinical Oncology Policy Statement Update: Genetic and Genomic Testing for Cancer Susceptibility. *J Clin Oncol*, *33*(31), 3660-3667. https://doi.org/10.1200/jco.2015.63.0996

Robson, M. E., Storm, C. D., Weitzel, J., Wollins, D. S., & Offit, K. (2010). American Society of Clinical Oncology policy statement update: genetic and genomic testing for cancer susceptibility. *J Clin Oncol*, *28*(5), 893-901. https://doi.org/10.1200/jco.2009.27.0660

Rumilla, K., Schowalter, K. V., Lindor, N. M., Thomas, B. C., Mensink, K. A., Gallinger, S., Holter, S., Newcomb, P. A., Potter, J. D., Jenkins, M. A., Hopper, J. L., Long, T. I., Weisenberger, D. J., Haile, R. W., Casey, G., Laird, P. W., Le Marchand, L., & Thibodeau, S. N. (2011). Frequency of deletions of EPCAM (TACSTD1) in MSH2-associated Lynch syndrome cases. *J Mol Diagn*, *13*(1), 93-99. https://doi.org/10.1016/j.jmoldx.2010.11.011

Salvador, M. U., Truelson, M. R. F., Mason, C., Souders, B., LaDuca, H., Dougall, B., Black, M. H., Fulk, K., Profato, J., Gutierrez, S., Jasperson, K., Tippin-Davis, B., Lu, H. M., Gray, P., Shah, S., Chao, E. C., Ghahramani, N., Landsverk, M., Gau, C. L., . . . Pronold, M. (2019). Comprehensive Paired Tumor/Germline Testing for Lynch Syndrome: Bringing Resolution to the Diagnostic Process. *J Clin Oncol*, *37*(8), 647-657. https://doi.org/10.1200/jco.18.00696

Sepulveda, A. R., Hamilton, S. R., Allegra, C. J., Grody, W., Cushman-Vokoun, A. M., Funkhouser, W. K., Kopetz, S. E., Lieu, C., Lindor, N. M., Minsky, B. D., Monzon, F. A., Sargent, D. J., Singh, V. M., Willis, J., Clark, J., Colasacco, C., Rumble, R. B., Temple-Smolkin, R., Ventura, C. B., & Nowak, J. A. (2017). Molecular Biomarkers for the Evaluation of Colorectal Cancer: Guideline From the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology. *J Mol Diagn*, *19*(2), 187-225. https://doi.org/10.1016/j.jmoldx.2016.11.001

Stinton, C., Fraser, H., Al-Khudairy, L., Court, R., Jordan, M., Grammatopoulos, D., & Taylor-Phillips, S. (2021). Testing for lynch syndrome in people with endometrial cancer using immunohistochemistry and microsatellite instability-based testing strategies - A systematic review of test accuracy. *Gynecol Oncol*, *160*(1), 148-160. https://doi.org/10.1016/j.ygyno.2020.10.003

Stjepanovic, N., Moreira, L., Carneiro, F., Balaguer, F., Cervantes, A., Balmaña, J., & Martinelli, E. (2019). Hereditary gastrointestinal cancers: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. *Ann Oncol*, *30*(10), 1558-1571. https://doi.org/10.1093/annonc/mdz233

Stoffel, E. M., Mangu, P. B., Gruber, S. B., Hamilton, S. R., Kalady, M. F., Lau, M. W., Lu, K. H., Roach, N., & Limburg, P. J. (2015). Hereditary colorectal cancer syndromes: American Society of Clinical Oncology Clinical Practice Guideline endorsement of the familial risk-colorectal cancer: European Society for Medical Oncology Clinical Practice Guidelines. *J Clin Oncol*, *33*(2), 209-217. https://doi.org/10.1200/jco.2014.58.1322

Syngal, S., Brand, R. E., Church, J. M., Giardiello, F. M., Hampel, H. L., & Burt, R. W. (2015). ACG clinical guideline: Genetic testing and management of hereditary gastrointestinal cancer syndromes. *Am J Gastroenterol*, *110*(2), 223-262; quiz 263. https://doi.org/10.1038/ajg.2014.435

Umar, A., Boland, C. R., Terdiman, J. P., Syngal, S., de la Chapelle, A., Ruschoff, J., Fishel, R., Lindor, N. M., Burgart, L. J., Hamelin, R., Hamilton, S. R., Hiatt, R. A., Jass, J., Lindblom, A., Lynch, H. T., Peltomaki, P., Ramsey, S. D., Rodriguez-Bigas, M. A., Vasen, H. F., . . . Srivastava, S. (2004). Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst*, *96*(4), 261-268. https://doi.org/10.1093/jnci/djh034

Vasen, H. F., Mecklin, J. P., Khan, P. M., & Lynch, H. T. (1991). The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). *Dis Colon Rectum*, *34*(5), 424-425. https://doi.org/10.1007/BF02053699

Vasen, H. F., Watson, P., Mecklin, J. P., & Lynch, H. T. (1999). New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. In *Gastroenterology* (Vol. 116, pp. 1453-1456). https://doi.org/10.1016/s0016-5085(99)70510-x

Vikas, P., Messersmith, H., Compton, C., Sholl, L., Broaddus, R. R., Davis, A., Estevez-Diz, M., Garje, R., Konstantinopoulos, P. A., Leiser, A., Mills, A. M., Norquist, B., Overman, M. J., Sohal, D., Turkington, R. C., & Johnson, T. (2023). Mismatch Repair and Microsatellite Instability Testing for Immune Checkpoint Inhibitor Therapy: ASCO Endorsement of College of American Pathologists Guideline. *Journal of Clinical Oncology*, *41*(10), 1943-1948. https://doi.org/10.1200/jco.22.02462

Weissman, S. M., Burt, R., Church, J., Erdman, S., Hampel, H., Holter, S., Jasperson, K., Kalady, M. F., Haidle, J. L., Lynch, H. T., Palaniappan, S., Wise, P. E., & Senter, L. (2012). Identification of individuals at risk for Lynch syndrome using targeted evaluations and genetic testing: National Society of Genetic Counselors and the Collaborative Group of the Americas on Inherited Colorectal Cancer joint practice guideline. *J Genet Couns*, *21*(4), 484-493. https://doi.org/10.1007/s10897-011-9465-7

Win, A. K., Parry, S., Parry, B., Kalady, M. F., Macrae, F. A., Ahnen, D. J., Young, G. P., Lipton, L., Winship, I., Boussioutas, A., Young, J. P., Buchanan, D. D., Arnold, J., Le Marchand, L., Newcomb, P. A., Haile, R. W., Lindor, N. M., Gallinger, S., Hopper, J. L., & Jenkins, M. A. (2013). Risk of metachronous colon cancer following surgery for rectal cancer in mismatch repair gene mutation carriers. *Ann Surg Oncol*, *20*(6), 1829-1836. https://doi.org/10.1245/s10434-012-2858-5

Yilmaz, A., Mirili, C., Bilici, M., & Tekin, S. B. (2020). Colorectal cancer in Lynch syndrome associated with PMS2 and MSH6 mutations. *Int J Colorectal Dis*, *35*(2), 351-353. https://doi.org/10.1007/s00384-019-03454-4

Yurgelun, M. B., & Hampel, H. (2018). Recent Advances in Lynch Syndrome: Diagnosis, Treatment, and Cancer Prevention. *American Society of Clinical Oncology Educational Book*(38), 101-109. https://doi.org/10.1200/edbk_208341

Microsatellite Instability and Tumor Mutational Burden Testing

Policy Number: AHS – M2178 – Microsatellite Instability and Tumor Mutational Burden Testing	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Tumor mutational burden (TMB) is the genetic characteristic of non-inherited mutations within tumor tissue, often reported as the total number of DNA mutations per one million bases (megabase). Original studies calculated TMB based on whole-exome sequencing and reported TMB as the number of mutations that exist within the exome. However, TMB testing has expanded to targeted gene sequencing panels that do not cover the entire exome. TMB may serve as a biomarker to identify patients likely to have a favorable response to immunotherapy, as high TMB levels correlate with objective response rates to immunotherapy in several different cancer types (Ritterhouse, 2019; C. Willis et al., 2019).

Microsatellites are short, repetitive segments of DNA that are highly prone to mutation. Microsatellite instability (MSI) in tumor DNA is defined as the presence of alternate sized repetitive DNA sequences that are not present in the corresponding germline DNA (Nojadeh et al., 2018). Tumors with high microsatellite instability (MSI-H) are more immunogenic and may therefore respond to drugs that activate the immune system.

II. Related Policies

Policy Number	Policy Title
AHS-G2054	Liquid Biopsy
AHS-M2004	Lynch Syndrome
AHS-M2026	Testing for Colorectal Cancer Management
AHS-M2030	Testing for Targeted Therapy of Non-Small-Cell Lung Cancer
AHS-M2065	Molecular Profiling for Cancers of Unknown Primary Origin
AHS-M2146	General Genetic Testing, Somatic Disorders
AHS-M2168	Proteogenomic Testing of Individuals with Cancer

AHS-M2171	Esophageal Pathology Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals being considered for immune checkpoint inhibitor (ICI) targeted therapy, tissue-based tumor mutational burden (TMB) **and/or** microsatellite instability (MSI) testing (Note 1) for the solid tumors outlined in Note 2 **MEETS COVERAGE CRITERIA**.

2) When tissue-based testing is infeasible (i.e., quantity not sufficient for tissue-based test or invasive biopsy is medically contraindicated) for an individual being considered for ICI targeted therapy, liquid biopsy based TMB **and/or** MSI testing for the solid tumors outlined in Note 2 **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

3) TMB **and/or** MSI testing for all other tumors and in any other situations not listed above **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: NGS panels designed to provide a TMB score are allowed with no restrictions on the number of genes being tested, so long as the panel is designed for TMB assessment.

Note 2: Table of all solid tumors that will be allowed for TMB or MSI testing.

Condition	TMB/MSI Targeted Therapy Indications
Ampullary adenocarcinoma	MSI/MMR and TMB testing are recommended for individuals with locally advanced/metastatic adenocarcinoma who are candidates for anti-cancer therapy.
Biliary tract cancers (gallbladder, intrahepatic cholangiocarcinoma [CCA], extrahepatic CCA)	MSI/MMR and TMB testing are recommended for unresectable or metastatic biliary tract cancers (gallbladder, intrahepatic cholangiocarcinoma [CCA], extrahepatic CCA).

Condition	TMB/MSI Targeted Therapy Indications
Bone cancers (metastatic chondrosarcoma, chordoma, Ewing sarcoma, and osteosarcoma, NOT Giant Cell Tumor of Bone)	Recommended to consider TMB or MSI testing to inform treatment.
Breast cancer	TMB and MSI/dMMR are recommended to guide treatment options for individuals with recurrent unresectable (local or regional) or stage IV (M1) disease.
Cervical cancers (squamous cell carcinoma, adenocarcinoma, or adenosquamous carcinoma)	MSI testing is recommended for recurrent, progressive, or metastatic squamous cell carcinoma, adenocarcinoma, or adenosquamous carcinoma. Comprehensive molecular profiling should be considered for squamous cell carcinoma, adenocarcinoma, or adenosquamous carcinoma.
Colon cancer	MSI/MMR testing advised in all newly diagnosed patients, as a workup in pedunculated or sessile colon polylps (adenoma), in colon cancer appropriate for resection (non-metastatic), in documented metachronous metastases by CT, MRI, and/or biopsy, and in suspected or proven metastatic synchronous adenocarcinoma (any T, any N, M1) if not already done. Stage II MSI-H patients may have a good prognosis and do not benefit from 5-FU adjuvant therapy. Adjuvant therapy should not be given to patients with low-risk stage II MSI-H tumors. MMR/MSI testing should be done during initial workup to help with the diagnosis of Lynch syndrome and inform treatment decision-making if adjuvant therapy is later indicated.
Esophageal and Esophagogastric junction (EGJ) cancers	MSI/MMR is recommended as a universal test in all individuals who have been newly diagnosed with esophageal and EGJ cancers. TMB is considered one of the targeted biomarkers for individuals with esophageal or EGJ cancer.
Gastric cancers	TMB and MSI/dMMR testing are recommended for the clinical management of advanced gastric cancer.
Head and Neck Cancers (nasopharyngeal, non-nasopharyngeal, salivary gland tumors)	TMB is recommended to guide treatment options for individuals with recurrent, unresectable, oligometastatic, or metastatic nasopharyngeal cancer. TMB and MSI/dMMR are recommended to guide treatment options for individuals with non-nasopharyngeal cancers (oral cavity [including mucosal lip], oropharynx, hypopharynx, glottic larynx, supraglottic larynx, ethmoid sinus, maxillary sinus, and occult primary). TMB is recommended as part of follow-up assessment for salivary gland tumors for recurrence with distance metastases. TMB and MSI/dMMR are recommended to guide therapy options in recurrent, unresectable, or metastatic salivary gland tumors.
Neuroendocrine/Adrenal cancers (Grade 3 neuroendocrine tumors, adrenocortical carcinoma, and for poorly differentiated neuroendocrine carcinoma/large or small cell)	TMB and MSI testing should be considered for adrenocortical carcinoma and poorly differentiated neuroendocrine carcinoma/large or small cell and TMB testing should be considered for well-differentiated Grade 3 neuroendocrine tumors. TMB and MSI testing are an appropriate evaluation when the tumor type is poorly differentiated neuroendocrine carcinoma, large or small cell carcinoma (other than the lung), or unknown primary (poorly differentiated).
Non-small cell lung cancer	Biomarker panel testing for actionable driver mutations (with or without TMB/MSI) is allowed.

Condition	TMB/MSI Targeted Therapy Indications
Occult primary cancers (cancer of unknown primary [CUP])	Suspected metastatic malignancy should undergo TMB determination or MSI/MMR testing during the workup of the tumor. Of note, the population of patients with MSI-H/dMMR occult primary tumors is low.
Ovarian cancers (epithelial ovarian, endometrioid carcinoma, low-grade serous carcinoma, fallopian tube, primary peritoneal cancers)	TMB and MSI/dMMR is recommended as part of validated molecular testing in Stage I, II, III, and IV epithelial ovarian cancer, fallopian tube cancer, and primary peritoneal cancer when disease is recurrent and CA-125 is rising or there is clinical relapse. TMB and MSI/dMMR are recommended for ovarian cancer, fallopian tube cancer, and primary peritoneal cancer as part of tumor molecular analysis in the recurrence setting. MSI/dMMR testing is recommended for all patients with endometrioid carcinoma. TMB and MSI/dMMR are recommended as part of tumor molecular analysis for monitoring and follow up for recurrence in low-grade serous carcinoma.
Pancreatic cancer (adenocarcinoma)	TMB and MSI/dMMR testing is recommended in locally advanced disease when adenocarcinoma is confirmed, in metastatic disease, and in tumors with recurrence after resection.
Pediatric central nervous system cancers (high-grade gliomas EXCEPT oligodendroglioma)	TMB is recommended to guide targeted therapy of recurrent and progressive disease for diffuse high-grade gliomas EXCEPT oligodendroglioma, *IDH*-mutant and 1p/19q codeleted or astrocytoma *IDH*-mutant.
Penile cancer	TMB and MSI/dMMR are recommended to guide treatment options for individuals with metastatic or recurrent disease.
Prostate cancers (castration-resistant prostate cancer [CRPC], adenocarcinoma)	TMB and MSI/dMMR testing should be considered when CRPC imaging studies are positive for metastases. Tumor testing recommended in MSI-H or dMMR among patients with metastatic CRPC and may be considered in patients with regional or castration-naïve metastatic prostate cancer. TMB may be considered in patients with metastatic CRPC.
Rectal cancers	Universal MSI/MMR testing is recommended in all newly diagnosed patients with rectal cancer. In addition, MSI/MMR testing is recommended as an initial workup in pedunculated polyps or sessile polyps (adenoma) with invasive cancer, rectal cancer appropriate for resection, and in suspected or proven metastatic synchronous adenocarcinoma (any T, any N, M1) if not previously done.
Small bowel cancer (adenocarcinoma)	TMB testing should be considered as part of the workup for metastatic and/or recurrent adenocarcinoma. Universal MMR or MSI testing is recommended in all newly diagnosed patients with small bowel adenocarcinoma. When not part of the initial workup, MSI testing is recommended as a workup when the tumor is in the duodenum or the jejunum/ileum and should be part of the workup for metastatic and/or recurrent adenocarcinoma.
Soft tissue sarcoma	TMB and MSI/dMMR are recommended to guide treatment options for individuals with unresectable or metastatic soft tissue sarcoma.
Testicular cancers (nonseminoma, pure seminoma)	TMB and MSI testing are recommended as a third-line therapy in nonseminoma and pure seminoma if there is progression after high-dose chemotherapy or other third-line therapies.
Thyroid cancer (anaplastic thyroid carcinoma)	TMB and MSI/dMMR are recommended as part of initial diagnostic molecular testing for anaplastic thyroid carcinoma.

Condition	TMB/MSI Targeted Therapy Indications
Thyroid cancers (papillary carcinoma, follicular carcinoma, and oncocytic carcinoma)	TMB and MSI/dMMR testing are recommended for papillary carcinoma, follicular carcinoma, and oncocytic carcinoma for advanced, progressive, or threatening disease when tumor is locally recurrent, advanced, and/or metastatic disease that is not amenable to RAI therapy.
Thyroid cancer (medullary carcinoma)	TMB testing is recommended for medullary carcinoma for recurrent or persistent locoregional disease or for symptomatic disease or progression of disease for recurrent or persistent disease with distance metastases.
Uterine cancers (endometrial carcinoma, uterine sarcoma)	MSI testing is recommended if MMR results are equivocal in endometrial carcinoma. TMB testing should be considered for endometrial carcinoma. For sarcoma, comprehensive genomic profiling (at minimum TMB/MSI, and *NTRK*) in a setting of metastatic disease is informative for predicting rare pan-tumor targeted therapy opportunities.
Vulvar cancer (squamous cell carcinoma)	MSI and TMB testing may be considered for treatment planning purposes in patients with recurrent, progressive, or metastatic disease.

MSI-H (MSI-high; MSI-positive); NCCN defines TMB-H as a TMB value of ≥10 mutations/megabase

IV. Table of Terminology

Term	Definition
5-FU	5-fluorouracil
ALK	*Anaplastic lymphoma kinase*
ANTI-PD1	Anti-programmed cell death 1
BRAF	*B-Raf proto-oncogene, serine/threonine kinase*
BRCA1	*Breast cancer 1*
BRCA2	*Breast cancer 2*
bTMB	Blood tumor mutational burden
CA-125	Cancer antigen 125
CAPEOX	Capecitabine and oxaliplatin
CCA	Cholangiocarcinoma
cfDNA	Circulating cell-free deoxyribonucleic acid
CGP	Comprehensive genomic profiling
CMS	Centers for Medicare and Medicaid Services
CNA	Copy number alteration
CNS	Central nervous system
CNV	Copy number variation
CPS	Combined positive score
CRM	Circumferential resection margin
CRPC	Castration-resistant prostate cancer
CT	Computerized tomography

ctDNA	Circulating tumor deoxyribonucleic acid
CTLA-4	Cytotoxic t-lymphocyte-associated protein 4
CUP	Cancer of unknown primary
DCB	Durable clinical benefit
dMMR	Deoxyribonucleic acid mismatch repair
DNA	Deoxyribonucleic acid
DRUP	Drug rediscovery protocol
EGFR	*Epidermal growth factor receptor*
EGJ	Esophagogastric junction
ESMO	European Society for Medical Oncology
F1CDx	FoundationOne CDx™
FBXW7	*F-box and WD40 repeat domain containing-7*
FDA	Food and Drug Administration
FFPE	Formalin-fixed, paraffin-embedded
FOLFOX	Leucovorin calcium, fluorouracil, oxaliplatin
GEA	Gastroesophageal adenocarcinoma
HLA	Human leukocyte antigen
ICI	Immune checkpoint inhibitor
IHC	Immunohistochemistry
INDELS	Insertion and deletions
iRECIST	RECIST 1.1 for immune-based therapeutics
LCD	Local coverage determination
LDT	Laboratory-developed test
LOH	Loss of heterozygosity
mCRC	Metastatic colorectal cancer
MLH1	MutL homolog 1
MMR	Mismatch repair
MRI	Magnetic resonance imaging
MSH2	MutS homolog 2
MSH6	MutS homolog 6
MSI	Microsatellite instability
MSI-H	High microsatellite instability
MSI-L	Low microsatellite instability
MSK	Memorial Sloan Kettering Cancer Center
MSS	Microsatellite stable
NCCN	National Comprehensive Cancer Network
NCD	National coverage determination
NCI	National Cancer Institute
NDB	No durable benefit

NET	Neuroendocrine tumor
NGS	Next-generation sequencing
NSCLC	Non-small cell lung carcinoma
NTRK	Neurotrophic tyrosine receptor kinase
ORR	Objective response rate
OS	Overall survival
PCR	Polymerase chain reaction
PD-1	Programmed cell death protein
PD-L1	Programmed cell death protein 1
PFS	Progression-free survival
PMS2	Mismatch repair endonuclease PMS2
POLD1	Deoxyribonucleic acid polymerase delta catalytic subunit
POLE1	Deoxyribonucleic acid polymerase epsilon catalytic subunit isoform b
PS	Performance status
R^2	Correlation
RAI	Radioactive iodine
RAS	*Retrovirus-associated deoxyribonucleic acid sequences*
RECIST	Response evaluation criteria in solid tumors
RNA	Ribonucleic acid
ROS1	*Receptor tyrosine kinase*
SBA	Small bowel adenocarcinoma
SCL	Small-cell lung cancer
SMAD4	*SMAD family member 4*
SNV	Single nucleotide variant
TF	Tumor fraction
TMB	Tumor mutational burden
TMB-H	Tumor mutational burden – high
TML	Tumor mutational load
TRAE	Treatment-related adverse events
TSO500	TruSight Oncology 500
tTMB	Tissue tumor mutation burden
TTP	Time to progression
WES	Whole exome sequencing
WTS	Whole transcriptome sequencing

V. Scientific Background

Tumor mutational burden (TMB) is an emerging biomarker associated with predicting the response to immune checkpoint inhibitors (ICIs). ICIs are therapies that have made

significant progress in helping to treat certain advanced cancers. ICIs work by releasing the brakes on the immune system's antitumor response. ICI therapy has proven most effective on tumor types with a high TMB, whereby a high TMB value indicates better treatment outcomes (Yarchoan et al., 2017).

Clinical biomarkers are widely used for making personalized and actionable decisions for cancer treatment. Many mutations result in production of numerous altered peptides—the more mutations present, the larger the number of altered peptides is produced. A subset of these peptides is expressed and processed by the major histocompatibility complex, resulting in neoantigens. These neoantigens can be recognized by the immune system to generate an antitumor response. The recognition of these neoantigens is thought to be largely stochastic and a higher number of DNA mutations means a higher number of candidate peptides being produced, resulting in a greater likelihood of these neoantigens being recognized by the immune system (Ritterhouse, 2019; Yarchoan et al., 2019).

This phenomenon is observed in patients with melanoma, non-small-cell lung carcinoma (NSCLC), small cell lung cancer, bladder cancer, colorectal cancer, microsatellite instability cancers, and pan-tumors who are on inhibitors of programmed cell death protein (PD-1) and the ligand for programmed cell death protein 1 (PD-L1). High TMB has also been associated with improved outcomes in patients on a combination of PD-1/PD-L1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitors (Hellmann, Callahan, et al., 2018; Hellmann, Nathanson, et al., 2018; Le et al., 2015; Merino et al., 2020; Rizvi et al., 2015; Snyder et al., 2014; Van Allen et al., 2015).

Originally, TMB was measured with whole-exome sequencing (WES). In theory, WES is the best measure of TMB, as it covers every coding sequence within the tumor, ensuring that every mutation contributes to the TMB score. Recently, studies have shown that TMB measured using targeted panels which analyze mutations across specific genes (rather than the whole exome) correlates well with TMB measured by WES (Chalmers et al., 2017; Quy et al., 2019; Wu et al., 2019). WES method has limited clinical utility as it is considered costly, has a high turnaround time (a roughly six to eight week sequencing period), and is difficult for many clinical laboratories to use for routine patient care (Ritterhouse, 2019). Hence, targeted next-generation sequencing (NGS)-based panels that focus analysis on a subset of genes implicated in cancer biology offer several advantages for TMB estimation (Garofalo et al., 2016).

There are FDA-approved products for calculating TMB, including the FoundationOne CDx™ (F1CDx) assay and the FoundationOne Liquid CDx assay (Foundation Medicine Inc.) (FDA, 2017c, 2020b), as well as an additional FDA 510(k) authorized assay, MSK-

IMPACT (Memorial Sloan Kettering Cancer Center) (FDA, 2017a, 2017b). These tests, referred to collectively as comprehensive genomic profiling (CGP), can identify all types of "molecular alterations (i.e., single nucleotide variants, small and large insertion-deletion alterations, copy number alterations, and structural variants) in cancer-related genes, as well as genomic signatures such as microsatellite instability (MSI), loss of heterozygosity [LOH], and TMB" (Klempner et al., 2020). Studies show that TMB calculated from CGP has high concordance with TMB measured from WES. On June 16, 2020, the FDA approved pembrolizumab for the treatment of adult and pediatric patients with a TMB value of greater than 10 mutations per megabase as determined by the FoundationOne CDx™ assay (FDA, 2020a).

Microsatellites are short, repetitive segments of DNA that are highly prone to mutation. The presence of alternate sized repetitive DNA sequences that are not present in the corresponding germline DNA is defined as microsatellite instability (MSI) (Nojadeh et al., 2018). When a tumor has a high degree of microsatellite instability (MSI-H), it is more immunogenic and thus may respond to drugs that activate the immune system, such as ICIs (Zhao et al., 2019).

The National Cancer Institute (NCI) recommends two mononucleotide repeats (BAT-25 and BAT-26) and three dinucleotide repeats (D5S346, D2S123, and D17S250) as the standard sites/markers in panels for MSI testing (Boland et al., 1998). Per the NCI guidelines, when a tumor shows instability in two or more of the five markers, it is defined as MSI-H. However, this definition may vary by source and markers used (NCI, n.d.). When only a single marker shows instability, tumors may be classified as low-frequency MSI (MSI-L) and tumors lacking any instability are often classified as microsatellite stable (MSS) (Boland et al., 1998).

Microsatellite instability has been identified in many cancer types, with the highest prevalence in uterine corpus endometrial carcinoma, colon adenocarcinoma, stomach adenocarcinoma, and rectal adenocarcinoma. In studies by Bonneville et al. (2017) and Zhao et al. (2019), MSI was undetectable (or at the borderline of detection) in 12 out of 39 cancer types (Bonneville et al., 2017; Zhao et al., 2019).

Proprietary Testing

Caris MI Tumor Seek

Caris Life Sciences has developed MI Tumor Seek™, an NGS-based tumor profiling service that combines WES analysis of DNA for mutations, copy number alterations, insertions/deletions, and genomic signatures (MSI, TMB, and LOH) with Whole Transcriptome Sequencing (WTS) analysis for RNA fusions and variant transcripts. WES covers about 23,000 genes with 200-500x depth of coverage, while WTS covers about

23,000 genes with 60 million reads/sample. DNA and RNA are isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue "with a minimum of 20% malignant origin for DNA and 10% malignant origin for RNA. Needle biopsy is also acceptable (4-6 cores)" (Caris Life Sciences, 2024).

FoundationOne CDx™

FoundationOne CDx™ is a single-site assay that is "a next generation sequencing based in vitro diagnostic device for detection of substitutions, insertion and deletion alterations (indels) and copy number alterations (CNAs) in 324 genes and select gene rearrangements, as well as genomic signatures including microsatellite instability (MSI) and TMB using DNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue specimens." It is intended for use by health care professionals as a companion diagnostic to identify patients with solid malignant neoplasms who may benefit from treatment with targeted ICIs (FDA, 2017c).

FoundationOne Liquid CDx

The FoundationOne Liquid CDx assay uses circulating cell-free DNA (cfDNA) that is isolated from plasma from patients with solid malignant neoplasms. The assay uses a "single DNA extraction method to obtain cfDNA from plasma from whole blood. Extracted cfDNA undergoes whole-genome shotgun library construction and hybridization-based capture of 324 cancer-related genes. All coding exons of 309 genes are targeted; select intronic or non-coding regions are targeted in BRCA1 and BRCA2 … Hybrid-capture selected libraries are sequenced with deep coverage using the NovaSeq® 6000 platform. Sequence data are processed using a custom analysis pipeline designed to detect genomic alterations, including base substitutions and indels in 311 genes, and copy number variants and genomic rearrangements in BRCA1 and BRCA2. A subset of targeted regions in 75 genes is baited for increased sensitivity" (FDA, 2020b).

Guardant360® TissueNext

Guardant Health offers Guardant360® CDx, a liquid biopsy that provides comprehensive genomic results from a simple blood draw. This test "uses targeted high throughput hybridization-based capture technology for detection of single nucleotide variants (SNVs), insertions and deletions (indels) in 55 genes, copy number amplifications (CNAs) in two (2) genes, and fusions in four (4) genes. Guardant360® CDx utilizes circulating cell-free DNA (cfDNA) from plasma of peripheral whole blood." It is an FDA-approved liquid biopsy that can be used for tumor mutation profiling across all solid cancers and as a companion diagnostic to identify NSCLC patients who may benefit from Tagrisso® (10ealth10inib), RYBREVANT™ (amivantamab-vmjw), or LUMAKRAS™ (sotorasib). To compliment Guardant360 CDx, Guardant Health also

offers Guardant360® TissueNext. Guardant360 TissueNext assesses TMB either from an FFPE tumor specimen or from a blood sample initially processed with Guardant360® CDx. If an actionable biomarker is found in a Guardant360® CDx sample, it is then processed with the Guardant360® TissueNext assay (FDA, 2021; Guardant, 2024a, 2024b).

MSK-IMPACT

The MSK-IMPACT assay is a single-site assay performed at Memorial Sloan Kettering Cancer Center (MSKCC) and is designed to compare two samples from a single patient—a normal specimen and a specimen from a solid malignant neoplasm—allowing the assay to detect tumor gene alterations in a broad multi gene panel (FDA, 2017a). MSK-IMPACT analyzes 505 genes chosen for their critical role in the development and behavior of tumors. To "take full advantage of MSK-IMPACT, MSK doctors and researchers developed a knowledge base called OncoKB®. This system includes information about the clinical and biological effects of more than 4,000 genomic changes. That information is based on public databases, scientific literature, and clinical guidelines" (MSKCC, 2024). The assay is intended to provide information on both somatic mutations and microsatellite instability and is not conclusive or prescriptive for labeled use of any specific therapeutic product (FDA, 2017a).

QIAseq TMB

Qiagen offers QIAseq Tumor Mutational Burden Panels. These new panels collectively create a comprehensive profile of TMB and MSI status by "achieving high analytical sensitivity, with lower false and negative rates, while still maintaining >95% correlation with whole exome datasets." The QIAseq TMB Panel covers 486 genes and can be boosted to add 27 MSI markers, with sample preparation coming from FFPE of solid tumors, patient's plasma/serum, fresh or frozen tissue, or from cell lines in a research setting. This assay has been shown to correlate well with WES and established TMB panels. It uses enzymatic fragmentation for easy workflow, employs robust analysis modules, and incorporates unique molecular indices to correct for polymerase chain reaction (PCR) and sequencing errors (Qiagen, 2024).

TruSight Oncology 500

Illumina offers the TruSight Oncology 500 (formerly Illumina TSO500), a NGS assay that enables in house CGP of tumor samples, supports the identification of relevant DNA and RNA variants implicated in various solid tumor types, and accurately measure current immune-oncology biomarkers: MSI and TMB. This assay assesses both DNA (523 genes) and RNA (55 genes) for assessment of all variant types in addition to MSI and TMB. The TruSight Oncology 500 assay is intended for multiple solid tumor types and detects CNVs, gene fusions, insertions-deletions, single nucleotide variants, and

transcript variants. It provides stealth-oncology biomarker coverage, including biomarkers of TMB and MSI, and is inclusive of human leukocyte antigen (HLA) regions, DNA polymerase epsilon catalytic subunit isoform b (POLE1), and DNA polymerase delta catalytic subunit (POLD1) (Illumina, 2024).

Analytical Validity

Wu et al. (2019) noted that targeted NGS panels often use correlation (R^2) between panel- and WES- based TMB to validate TMB estimation, which can be distorted by cases where a tumor within a certain cancer type has a relatively ultra-high TMB. Thus, the authors proposed using accuracy (the proportion of cases correctly identified as TMB-high or TMB-low using panel based TMB) as a more robust indicator of panel performance, suggesting that accuracy and cancer type individualization are key factors to consider when designing panels for TMB estimation. The authors examined the TMB estimation from five available NGS panels for TMB determination (F1CDx, MSK-IMPACT, Illumina TSO500 [now TruSight Oncology 500], Oncomine TML, QIAseq TMB) to compare correlation to accuracy. F1CDx seemed to accurately assess TMB ($R^2 \geq 0.75$) in at least 24 out of 33 cancer types. However, when the cutoff point for high TMB was defined as the top 20% in each cancer type, the accuracy within these 24 cancer types ranged largely (56-99%), with only seven cancer types having satisfactory accuracy. These results indicate that F1CDx-based TMB estimation is only reliable in certain cancer types (cervical squamous cell carcinoma and endocervical adenocarcinoma, colon adenocarcinoma, head and neck squamous cell carcinoma, lung adenocarcinoma, skin cutaneous melanoma, stomach adenocarcinoma, and uterine corpus endometrial carcinoma). In the other 17 cancer types, correlation overestimated TMB status misclassifying a considerable number of TMB-low patients as TMB-high. This overestimation by correlation occurs because correlation is vulnerable to distortion by the common presence of cases with relatively ultra-high TMB within each cancer type. When removing cases with these relatively ultra-high TMB (top five percent within a particular cancer type), a dramatic decline in correlation between F1CDx- and WES-based TMB was observed in half of the 24 cancer types. In contrast, removing the top five percent of cases in each cancer type did not, in general, affect the accuracy within these 24 cancer types. Accuracy was also superior to correlation in the other four NGS panels examined. The authors conclude that the five available NGS panels can assess TMB accurately only in the seven cancer types aforementioned and note that for relatively ultra-high TMB, correlation is an unreliable evaluation of panel based TMB estimation performance in most cancer types. Instead, they find that accuracy is a superior index in this situation. They conclude that individualized panels by cancer type might be a better strategy to guarantee the most robust TMB estimation and provide better power in detecting the predictive function of TMB in these cancer types (Wu et al., 2019).

J. Willis et al. (2019) aimed to evaluate the validity of cell-free (cf) DNA to detect microsatellite instability (MSI). The authors validated the Guardant360 MSI detection with 1145 cf-DNA samples and further investigated the landscape of cf-DNA based MSI across 28459 plasma samples. Circulating tumor (ct, used as a proxy for cf-DNA) MSI assessment was compared to current standard of care tissue testing (a combination of immunohistochemistry, PCR, and NGS). The study included 949 patients that were considered unique and evaluable. The ctDNA method detected MSI-high values (82% accuracy) in 71 of 82 patients. A total of 863 of 867 patients reported as MSI-stable (99.5% accuracy) for a total of 98.4% accuracy amongst all MSI-reported groups. The positive predictive value was found to be 95%. Finally, the authors examined the clinical outcomes of 16 patients with MSI-H gastric cancer that were treated with immunotherapy. The authors found that 10 of 16 patients achieved "complete or partial remission with sustained clinical benefit" after being treated with immunotherapy (J. Willis et al., 2019).

Georgiadis et al. (2019) aimed to validate a noninvasive approach for detection of MSI and TMB. The authors used "a hybrid-capture–based 98-kb pan-cancer gene panel, including targeted microsatellite regions" as well as a "novel peak-finding algorithm…established to identify rare MSI frameshift alleles in cell-free DNA." The authors evaluated 163 healthy patients with this assay, identifying a specificity of 99.4% (one false positive, which was considered a lower bound by the authors). The authors also evaluated 23 MSI-H cases (from patients with metastatic cancers) and six MSI-stable cases with the algorithm. Using cf-DNA, the algorithm detected MSI in 18 of the 23 MSI-H samples (78.3%) and correctly identified all six of the MSI-stable cases. Further, this approach was also applied to TMB (using a cutoff of 51 mutations/Mbp), of which the algorithm correctly detected 10 of the 15 samples identified as TMB-high (67%) (Georgiadis et al., 2019).

Woodhouse et al. (2020) evaluated the analytical performance of FoundationOne Liquid CDx assay to detect genomic alterations in cancer patients. The assay was evaluated across more than 30 different cancer types in over 300 genes and greater than 30,000 gene variants. "Results demonstrated a 95% limit of detection of 0.40% variant allele fraction for select substitutions and insertions/deletions, 0.37% variant allele fraction for select rearrangements, 21.7% tumor fraction (Marabelle, Le, et al.) for copy number amplifications, and 30.4% TF for copy number losses. The false positive variant rate was 0.013% (approximately one in 8,000). Reproducibility of variant calling was 99.59%" (Woodhouse et al., 2020). In comparison to in situ hybridization and immunohistochemistry, FoundationOne had an overall 96.3% positive percent agreement and >99.9% negative percent agreement. "These study results demonstrate that FoundationOne Liquid CDx accurately and reproducibly detects the major types of genomic alterations in addition to complex biomarkers such as microsatellite

instability, blood tumor mutational burden, and tumor fraction" (Woodhouse et al., 2020).

Using data from the phase III MYSTIC trial, Si et al. (2021) validated the use of blood TMB (bTMB) using circulating cell-free tumor DNA (ctDNA) through comparisons with TMB measurements from tumor tissue (tTMB). Patients with metastatic NSCLC were treated either with first-line durvalumab (anti–PD-L1 antibody) ± tremelimumab (anticytotoxic T-lymphocyte-associated antigen-4 antibody) or chemotherapy, then bTMB and tTMB were evaluated using the GuardantOMNI and the FoundationOne CDx assay, respectively. To identify the optimal bTMB cutoff, the authors used a Cox proportional hazards model and minimal p value cross-validation. In the majority of patients in the MYSTIC study, somatic mutations were detected in ctDNA extracted from plasma samples, which allowed for the subsequent calculation of bTMB. The authors found that the success rate for obtaining valid TMB scores was higher for bTMB (81%) than for tTMB (63%) and that with minimal p value cross-validation analysis, they suggest a bTMB ≥20 mutations per megabase (mut/Mb) as the optimal cutoff for clinical benefit with durvalumab + tremelimumab. Overall, their study demonstrated that use of bTMB from plasma samples was feasible and provided accurate and reproducible TMB detection using the GuardantOMNI ctDNA platform. They also state that using "the new bTMB algorithm and an optimal bTMB cutoff of ≥20 mut/Mb, high bTMB was predictive of clinical benefit with durvalumab + tremelimumab versus chemotherapy" (Si et al., 2021).

Clinical Utility and Validity

Overman et al. (2017) examined MSI-H/dMMR colorectal cancer individual response to nivolumab, a PD-1 ICI. In this phase two trial, the authors assessed adult individuals with histologically confirmed recurrent or mCRC locally assessed as dMMR/MSI-H who had progressed either while still on or after, or who had been intolerant of, at least one prior line of treatment, including fluoropyrimidine and oxaliplatin or irinotecan. The primary endpoint of this study was investigator-assessed ORR. This study ran from March 12, 2014 to March 16, 2016 and included 74 individuals, most of whom (54.1%), had received more than three prior therapies. Twelve months following initiation of nivolumab treatment, 31.1% (23) of the individuals had an investigator-assessed ORR, which 68.9% of the individuals had disease control for more than 12 weeks. The median duration of response was not reached, as all responders were alive and eight of them had responses greater than 12 months. Twenty-three of the individuals died during the study, though none of the deaths were considered treatment-related. The authors concluded that nivolumab provided durable responses and disease control in dMMR/MSI-H patients that had previously received other forms of treatment for their

colorectal cancer and they also note that nivolumab is a relevant treatment option for these patients (Overman et al., 2017).

Hellmann, Ciuleanu, et al. (2018) conducted an open-label, multipart, phase three trial to examine progression-free survival in non-small-cell lung cancer patients with a high TMB (TMB-H) that were being treated with nivolumab plus ipilimumab or with chemotherapy. TMB was determined by the FoundationOne CDx™ assay. Here, they found that progression-free survival in TMB-H patients was significantly longer with nivolumab plus ipilimumab (42.6%) compared with chemotherapy (13.2%). This discovery was found to be accurate irrespective of PD-L1 expression level and validated that a treatment of nivolumab and ipilimumab for NSCLC elicited a favorable outcome for select patients, as well as determining that tumor mutational burden was an important biomarker for patient selection when it comes to drug therapy (Hellmann, Ciuleanu, et al., 2018).

To examine the association between TMB and clinical response to ICIs in select cancer types, Samstein et al. (2019) analyzed the clinical and genomic data of 1,662 advanced cancer patients treated with ICI compared to 5,371 patients not treated with ICI. Tumors from these patients underwent targeted NGS with MSK-IMPACT. The authors found that among all patients, higher somatic TMB (highest 20% in each cancer type, consistent with many MSI-high colorectal tumors receiving ICI treatment) was associated with better overall survival. However, the TMB cutoffs associated with improved survival varied markedly between the different cancer types studied, suggesting that while TMB is associated with improved survival in patients receiving ICI across a variety of cancer types, there may not be a single, universal definition of high TMB (Samstein et al., 2019).

Ready et al. (2019) conducted an open-label phase II trial to evaluate the efficacy and safety of nivolumab plus low-dose ipilimumab as first-line treatment of advanced/metastatic NSCLC and assessed the treatment efficacy associated with PD-L1 expression and TMB. For this trial, "the primary end point was objective response rate (ORR) in patients with 1% or more and less than 1% tumor PD-L1 expression. Efficacy on the basis of TMB (FoundationOne CDx™ assay) was a secondary end point." They found that regardless of PD-L1 expression, ORRs were higher in TMB-H patients versus patients with tumors below the threshold for TMB-H status and progression-free survival was longer in patients with TMB-H. They concluded that "nivolumab plus low-dose ipilimumab was effective and tolerable as a first-line treatment of advanced/metastatic NSCLC" and a high TMB status was associated with both improved response and prolonged progression-free survival irrespective of PD-L1 status. This identifies TMB-H as a potentially relevant cutoff in the assessment of TMB as a biomarker for first-line nivolumab plus ipilimumab (Ready et al., 2019).

A comprehensive study by Singal et al. (2019) examined the electronic health records of 4064 individuals with NSCLC from 275 different oncology practices to explore the associations between tumor genomics and patient characteristics with clinical outcome. The authors note that 21.4% of these individuals had a mutation in *EGFR, ALK, or ROS1* and that patients with driver mutations who received targeted therapies had significantly improved overall survival times than individuals who did not receive targeted therapies (median of 18.6 versus 11.4 months, respectively). Moreover, a TMB of 20 or higher was associated with improved overall survival for patients on PD-L1-targeted therapy compared to patients with a TMB less than 20. TMBs measure the quality of a mutation in a tumor, suggesting whether a patient will benefit from immunology-based cancer therapies or not. The authors concluded that similar associations from previous research were replicated "between clinical and genomic characteristics, between driver mutations and response to targeted therapy, and between TMB and response to immunotherapy" (Singal et al., 2019).

Yarchoan et al. (2019) examined the relationship between the expression of PD-L1, a widely used biomarker for selecting patients for ICI, and TMB across the entire spectrum of ICI-responsive human cancers. They sought to use these predictive biomarkers to provide a broad definition to the immunologic subtypes of cancers and identify opportunities for the development of therapeutics. Their clinical cohort contained 9,887 unique clinical samples with paired CGP and PD-L1 expression obtained during standard clinical care. They found that 15.2% of samples had a positive expression of PD-L1 and that the highest occurrence of PD-L1 positivity occurred in thymic cancer and diffuse large B cell lymphoma, while there were no adenoid cystic or appendiceal samples with positive PD-L1. A total 3.6% of sample specimens were identified as having a high PD-L1 positivity (defined as greater than 50% of tumor cells staining positive). In addition, the median tumor mutational burden for all specimens was 3.48 mutations per megabase. One group of sampled tumors (16.4% of the cancers) had a TMB of greater than 10 mutations per megabase and 7.3% of tumors analyzed had a TMB of more than 20 mutations per megabase, indicating that the overall TMB profiles were comparable to prior cancer studies of a similar nature (including whole-exome studies) that estimated TMB biomarkers in solid tumors. This study found a relative independence of PD-L1 expression and TMB, and that the biomarkers were important indicators of patient response to ICI therapy. The study also defined which biomarkers may help identify future therapies through their ability to segment and profile different tumor types. In conclusion, they found that PD-L1 and TMB status may help determine whether the use of ICI therapy is indicated as well as pinpoint tumor types that would demonstrate a beneficial response to immune checkpoint inhibitor therapy (Yarchoan et al., 2019).

Alborelli et al. (2020) investigated the predictive power of TMB for patients treated with ICIs. Seventy-six NSCLC patients treated with ICIs were included and TMB was evaluated with the Oncomine™ Tumor Mutational Load (TML) sequencing assay. Patients were separated into cohorts of "durable clinical benefit" (DCB) or "no durable benefit" (NDB). TMB was found to be higher in the DCB cohort (median TMB of 8.5 mutations / Mb compared to six mutations / Mb in NDB). Sixty-four percent of patients in the highest one third of TMB were responders, compared to 33% and 29% in the middle and lowest thirds, respectively. TMB-H patients were also found to have higher progression-free survival and overall survival. Overall, the authors concluded that the TML panel was an effective tool to stratify patients for ICI treatment and suggested that "a combination of biomarkers might maximize the predictive precision for patient stratification." Further, the authors remarked that their data "supports TMB evaluation through targeted NGS in NSCLC patient samples as a tool to predict response to ICI therapy" (Alborelli et al., 2020).

Marabelle, Fakih, et al. (2020) prospectively explored the association of a high tumor mutational burden with outcomes in ten tumor-type-specific cohorts from the phase two KEYNOTE-158 study. The study assessed use of the anti–PD-1 monoclonal antibody pembrolizumab in patients with select, previously treated, advanced solid tumors. Patients for this study were from 81 academic facilities across 21 countries in Africa, the Americas, Asia, and Europe, were over 18 years of age, and had an established tumor of anal, biliary, cervical, endometrial, mesothelioma, neuroendocrine, salivary, small-cell lung, thyroid, or vulvar origin. In addition, patients showed continued tumor progression or an inability to tolerate standard therapy and continued measurable disease (based on the Response Evaluation Criteria in Solid Tumors). The treatment course for this study was pembrolizumab 200 mg intravenously every three weeks for up to thirty-five cycles, with tissue TMB assessed in formalin-fixed, paraffin-embedded tumor samples using the FoundationOne CDx assay. The authors report that 13% of the participants showed a high number of tumor mutations (≥ 10 mutations per megabase) while 87% of the participants had a TMB status lower than this threshold. From this study, they found that a high TMB status characterized a small group of patients who might have a beneficial response to pembrolizumab therapy; those with previously treated recurrent or advanced solid tumors. As such, tissue TMB may be both a "novel and useful predictive biomarker" for therapy indications (Marabelle, Fakih, et al., 2020).

Data from the KEYNOTE-158 study was also analyzed by to examine the efficacy of pembrolizumab as an ICI treatment for MSI-H.dMMR cancer. The authors examined the efficacy of pembrolizumab in patients with previously treated, advanced noncolorectal MSI-H/DMMR cancer from the phase II KEYNOTE-158 study. In the 233 enrolled patients, 27 tumor types were represented (endometrial, gastric,

cholangiocarcinoma, and pancreatic cancers were the most common). ORR was 34.3%, median PFS was 4.1 months, and median OS was 23.5 months. Adverse, treatment-related events occurred in 64.8% of patients (151) and 14.6% (34) patients had grade three to five treatment-related fatal adverse events. They report that pembrolizumab is clinically beneficial in patients with previously treated, unresectable or metastatic MSI-H/dMMR noncolorectal cancer and that toxicity of pembrolizumab monotherapy in these patients was consistent with previous data (Marabelle, Le, et al., 2020).

A study by Stahler et al. (2020) explored the ORR, PFS, and OS in the FIRE-3 clinical trial on metastatic colorectal cancer patients who were identified by the FoundationOne CDx panel to have SNVs, copy number alterations, TMB-H, or MSI-H tumors. Of the 752 patients in the FIRE-3 trial, 373 provided material for this specific analysis. They found that MSI-H (30%) and TMB-H (17.3%) tumors were enriched by *FBXW7* mutations a frequent SNV found in the analysis. The authors found that "*RAS, BRAF V600E* and *SMAD4* mutations were identified as poor prognostic biomarkers in patients of the FIRE-3 trial, whereas improved outcome was observed for *BRAF* non-V600E mutation. *SMAD4* mutation might provide predictive relevance for cetuximab efficacy. MSI-H tumours showed numerically lower ORR, OS and PFS" (Stahler et al., 2020).

While ICIs are highly effective in patients with MSI/dMMR metastatic colorectal cancer (mCRC), Cohen et al. (2020) predicted that the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria was underestimating the response to ICIs due to the pseudoprogression phenomenon. Using data from the GERCOR NIPICOL phase II study, the authors aimed to evaluate the frequency of pseudoprogressions in patients with MSI/dMMR mCRC that were treated with nivolumab and ipilimumab. Nivolumab was given to MSI/dMMR mCRC patients previously treated with fluoropyrimidines, oxaliplatin, and irinotecan with/without targeted therapies. The primary endpoint of the study was disease control rate at 12 weeks according to RECIST 1.1 and iRECIST by central review. Fifty-seven patients were included in the study, with 48% having received ≥ three prior lines of chemotherapy, 18% with *BRAF* mutations, and 56% with Lynch syndrome-related cancer. Of these 57 patients, seven discontinued due to adverse events and one died due to a treatment-related adverse events (TRAE). The authors found that at 12 weeks, the disease control rate was 86% with RECIST 1.1 and 87.7% with iRECIST and two pseudoprogressions were observed (one at week 6, one at week 36). These two pseudoprogressions represented 18% of patients with disease progression per RECIST 1.1 criteria. The 12-month PFS rate was 72.9% with RECIST 1.1 and 76.5% with iRECIST and the 12-month OS rate was 84%. The ORR was 59.7% with both criteria. The authors concluded that pseudoprogression is rare in patients with MSI/dMMR mCRC who are treated with nivolumab and ipilimumab and that this combined ICI therapy confirms impressive disease control rate and survival outcomes in these MSI/dMMR patients (Cohen et al., 2020).

Andre et al. (2020) examined the efficacy of first-line pembrolizumab therapy as compared to first-line chemotherapy therapy for dMMR/MSI-H advanced or metastatic colorectal cancer. In this phase three, open-label clinical trial, 307 patients with metastatic MSI/dMMR colorectal cancer who had not received prior cancer treatment were randomly assigned to pembrolizumab or chemotherapy as a primary treatment and PFS and OS were assessed. Patients that received chemotherapy were able to cross over to pembrolizumab therapy if their disease progressed. The authors found that pembrolizumab treatment in MSI-H/dMMR colorectal cancer patients was superior to chemotherapy with respect to PFS. At the time of cutoff, 56 members of the pembrolizumab group and 69 chemotherapy group patients had died. At the time of the report, OS data were still evolving (they report that 66% of required events had occurred). An overall response (complete or partial) was observed in 43.8% of pembrolizumab patients as compared to only 33.1% of chemotherapy patients. Among those patients with an overall response, 83% of the pembrolizumab group, as compared to just 35% of the chemotherapy group, had an ongoing response at 24 months. TRAEs occurred in just 22% of pembrolizumab patients as compared to 66% of chemotherapy patients (including one death). The authors conclude that pembrolizumab is superior to chemotherapy as a first-line therapy in MSI-H/dMMR metastatic colorectal cancer, with fewer TRAE (Andre et al., 2020).

Wei et al. (2022) evaluated Illumina's TruSight Oncology 500 (TSO500) Assay for its ability to assess tumor mutational burden and the clinical utility of predicting response to pembrolizumab. Using samples collected from approximately 300 patients with "at least twelve different types of advanced solid tumors enrolled in eight clinical trials of pembrolizumab monotherapy," and using FoundationOneCDx and WES as reference methods for TMB, the researchers found that the TSO500 was able to properly classify the status of high tumor mutational burden, a reproducible finding with subsequent use of the assay. The researchers also found that "similar to the validated and FDA-approved TMB cut-point of 10 mut/Mb assessed by FoundationOneCDx, the TSO500 TMB cut-point of 10 mut/Mb is predictive of response to pembrolizumab monotherapy." Ensuring the comparability of this assay to current technologies in the necessary settings may increase access to immunotherapy for patients with solid tumors and TMB-H (Wei et al., 2022).

Aggarwal et al. (2023) completed a clinical validation study of a TMB biomarker in the aim of assessing the association between TMB and clinical outcomes. The study included 674 patients with solid tumors from eight different cancer types. Patients were collected from 300 cancer sites between 2018 and 2022. All patients were sequenced with Tempus xT and treated with ICIs. The authors studied the association between the TMB binary category, high or low, and overall survival. Overall, "high TMB (TMB-H) cancers (206 patients [30.6%]) were significantly associated with longer OS

than low TMB (TMB-L) cancers." Additionally, "an overall survival benefit was seen regardless of the type of ICI used." The authors concluded that "TMB-H cancers were significantly associated with improved clinical outcomes compared with TMB-L cancers" (Aggarwal et al., 2023).

VI. Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

The NCCN includes TMB and MSI testing in several of their guidelines for different types of cancers. Across these guidelines, TMB-high (TMB-H) is defined as tumors with a TMB value of ≥10 mutations/megabase.

The NCCN has clinical practice guidelines for NSCLC. In 2020 the NCCN deleted TMB as an emerging immune biomarker based on clinical trial data and other issues, though was previously considered potentially useful for selecting patients to be treated with nivolumab with or without ipilimumab. However, recent data showed that overall survival was improved with nivolumab/ipilimumab regardless of TMB status. They conclude that PD-L1 expression level is a more useful immune biomarker than TMB for deciding how to prescribe immunotherapy in NSCLC patients in some instances. For example, "Testing for PD-L1 expression levels is recommended before prescribing pembrolizumab monotherapy", but "is not required for prescribing subsequent therapy with atezolizumab or nivolumab", though it may still provide useful information. Moreover, the NCCN guidelines "do not recommend measurement of TMB levels before deciding whether to use nivolumab plus ipilmumab regimens or to use other ICIs, such as pembrolizumab" (NCCN, 2024l).

The NCCN clinical practice guidelines for cervical cancer advise that for stage IVB or distant metastases, "comprehensive molecular profiling as determined by an FDA-approved assay, or a validated test performed in a CLIA-certified laboratory" should be considered. They repeat this under their section for principles of pathology for squamous cell carcinoma, adenocarcinoma, and adenosquamous carcinoma, and add on that they recommend "PD-L1 testing for patients with recurrent, progressive, or metastatic disease". The NCCN also recommend "mismatch repair (MMR)/microsatellite instability (MSI) testing for patients with recurrent, progressive, or metastatic cervical carcinoma" and/or "*NTRK* gene fusion testing for patients with cervical sarcoma". For systemic cervical cancer therapy, the NCCN reports that pembrolizumab is a preferred regimen as a second-line or subsequent therapy for TMB-H, PD-L1–positive, or MSI-H/dMMR tumors that are recurrent or metastatic squamous cell carcinoma, adenocarcinoma, or adenosquamous carcinoma (NCCN, 2024g).

The NCCN clinical practice guidelines for breast cancer note that pembrolizumab is indicated for the treatment of patients with either unresectable or metastatic, MSI-high or mismatch repair-deficient (dMMR) solid tumors, or TMB-H breast tumors that have progressed following prior treatment and have no satisfactory alternative treatment options. TMB-H status is detected via NGS and MSI is detected via immunohistochemistry, NGS, or PCR. The NCCN finds using TMB and MSI to guide pembrolizumab treatment is associated with category 2A evidence (based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate). Dostarlimab-gxly is indicated for adult patients with MSI-H/dMMR unresectable or metastatic tumors and (category 2A) solid tumors or for TMB-H tumors that have progressed following prior treatment and with no satisfactory alternative treatment options. (NCCN, 2024f).

In their clinical practice guidelines for uterine neoplasms, the NCCN recommends that one should "Consider tumor mutational burden (TMB) testing through an FDA-approved assay, or a validated test performed in a CLIA-certified laboratory" as one of their principles of molecular analysis. The NCCN also notes the use of pembrolizumab for TMB-H or MSI-H/deficient DNA mismatch repair (dMMR) tumors as "useful in certain circumstances" as a biomarker-directed systemic therapy for uterine sarcoma and endometrial carcinoma. There, the NCCN also "recommends TMB-H testing if not previously done. Pembrolizumab is indicated for patients with unresectable or metastatic tumors with TMB-H [≥10 mutations/ megabase (mut/Mb)], as determined by an FDA-approved assay, or a validated test performed in a CLIA-certified laboratory, whose disease has progressed following prior treatment and who have no satisfactory alternative treatment options." In the molecular analysis of sarcoma, it is recommended that "Comprehensive genomic profiling in setting of metastatic disease as determined by an FDA-approved assay, or a validated test performed in a CLIA-certified laboratory, is informative for predicting rare pan-tumor targeted therapy opportunities and should include at least *NTRK*, MSI, and TMB"(NCCN, 2024t).

The NCCN guidelines for colon cancer state that "determination of tumor gene status for KRAS/NRAS and BRAF mutations, as well as HER2 amplifications and MSI/MMR status (if not previously done), are recommended for patients with mCRC." They note that "Testing may be carried out for individual genes or as part of an NGS panel, although no specific methodology is recommended." However, NGS panels do have an advantage in that they are "able to pick up rare and actionable genetic alterations, such as neurotrophic tyrosine receptor kinase (NTRK) and rearranged during transfection (RET) fusions and may be carried out using either a tissue or blood-based (eg, liquid) biopsy" (NCCN, 2024h). The NCCN advises that "As with other conditions in which stage IV disease is diagnosed, a tumor analysis (metastases or original primary) for KRAS/NRAS and BRAF mutations and HER2 amplifications, as well as MSI/MMR

testing if not previously done, should be performed to define whether targeted therapies can be considered among the potential options". In pedunculated or sessile colon polyps (adenoma) with invasive cancer, MSI/MMR testing can be part of the workup. Colon cancer appropriate for resection (non-metastatic) will "require a complete staging workup, including biopsy, pathologic tissue review, total colonoscopy, CBC, chemistry profile, CEA determination, and baseline CT scans of the chest, abdomen, and pelvis", while "Testing for MMR/MSI should be done at diagnosis to help with detection of Lynch syndrome and to inform treatment decision-making." When evaluating the recurrence of documented metachronous metastases by CT, MRI, and/or biopsy, dMMR and MSI-H can be considered endpoints for workups (NCCN, 2024h).

In the NCCN clinical practice guidelines for head and neck cancer note that in cancer that occurs in the oral-cavity, image-guided (ultrasound [US] or CT) needle biopsy of cystic neck nodes may offer better diagnostic yield than fine-needle aspiration (FNA) by palpation alone for initial diagnosis. For unresectable or metastatic disease where there is a plan for systemic therapy, a core biopsy would allow for ancillary immune-genomic testing." It is a requirement for cancer that occurs in the oropharynx be tested for tumor human papillomavirus (HPV) by p16 immunohistochemistry (IHC). Biopsy of primary site or FNA of the neck is recommended (NCCN, 2024w). For cancer that occurs in the Nasopharynx with nonkeratinizing or undifferentiated histology, it is recommended to test for EBV in tumor and blood. Common means for detecting EBV in pathologic specimens include ISH for EBV-encoded RNA or immunohistochemical staining for latent membrane protein (LMP). The EBV DNA load within the serum or plasma may be quantified using PCR targeting genomic sequences of the EBV DNA such as BamHI-W, Epstein-Barr virus nuclear antigen (EBNA), or LMP; these tests vary in their sensitivity. The EBV DNA load may reflect prognosis and change in response to therapy. Cancer that occurs in the salivary glands, "use NGS profiling and other appropriate biomarker testing to check status of androgen receptor (AR), HER2, NTRK, HRAS, PIK3CA, and tumor mutational burden (TMB) prior to treatment"(NCCN, 2024w).

In the NCCN clinical practice guidelines for esophageal and EGJ cancers, the panel outlines their principles of pathologic review and biomarker testing. There, they assert that "Immunohistochemistry/in situ hybridization/targeted PCR should be considered first for the identification of biomarkers, followed by NGS testing. If limited tissue is available, or the patient is unable to undergo a traditional biopsy, sequential testing of single biomarkers/limited molecular diagnostic panels will exhaust the sample. In these scenarios, or at the discretion of the treating physician, comprehensive genomic profiling via a validated NGS assay performed in a CLIA-approved laboratory should be considered". Their list of targeted biomarkers include HER2 overexpression/amplification, PD-L1 expression by immunohistochemistry,

microsatellite instability, tumor mutational burden, *NTRK* gene fusion, *RET* gene fusion, and *BRAF V600E* mutation (NCCN, 2024i). Furthermore, during workup for esophageal and esophagogastric junction cancers, "Universal testing for microsatellite instability (MSI) by PCR/next-generation sequencing (NGS) or MMR by IHC is recommended in all newly diagnosed patients". They also recommend "Programmed death ligand 1 (PD-L1) testing if advanced/ metastatic disease is documented/suspected", "HER2 testing if metastatic adenocarcinoma is documented/ suspected", and that "NGS should be considered". Universal testing for MSI by PCR/NGS or MMR by IHC is recommended in all newly diagnosed patients for biopsy specimen types, endoscopic resection, and esophagogastrectomy, without prior chemoradiation (if MSI and MMR has not been previously performed) (NCCN, 2024i). In their assessment of overexpression or amplification of HER2 in esophageal and EGJ cancers, the NCCN recommends that "The use of IHC/ISH should be considered first, followed by NGS testing as appropriate. Repeat biomarker testing may be considered at clinical or radiologic progression for patients with advanced/metastatic esophageal/EGJ adenocarcinoma" (NCCN, 2024i).

The NCCN clinical practice guidelines for occult primary (cancer of unknown primary [CUP]) note that "MSI/mismatch repair (MMR) testing is indicated for patients with CUP; however, it should be noted that the population of patients with MSI-high/MMR-deficient (MSI-H/dMMR) occult primary tumors is generally low." Moreover, they remark that determination of TMB by a validated and/or FDA-approved assay is a category 2B recommendation (2B: based on lower-level evidence, there is NCCN consensus that the intervention is appropriate) and a biopsy from a suspected metastatic malignancy should undergo TMB testing during the workup of the tumor. The NCCN also asserts that during workup "Gene sequencing to predict tissue of origin is not recommended" and "Molecular profiling of tumor tissue using next-generation sequencing (NGS) (or other technique to identify gene fusions) can be considered after an initial determination of histology has been made". Again, the NCCN cautions that "The population of patients with microsatellite instability-high/mismatch repair-deficient (MSI-H/dMMR) occult primary tumors is low. Use immunohistochemistry (IHC) for MMR or polymerase chain reaction (PCR) for MSI, which are different assays measuring the same biological effect" (NCCN, 2024m). For occult primary tumors, dostarlimab-gxly is recommended as "useful in certain circumstances" only in dMMR/MSI-H tumors, while pembrolizumab may be extended to tumors with either TMB-H or dMMR/MSI-H (NCCN, 2024m).

In the NCCN clinical practice guidelines for gastric cancer, universal testing, no matter the specimen type, for MSI by PCR/NGS or MMR by IHC is recommended in all newly diagnosed patients in the biopsy stage, endoscopic mucosal resection, gastrectomy without prior chemoradiation (if not previously performed). In the palliative

management of unresectable locally advanced, locally recurrent, or metastatic disease, "Perform HER2, programmed death ligand 1 (PD-L1), and microsatellite testing (if not done previously) if metastatic cancer is documented or suspected" and "NGS may be considered via a validated assay". Tumors should be interpreted as MSI-stable, MSI-low (1-29% of markers exhibit instability, one of the five NCI or mononucleotide markers exhibits instability), or MSI-H (≥30% of the markers exhibit instability, two or more of the five NCI or mononucleotide markers exhibit instability). They also state that "If limited tissue is available, or the patient is unable to undergo a traditional biopsy, sequential testing of single biomarkers/limited molecular diagnostic panels will exhaust the sample. In these scenarios, or at the discretion of the treating physician, comprehensive genomic profiling via a validated NGS assay performed in a CLIA-approved laboratory should be considered." Their list of targeted biomarkers includes HER2 overexpression/amplification, PD-L1 expression by immunohistochemistry, microsatellite instability, tumor mutational burden, *NTRK* gene fusion, *RET* gene fusion, and *BRAF V600E* mutation (NCCN, 2024j).

The NCCN clinical practice guidelines for hepatocellular carcinoma state that there is "no established role for microsatellite instability (MSI), mismatch repair (MMR), tumor mutational burden (TMB), or PD-L1 testing in [hepatocellular carcinoma] at this time". Immune checkpoint inhibition has shown clinical benefit leading to regulatory approvals in patients with HCC without selection for MSI, MMR, TMB, or PD-L1 status. Regarding therapies, the panel recommends nivolumab + ipilimumab (category 2B) as a first-line systemic therapy that is useful in certain circumstances and a subsequent-line systemic therapy if there is disease progression for individuals with TMB-H tumors. The NCCN also dostarlimab-gxly (category 2B) as a subsequent-line systemic therapy if there is disease progression for individuals with MSI-H/dMMR tumors (NCCN, 2024x).

The NCCN clinical practice guidelines for biliary tract cancers (BTCs) recommend germline testing and/or referral to a genetic councilor for patients with dMMR/MSI-H tumors or a family history suggestive of BRCA1/2 mutation" when presenting during intraoperative staging biopsy following an incidental finding of suspicious mass during surgery. Germline testing and/or referral to genetic counseling for patients with dMMR/MSI-H tumors or a family history suggestive of BRCA1/2 mutations should also be extended to situations where hepatobiliary surgery expertise is unavailable and postoperative workup is deemed unresectable, incidental finding on pathologic review with T1b or greater and/or T1a with positive margins deemed unresectable, unresectable mass on imaging, jaundice, metastatic disease, or isolated intrahepatic mass with unresectable or metastatic disease biopsy. The NCCN recommends that "Comprehensive molecular profiling is recommended for patients with unresectable or metastatic BTC who are candidates for systemic therapy". They also note that "Evidence remains insufficient for definitive recommendations regarding specific

criteria to guide genetic risk assessment in hepatobiliary cancers or for universal germline testing in these tumors" and, as such, "In BTCs, genetic counseling referral and potential germline testing should be considered in patients with any of the following characteristics: young age at diagnosis; a strong personal or family history of cancer; no known risk factors for liver disease; or presence of mutations identified during tumor testing that are suspected to be possible germline alterations. For patients who do harbor a known germline mutation associated with a cancer predisposing syndrome (ie, Lynch syndrome or hereditary breast and ovarian cancer syndrome), there is currently insufficient evidence to support screening for biliary tract malignancies" (NCCN, 2024d). The NCCN asserts that "testing for MSI or MMR deficiency is recommended in patients with unresectable or metastatic gallbladder cancer, intrahepatic CCA, or extrahepatic CCA" and that "Testing for TMB is recommended for patients with unresectable or metastatic gallbladder cancer, intrahepatic CCA, or extrahepatic CCA based upon clinical benefit observed across advanced solid tumors." They also outline the three possible tests to evaluate mismatch repair (MMR) protein deficiency or microsatellite status. First, an immunohistochemical staining for the *MLH1*, *MSH2*, *MSH6*, and *PMS2* gene products will establish protein retention or loss, followed by NGS to determine if there are inactivating mutations in the MMR genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*). Lastly, "microsatellite repeats of tumor DNA are examined by polymerase chain reaction (PCR)" and caution that "Tumor mutational burden (TMB) can be tested with a clinically validated NGS panel but has inherent platform variation" (NCCN, 2024d). In addition, the NCCN provides a helpful graphic to show their recommendations for molecular testing in unresectable or BTCs, captured below:

Recommended Molecular Testing	Anatomic Subsite		
	Gallbladder	Intrahepatic CCA	Extrahepatic CCA
***NTRK* gene fusion**	X	X	X
MSI-H/dMMR	X	X	X
TMB-H	X	X	X
***BRAF* V600E mutation**	X	X	X
***FGFR2* fusion or rearrangement**	–	X	X
***IDH1* mutation**	–	X	X
HER2 *(ERBB2)* overexpression and/or amplification	X	X	X
***RET* gene fusion**	X	X	X
***KRAS* G12C**	X	X	X

MSI-H: microsatellite instability-high
dMMR: mismatch repair deficient
TMB-H: tumor mutational burden-high

(NCCN, 2024d).

The NCCN clinical practice guidelines for neuroendocrine and adrenal tumors recommends that TMB and MSI testing should be considered as part of the workup for adrenocortical carcinoma. In the evaluation of poorly differentiated or large or small cell carcinomas or unknown primary, "Tumor biomarkers such as microsatellite instability (MSI), MMR, and TMB testing (by an FDA-approved test) should be considered as they can aid in assessing targeted therapy options". In well-differentiated grade three neuroendocrine tumors, "Tumor/somatic molecular profiling should be considered for patients with locoregional unresectable/metastatic disease who are candidates for anti-cancer therapy to identify actionable alterations. Testing on tumor tissue is preferred; however, cell-free DNA testing can be considered if tumor tissue testing is not feasible." The NCCN notes that "patients with ACC may also consider MSI, MMR, and TMB (by an FDA-approved test) testing" because some analyses suggest that "3% of patients with ACC have Lynch syndrome" (NCCN, 2024k).

The NCCN clinical practice guidelines for vulvar cancer state that "Mismatch repair (MMR), microsatellite instability (MSI), programmed death ligand 1 (PD-L1), neurotrophic tyrosine receptor kinase (*NTRK*) gene fusion, and tumor mutational burden (TMB) testing may also be considered for treatment planning purposes in patients with recurrent, progressive, or metastatic disease" (NCCN, 2024u).

The NCCN clinical practice guidelines for ovarian cancer identify TMB testing as a clinically indicated tumor molecular analysis in addition to NGS for *BRCA1/2* mutations and other somatic mutations. They also recommend using tumor molecular analysis "to identify potential benefit from targeted therapeutics that have tumor-specific or tumor-agnostic benefit including, but not limited to, HER2 status (by IHC), BRCA1/2, HRD status, microsatellite instability (MSI), mismatch repair (MMR), tumor mutational burden (TMB), *BRAF*, FRα (FOLR1), *RET*, and *NTRK* if prior testing did not include these markers" in patients with recurrent disease and rising CA-125, regardless of prior chemotherapy status if molecular testing was not done previously in epithelial ovarian cancer/fallopian tube cancer/primary peritoneal cancer. The NCCN guidelines also recommend tumor molecular testing with TMB and MSI if not previously done when following up or monitoring for the recurrence of low-grade serous carcinoma (NCCN, 2024n).

In the NCCN clinical practice guidelines for testicular cancer, the NCCN recommends TMB or MSI testing as a third-line therapy in nonseminoma if there is progression after high-dose chemotherapy or other third-line therapies. The guideline also notes that similar to many other cancers, pembrolizumab may be useful in certain circumstances as a third-line chemotherapy regimen for metastatic germ cell tumors when the tumor has a TMB-H or MSI-H status, regardless of prior high-dose chemotherapy status (NCCN, 2024r).

In the NCCN clinical practice guidelines for pancreatic adenocarcinoma, it is stated that "Tumor/somatic molecular profiling is recommended for patients with locally advanced/metastatic disease who are candidates for anti-cancer therapy to identify uncommon mutations. Consider specifically testing for potentially actionable somatic findings including, but not limited to: fusions (*ALK*, *NRG1*, *NTRK*, *ROS1*, *FGFR2*, and *RET*), mutations (*BRAF*, *BRCA1/2*, *KRAS*, and *PALB2*), amplifications (*HER2*), microsatellite instability (MSI), mismatch repair deficiency (dMMR), or tumor mutational burden (TMB) via an FDA-approved and/or validated next-generation sequencing (NGS)-based assay. RNA sequencing assays are preferred for detecting RNA fusions because gene fusions are better detected by RNA-based NGS. Testing on tumor tissue is preferred; however, cell-free DNA testing can be considered if tumor tissue testing is not feasible". This is true for locally advanced disease where adenocarcinoma is confirmed, in metastatic disease when tumor tissue is available, and in tumors with recurrence after resection (NCCN, 2024b).

In the NCCN clinical practice guidelines for penile cancer, pembrolizumab is recommended as a subsequent-line systemic therapy for metastatic/recurrent disease in MSI-H/dMMR tumors that have progressed following prior treatment and that have no satisfactory alternative treatment options (NCCN, 2024c).

The NCCN clinical practice guidelines for prostate cancer recommend that in CRPC, tumor testing for microsatellite instability (MSI-H) or DMMR and HRRm should be considered when CRPC imaging studies are positive for metastases, if the testing has not been previously performed. It also recommends tumor testing for MSI-H or dMMR among patients with metastatic CRPC and "may be considered in patients with regional or castration-sensitive metastatic prostate cancer", and that "TMB testing may be considered in patients with metastatic castration-resistant prostate cancer." The NCCN also provides tumor testing recommendations based on risk groups. These recommendations include

1. "Tumor testing for somatic homologous recombination gene mutations (eg, BRCA1, BRCA2, ATM, PALB2, FANCA, RAD51D, CHEK2, CDK12) can be considered in patients with regional (N1) prostate cancer and is recommended for those with metastatic disease."
2. "Tumor testing for MSI or dMMR can be considered in patients with regional or metastatic castration-naïve prostate cancer and is recommended in the metastatic CRPC setting."
3. "Tumor mutational burden (TMB) testing may be considered in patients with metastatic CRPC."
4. "Multigene molecular testing can be considered for patients with low-, intermediate-, and high-risk prostate cancer and life expectancy ≥10 years."
5. "The Decipher molecular assay is recommended to inform adjuvant treatment if adverse features are found post-radical prostatectomy, and can be considered as part of counseling for risk stratification in patients with PSA resistance/recurrence after radical prostatectomy" (NCCN, 2024o).

The NCCN advises that "If MSI testing is performed, testing using an NGS assay validated for prostate cancer is preferred" and states that "If MSI-H or dMMR is found, the patient should be referred for genetic counseling to assess for the possibility of Lynch syndrome" (NCCN, 2024o).

The NCCN clincal practice guidelines for rectal cancer state that universal MMR or MSI testing is recommended in all newly diagnosed patients with rectal cancer, noting that IHC for MMR and DNA analysis for MSI are different assays and measure different biological effects caused by deficient MMR function (NCCN, 2024p). The NCCN cautions that "The presence of a BRAF V600E mutation in the setting of MLH1 absence would preclude the diagnosis of Lynch syndrome (LS) in the vast majority of patients. However, approximately one percent of cancers with BRAF V600E mutations (and loss of *MLH1*) are LS. Caution should be exercised in excluding patients with a strong family history from germline screening in the case of BRAF V600E mutations." The further explain that "One of two different initial tests can be performed on CRC specimens to

identify individuals who might have Lynch syndrome: 1) immunohistochemical analysis for MMR protein expression, which is often diminished because of mutation; or 2) analysis for microsatellite instability (MSI), which results from MMR deficiency and is detected as changes in the length of repetitive DNA elements in tumor tissue caused by the insertion or deletion of repeated units". They also remind clinicians that "MMR or MSI testing should be performed only in CLIA-approved laboratories" and that "testing for MSI may be accomplished by polymerase chain reaction (PCR) or a validated NGS panel, the latter especially in patients with metastatic disease who require genotyping of *RAS* and *BRAF*". In the management of malignant polyps, "All patients with a malignant polyp should undergo MMR or MSI testing at diagnosis". Similarly, "All patients with rectal cancer should undergo MMR or MSI testing at diagnosis to aid in the diagnosis of Lynch syndrome and for clinical trial availability, especially related to checkpoint inhibitors as neoadjuvant therapy" (NCCN, 2024p). MMR and MSI testing is also recommended as part of the initial workup in pedunculated polyps or sessile polyps (adenoma) with invasive cancer and in rectal cancers with and without suspected or proven distant metastases (if not previously done in the latter). Notably, during pathologic review, "It has not been established if molecular markers (other than MSI-H/dMMR) are useful in treatment determination (predictive markers) and prognosis" (NCCN, 2024p).

The NCCN clinical practice guidelines for small bowel adenocarcinoma recommend MSI testing as a workup when the tumor is in the duodenum or the jejunum/ileum, as well as in metastatic and/or recurrent tumors such as metastatic adenocarcinoma. Some general principles of pathologic review include the following:

- "Universal MMR or MSI testing is recommended in all newly diagnosed patients with SBA"
- "Patients with stage II dMMR/MSI-H may have improved survival compared to patients with proficient MMR (pMMR)/microsatellite stable (MSS); however, this has not been confirmed in the SBA population and is extrapolated from colorectal cancer data"
- "MMR or MSI testing should be performed only in Clinical Laboratory Improvement Amendments (CLIA)-certified laboratories"
- "Testing for MSI may be performed by validated next-generation sequencing (NGS) panels" (NCCN, 2024q).

The NCCN clinical practice guidelines for thyroid carcinoma suggest that treatment of structurally persistent/recurrent locoregional or distant metastatic disease not amenable to RAI therapy, "somatic testing to identify actionable mutations (including *ALK*, *NTRK*, *BRAF*, and *RET* gene fusions), mismatch repair deficiency (dMMR), microsatellite instability (MSI), and tumor mutational burden (TMB)" should be

considered for advanced, progressive, or threatening disease. Wh en FNA or core biopsy finds that it is anaplastic thyroid carcinoma, molecular testing is recommended as diagnostic molecular testing for actionable mutations, which they propound as including "BRAF, NTRK, ALK, RET, MSI, dMMR, and tumor mutational burden. BRAF IHC testing is recommended due to faster turnaround compared to genetic testing." This extends to the aggressive therapy approach for stage IVC (NCCN, 2024s).

The NCCN clinical practice guidelines for bone cancer recommend considering TMB or MSI testing as determined by a validated and/or FDA-approved assay to inform the use of treatment options in patients with metastatic chondrosarcoma, chordoma, Metastatic Ewing sarcoma, and metastatic osteosarcoma at presentation (NCCN, 2024e).

The NCCN clinical practice guidelines for ampullary adenocarcinoma suggest that if adenocarcinoma is found to be metastatic, then "Tumor/somatic molecular profiling is recommended for patients with locally advanced/metastatic disease who are candidates for anti-cancer therapy to identify uncommon mutations", and recommend that clinicians "Consider specifically testing for potentially actionable somatic findings including, but not limited to: fusions (*ALK*, *NRG1*, *NTRK*, *ROS1*, *FGFR2*, and *RET*), mutations (*BRAF*, *BRCA1/2*, *KRAS*, and *PALB2*), amplifications (HER2), microsatellite instability (MSI), mismatch repair deficiency (dMMR), or tumor mutational burden (TMB) via an FDA-approved and/or validated next-generation sequencing (NGS)-based assay." The NCCN panel also explains that "RNA sequencing assays are preferred for detecting RNA fusions because gene fusions are better detected by RNA-based NGS"; regardless, "Testing on tumor tissue is preferred; however, cell-free DNA testing can be considered if tumor tissue testing is not feasible" (NCCN, 2024a).

The NCCN clinical practice guidelines for anal carcinoma note that "microsatellite instability (MSI)/mismatch repair (MMR) testing is not required" during second-line treatment of metastatic anal cancer because "MSI is uncommon in anal cancer; [however], anal cancers may be responsive to PD-1/PD-L1 inhibitors because they often have high PD-L1 expression and/or a high tumor mutational load despite being microsatellite stable (MSS) " (NCCN, 2024v).

The NCCN clinical practice guidelines for hepatocellular carcinoma note that "There is no established role for microsatellite instability (MSI), mismatch repair (MMR), tumor mutational burden (TMB), or programmed death ligand 1 (PD-L1) testing in HCC at this time. Immune checkpoint inhibition has shown clinical benefit leading to regulatory approvals in patients with HCC without selection for MSI, MMR, TMB, or PD-L1 status" (NCCN, 2024x).

The NCCN clinical practice guidelines for pediatric central nervous system cancers mention TMB in terms of palliative systemic therapy for recurrent or progressive disease, noting that "targeted therapy based on the molecular composition of the tumor is recommended for patients with good performance status" including "checkpoint blockade for high tumor mutational burden (TMB) or personal or family history of cMMRD; RAF and MEK inhibition for tumors with BRAF V600E mutation, and TRK inhibitors for tumors with NTRK gene fusion" (NCCN, 2024y).

The NCCN clinical practice guidelines for soft tissue sarcoma recommends pembrolizumab as useful in certain circumstances as a subsequent line of therapy for advanced/metastatic disease for MSI-H or dMMR tumors (regardless of soft tissue sarcoma sub-type). The NCCN also recommends pembrolizumab or nivolumab ± ipilimumab as useful in certain circumstances as a subsequent line of therapy for advanced/metastatic disease for TMB-H (≥10 mutations/megabase [mut/Mb]) regardless of soft tissue sarcoma sub-type (NCCN, 2024z).

European Society for Medical Oncology (ESMO)

The ESMO has published clinical recommendations for MSI testing based on consensus decisions from the ESMO Translational Research and Precision Medicine Working Group. The recommended first action to assess MSI/dMMR, decided by a consensus with a strong agreement, is the use of immunohistochemistry for the mismatch repair proteins MLH1, MSH2, MSH6, and PMS2. The second method of MSI/dMMR testing, also with a consensus of strong agreement, is the use of PCR-based assessment of microsatellite alterations using five microsatellite markers (including at least BAT-25 and BAT-26). Based on a consensus with a very strong agreement, the ESMO notes that NGS coupling MSI and TMB analysis may represent "a decisive tool for selecting patients for immunotherapy, for common or rare cancers not belonging to the spectrum of Lynch syndrome." They note that the relationships between MSI, TMB, and PD-1/PD-L1 expression are both complex and different based on the type of tumor being assessed. They also note that the most important cancer types where MSI testing should be carried out using IHC to assess MSI-PCR or NGS are endometrial, intestinal (colorectal and small bowel), esophageal (adenocarcinomas and not squamous cell carcinoma), gastric, glioblastoma, and ovarian cancers (Luchini et al., 2019).

The ESMO has published clinical recommendations for the use of tumor multigene NGS in non-small cell lung cancer, cholangiocarcinoma, prostate, and ovarian cancers. They recommend to "test TMB in well- and moderately-differentiated neuroendocrine tumours (NETs), cervical, salivary, thyroid and vulvar cancers. . . as TMB-high predicted response to pembrolizumab in these cancers." They advise that a large panel of genes could be ordered but that the benefit for the patient and the cost for the public health

care system should be taken into consideration. Patient-specific recommendations are summarized in the table below (Mosele et al., 2020).

Tumour Types	General recommendations for daily practice	Special considerations for patients
Lung adenocarcinoma	Tumour multigene NGS to assess level I alterations. Larger panels can be used only on the basis of specific agreements with payers taking into account the overall cost of the strategy (drug included[a]) and if they report accurate ranking of alterations. NGS can either be done on RNA or DNA, if it includes level I fusions in the panel.	Using large panels of genes could lead to few clinically meaningful responders, not detected by small panels or standard testings. In this context and outside the diseases where large panels of genes are recommended, ESMO acknowledges that a patient and a doctor could decide together to order a large panel of genes, pending no extra cost for the public health care system, and if the patient is informed about the low likelihood of benefit.
Squamous cell lung cancers	No current indication for tumour multigene NGS	
Breast cancers	No current indication for tumour multigene NGS	
Colon cancers	Multigene tumour NGS can be an alternative option to PCR if it does not result in additional cost.	
Prostate cancers	Multigene tumour NGS to assess level I alterations. Larger panels can be used only on the basis of specific agreements with payers taking into account the overall cost of the strategy and if they report accurate ranking of alterations.	
Gastric cancers	No current indication for tumour multigene NGS	
Pancreatic cancers	No current indication for tumour multigene NGS	
Hepatocellular carcinoma	No current indication for tumour multigene NGS	
Cholangiocarcinoma	Multigene tumour NGS could be recommended to assess level I alterations. Larger panels can be used only on the basis of specific agreements with payers taking into account the overall cost of the strategy (drug includeda) and if they report accurate ranking of alterations. RNA-based NGS can be used.	
Others	Tumour multigene NGS can be used in ovarian cancers to	

	determine somatic BRCA1/2 mutations. In this latter case, larger panels can be used only on the basis of specific agreements with payers taking into account the overall cost of the strategy (drug includeda) and if they report accurate ranking of alterations. Large panel NGS can be used in carcinoma of unknown primary. It is recommended to determine TMB in cervical cancer, salivary cancer, thyroid cancers, well-to-moderately differentiated neuroendocrine tumours, vulvar cancer, pending drug access (and in TMB-high endometrial and SCL cancers if anti-PD1 antibody is not available otherwise).	

anti-PD1, anti-programmed cell death 1; DRUP, drug rediscovery protocol; ESMO, European Society for Medical Oncology; NGS, next-generation sequencing; SCL, small-cell lung cancer; TMB, tumour mutational burden.

[a] ESMO recommends using off-label drugs matched to genomics only if an access programme and a procedure of decision have been developed at the national or regional level, as illustrated by the DRUP programme.(Mosele et al., 2020)

College of American Pathologists (CAP)

In 2022, the College of American Pathologists issued an evidence-based clinical guideline on the use of mismatch repair and microsatellite instability testing for immune checkpoint inhibitor therapy. These recommendations include guidance for patients with Lynch syndrome, as well as for patients with colorectal, endometrial,

Guideline Statement	Strength of Recommendation
1. For patients with CRC being considered for immune checkpoint inhibitor therapy, pathologists should use MMR-IHC and/or MSI by PCR for the detection of DNA MMR defects. Although MMR-IHC or MSI by PCR are preferred, pathologists may use a validated MSI by NGS assay for the detection of DNA MMR defects. *Note:* MSI by NGS assay must be validated against MMR-IHC or MSI by PCR and must show equivalency.	Strong Recommendation
2. For patients with gastroesophageal and small bowel cancer being considered for immune checkpoint inhibitor therapy, pathologists should use MMR-IHC and/or MSI by PCR over MSI by NGS for the detection of DNA MMR defects. *Note:* This recommendation does not include esophageal squamous cell carcinoma.	Strong Recommendation
3. For patients with endometrial cancer being considered for immune checkpoint inhibitor therapy, pathologists should use MMR-IHC over MSI by PCR or NGS for the detection of DNA MMR defects.	Strong Recommendation
4. For patients with cancer types other than CRC, GEA, small bowel, and endometrial being considered for immune checkpoint inhibitor therapy, pathologists should test for DNA MMR, although the optimal approach for the detection of MMR defects has not been established. *Note:* Assays must be adequately validated for the specific cancer type being tested with careful consideration of performance characteristics of MMR-IHC and MSI by NGS or PCR for the detection of DNA MMR defects.	Conditional Recommendation
5. For all cancer patients being considered for immune checkpoint inhibitor therapy based upon defective MMR, pathologists should **NOT** use TMB as a surrogate for the detection of DNA MMR defects. If a tumor is identified as TMB-high, pathologists may perform IHC and/or MSI by PCR to determine if high TMB is secondary to MMR deficiency.	Strong Recommendation
6. For cancer patients being considered for immune checkpoint inhibitor therapy, if an MMR deficiency consistent with Lynch Syndrome is identified in the tumor, pathologists should communicate this finding with the treating physician.	Strong Recommendation

Abbreviations: CRC, colorectal cancer; DNA, deoxyribonucleic acid; GEA, gastroesophageal adenocarcinoma; IHC, immunohistochemistry; MMR, mismatch repair; MSI, microsatellite instability; NGS, next generation sequencing; PCR, polymerase chain reaction; TMB, tumor mutation burden

© 2022 College of American Pathologists. All rights reserved.

gastroesophageal, small bowel, and other types of cancers. Their summary of recommendations is captured below (CAP, 2022).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state

coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

On November 30, 2017, the FDA approved FoundationOne CDx™ (Foundation Medicine, Inc.). This device is a next-generation sequencing based in vitro diagnostic device for detection of substitutions, insertion, and deletion alterations (indels), and copy number alterations (CNAs) in 324 genes and select gene rearrangements, as well as genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) using DNA isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue specimens. The test is intended as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies in accordance with the approved therapeutic product labeling. Additionally, F1CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms. The F1CDx assay is a single-site assay performed at Foundation Medicine, Inc. (FDA, 2017c).

On August 26, 2020, the FDA approved FoundationOne® Liquid CDx (Foundation Medicine, Inc.). This device is a qualitative next-generation sequencing based in vitro diagnostic test that uses targeted high throughput hybridization-based capture technology to detect and report substitutions, insertions, and deletions (indels) in 311 genes, including rearrangements and copy number losses only in BRCA1 and BRCA2, utilizing circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood of cancer patients. This device is intended to provide tumor mutation profiling for substitutions and indels to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms (FDA, 2020b).

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81301	Microsatellite instability analysis (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) of markers for mismatch repair deficiency (eg, BAT25, BAT26), includes comparison of neoplastic and normal tissue, if performed
81457	Solid organ neoplasm, genomic sequence analysis panel, interrogation for sequence variants; DNA analysis, microsatellite instability
81458	Solid organ neoplasm, genomic sequence analysis panel, interrogation for sequence variants; DNA analysis, copy number variants and microsatellite instability
81459	Solid organ neoplasm, genomic sequence analysis panel, interrogation for sequence variants; DNA analysis or combined DNA and RNA analysis, copy number variants, microsatellite instability, tumor mutation burden, and rearrangements
81463	Solid organ neoplasm, genomic sequence analysis panel, cell-free nucleic acid (eg, plasma), interrogation for sequence variants; DNA analysis, copy number variants, and microsatellite instability
81464	Solid organ neoplasm, genomic sequence analysis panel, cell-free nucleic acid (eg, plasma), interrogation for sequence variants; DNA analysis or combined DNA and RNA analysis, copy number variants, microsatellite instability, tumor mutation burden, and rearrangements
81479	Unlisted molecular pathology procedure
0037U	Targeted genomic sequence analysis, solid organ neoplasm, DNA analysis of 324 genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability and tumor mutational burden Proprietary test: FoundationOne CDx™ (F1CDx) Lab/Manufacturer: Foundation Medicine, Inc
0048U	Oncology (solid organ neoplasia), DNA, targeted sequencing of protein-coding exons of 468 cancer-associated genes, including interrogation for somatic mutations and microsatellite instability, matched with normal specimens, utilizing formalin-fixed paraffin-embedded tumor tissue, report of clinically significant mutation(s) Proprietary test: MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets) Lab/Manufacturer: Memorial Sloan Kettering Cancer Center
0211U	Oncology (pan-tumor), DNA and RNA by next-generation sequencing, utilizing formalin-fixed paraffin-embedded tissue, interpretative report for

	single nucleotide variants, copy number alterations, tumor mutational burden, and microsatellite instability, with therapy association Proprietary test: MI Cancer Seek™ - NGS Analysis Lab/Manufacturer: Caris MPI d/b/a Caris Life Sciences
0239U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free DNA, analysis of 311 or more genes, interrogation for sequence variants, including substitutions, insertions, deletions, select rearrangements, and copy number variations Proprietary test: FoundationOne® Liquid CDx Lab/Manufacturer: Foundation Medicine, Inc
0242U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free circulating DNA analysis of 55-74 genes, interrogation for sequence variants, gene copy number amplifications, and gene rearrangements Proprietary test: Guardant360® CDx Lab/Manufacturer: Guardant Health Inc
0244U	Oncology (solid organ), DNA, comprehensive genomic profiling, 257 genes, interrogation for single-nucleotide variants, insertions/deletions, copy number alterations, gene rearrangements, tumor-mutational burden and microsatellite instability, utilizing formalin-fixed paraffin-embedded tumor tissue Proprietary test: Oncotype MAP™ Pan-Cancer Tissue Test Lab/Manufacturer: Paradigm Diagnostics, Inc
0250U	Oncology (solid organ neoplasm), targeted genomic sequence DNA analysis of 505 genes, interrogation for somatic alterations (SNVs [single nucleotide variant], small insertions and deletions, one amplification, and four translocations), microsatellite instability and tumor-mutation burden Proprietary test: PGDx elio™ tissue complete Lab/Manufacturer: Personal Genome Diagnostics, Inc
0326U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free circulating DNA analysis of 83 or more genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability and tumor mutational Proprietary test: Guardant360® Lab/Manufacturer: Guardant Health, Inc
0329U	Oncology (neoplasia), exome and transcriptome sequence analysis for sequence variants, gene copy number amplifications and deletions, gene rearrangements, microsatellite instability and tumor mutational burden utilizing DNA and RNA from tumor with DNA from normal blood or saliva for subtraction, report of clinically significant mutation(s) with therapy associations

	Proprietary test: Oncomap™ ExTra Lab/Manufacturer: Exact Sciences, Inc, Genomic Health Inc
0334U	Oncology (solid organ), targeted genomic sequence analysis, formalin-fixed paraffin-embedded (FFPE) tumor tissue, DNA analysis, 84 or more genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability and tumor mutational burden Proprietary test: Guardant360 TissueNext™ Lab/Manufacturer: Guardant Health, Inc
0379U	Targeted genomic sequence analysis panel, solid organ neoplasm, DNA (523 genes) and RNA (55 genes) by next-generation sequencing, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability, and tumor mutational burden Proprietary test: Solid Tumor Expanded Panel Lab/Manufacturer: Quest Diagnostics®
0391U	Oncology (solid tumor), DNA and RNA by next-generation sequencing, utilizing formalin-fixed paraffin-embedded (FFPE) tissue, 437 genes, interpretive report for single nucleotide variants, splicesite variants, insertions/deletions, copy number alterations, gene fusions, tumor mutational burden, and microsatellite instability, with algorithm quantifying immunotherapy response score Proprietary test: Strata Select™ Lab/Manufacturer: Strata Oncology, Inc.
0409U	Oncology (solid tumor), DNA (80 genes) and RNA (36 genes), by next-generation sequencing from plasma, including single nucleotide variants, insertions/deletions, copy number alterations, microsatellite instability, and fusions, report showing identified mutations with clinical actionability Proprietary Test: LiquidHALLMARK ® Lab/Manufacturer: Lucene Health, Inc.
0473U	Oncology (solid tumor), nextgeneration sequencing (NGS) of DNA from formalin-fixed paraffinembedded (FFPE) tissue with comparative sequence analysis from a matched normal specimen (blood or saliva), 648 genes, interrogation for sequence variants, insertion and deletion alterations, copy number variants, rearrangements, microsatellite instability, and tumor-mutation burden Proprietary test: xT CDx Lab/Manufacturer: Tempus AI, Inc
0487U	Oncology (solid tumor), cell-free circulating DNA, targeted genomic sequence analysis panel of 84 genes, interrogation for sequence variants, aneuploidycorrected gene copy number amplifications and losses, gene rearrangements, and microsatellite instability

	Proprietary test: Northstar SelectTM Lab/Manufacturer: BillionToOne Laboratory
0512U	Oncology (prostate), augmentative algorithmic analysis of digitized whole-slide imaging of histologic features for microsatellite instability (MSI) status, formalin-fixed paraffinembedded (FFPE) tissue, reported as increased or decreased probability of MSI-high (MSI-H) Proprietary test: Tempus p-MSI Lab/Manufacturer: Tempus AI, Inc
0513U	Oncology (prostate), augmentative algorithmic analysis of digitized whole-slide imaging of histologic features for microsatellite instability (MSI) and homologous recombination deficiency (HRD) status, formalinfixed paraffin-embedded (FFPE) tissue, reported as increased or decreased probability of each biomarker Proprietary test: Tempus p-Prostate Lab/Manufacturer: Tempus AI, Inc
0530U	Oncology (pan-solid tumor), ctDNA, utilizing plasma, nextgeneration sequencing (NGS) of 77 genes, 8 fusions, microsatellite instability, and tumor mutation burden, interpretative report for single nucleotide variants, copynumber alterations, with therapy association Proprietary test: LiquidHALLMARK® Lab/Manufacturer: Lucence Health, Inc, Lucence Health, Inc

Current Procedural Terminology© American Medical Association. All Rights reserved.
Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Aggarwal, C., Ben-Shachar, R., Gao, Y., Hyun, S. W., Rivers, Z., Epstein, C., Kaneva, K., Sangli, C., Nimeiri, H., & Patel, J. (2023). Assessment of Tumor Mutational Burden and Outcomes in Patients With Diverse Advanced Cancers Treated With Immunotherapy. *JAMA Netw Open*, *6*(5), e2311181. https://doi.org/10.1001/jamanetworkopen.2023.11181

Alborelli, I., Leonards, K., Rothschild, S. I., Leuenberger, L. P., Savic Prince, S., Mertz, K. D., Poechtrager, S., Buess, M., Zippelius, A., Laubli, H., Haegele, J., Tolnay, M., Bubendorf, L., Quagliata, L., & Jermann, P. (2020). Tumor mutational burden assessed by targeted NGS predicts clinical benefit from immune checkpoint inhibitors in non-small cell lung cancer. *J Pathol*, *250*(1), 19-29. https://doi.org/10.1002/path.5344

Andre, T., Shiu, K. K., Kim, T. W., Jensen, B. V., Jensen, L. H., Punt, C., Smith, D., Garcia-Carbonero, R., Benavides, M., Gibbs, P., de la Fouchardiere, C., Rivera, F., Elez, E., Bendell, J., Le, D. T., Yoshino, T., Van Cutsem, E., Yang, P., Farooqui, M. Z. H., . . .

Investigators, K.-. (2020). Pembrolizumab in Microsatellite-Instability-High Advanced Colorectal Cancer. *N Engl J Med*, *383*(23), 2207-2218. https://doi.org/10.1056/NEJMoa2017699

Boland, C. R., Thibodeau, S. N., Hamilton, S. R., Sidransky, D., Eshleman, J. R., Burt, R. W., Meltzer, S. J., Rodriguez-Bigas, M. A., Fodde, R., Ranzani, G. N., & Srivastava, S. (1998). A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res*, *58*(22), 5248-5257. https://www.ncbi.nlm.nih.gov/pubmed/9823339

Bonneville, R., Krook, M. A., Kautto, E. A., Miya, J., Wing, M. R., Chen, H. Z., Reeser, J. W., Yu, L., & Roychowdhury, S. (2017). Landscape of Microsatellite Instability Across 39 Cancer Types. *JCO Precis Oncol*, *2017*. https://doi.org/10.1200/PO.17.00073

CAP. (2022). *Mismatch Repair and Microsatellite Instability Testing for Immune Checkpoint Inhibitor Therapy*. College of American Pathologists. https://www.cap.org/protocols-and-guidelines/cap-guidelines/current-cap-guidelines/mismatch-repair-and-microsatellite-instability-testing-for-immune-checkpoint-inhibitor-therapy

Caris Life Sciences. (2024). *MI Tumor Seek™*. https://www.carislifesciences.com/products-and-services/molecular-profiling/

Chalmers, Z. R., Connelly, C. F., Fabrizio, D., Gay, L., Ali, S. M., Ennis, R., Schrock, A., Campbell, B., Shlien, A., Chmielecki, J., Huang, F., He, Y., Sun, J., Tabori, U., Kennedy, M., Lieber, D. S., Roels, S., White, J., Otto, G. A., . . . Frampton, G. M. (2017). Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med*, *9*(1), 34. https://doi.org/10.1186/s13073-017-0424-2

Cohen, R., Bennouna, J., Meurisse, A., Tournigand, C., De La Fouchardiere, C., Tougeron, D., Borg, C., Mazard, T., Chibaudel, B., Garcia-Larnicol, M. L., Svrcek, M., Vernerey, D., Menu, Y., & Andre, T. (2020). RECIST and iRECIST criteria for the evaluation of nivolumab plus ipilimumab in patients with microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: the GERCOR NIPICOL phase II study. *J Immunother Cancer*, *8*(2). https://doi.org/10.1136/jitc-2020-001499

FDA. (2017a). *EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets)*. https://www.accessdata.fda.gov/cdrh_docs/reviews/DEN170058.pdf

FDA. (2017b, November 15, 2017). *FDA unveils a streamlined path for the authorization of tumor profiling tests alongside its latest product action* https://www.fda.gov/news-events/press-announcements/fda-unveils-streamlined-path-authorization-tumor-profiling-tests-alongside-its-latest-product-action

FDA. (2017c). *FoundationOne CDx* https://www.accessdata.fda.gov/cdrh_docs/pdf17/P170019B.pdf

FDA. (2020a). FDA approves pembrolizumab for adults and children with TMB-H solid tumors. https://www.fda.gov/drugs/drug-approvals-and-databases/fda-approves-pembrolizumab-adults-and-children-tmb-h-solid-tumors
FDA. (2020b). *FoundationOne Liquid CDx (F1 Liquid CDx)*. https://www.accessdata.fda.gov/cdrh_docs/pdf19/P190032B.pdf
FDA. (2021, May 21, 2021). *SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)* Retrieved October 28, 2021 from https://www.accessdata.fda.gov/cdrh_docs/pdf20/P200010S001B.pdf
Garofalo, A., Sholl, L., Reardon, B., Taylor-Weiner, A., Amin-Mansour, A., Miao, D., Liu, D., Oliver, N., MacConaill, L., Ducar, M., Rojas-Rudilla, V., Giannakis, M., Ghazani, A., Gray, S., Janne, P., Garber, J., Joffe, S., Lindeman, N., Wagle, N., . . . Van Allen, E. M. (2016). The impact of tumor profiling approaches and genomic data strategies for cancer precision medicine. *Genome Med*, *8*(1), 79. https://doi.org/10.1186/s13073-016-0333-9
Georgiadis, A., Durham, J. N., Keefer, L. A., Bartlett, B. R., Zielonka, M., Murphy, D., White, J. R., Lu, S., Verner, E. L., Ruan, F., Riley, D., Anders, R. A., Gedvilaite, E., Angiuoli, S., Jones, S., Velculescu, V. E., Le, D. T., Diaz, L. A., & Sausen, M. (2019). Noninvasive Detection of Microsatellite Instability and High Tumor Mutation Burden in Cancer Patients Treated with PD-1 Blockade. *Clinical Cancer Research*. https://doi.org/10.1158/1078-0432.CCR-19-1372
Guardant. (2024a). *Blood-First, TissueNext Streamlined Biomarker Testing*. Retrieved November 1, 2021 from https://guardant360cdx.com/tissuenext/
Guardant. (2024b). *TREATMENT SELECTION AND RESPONSE MONITORING FOR PATIENTS WITH ADVANCED CANCER*. Retrieved October 28, 2021 from https://guardanthealth.com/solutions/#lunar-2
Hellmann, M. D., Callahan, M. K., Awad, M. M., Calvo, E., Ascierto, P. A., Atmaca, A., Rizvi, N. A., Hirsch, F. R., Selvaggi, G., Szustakowski, J. D., Sasson, A., Golhar, R., Vitazka, P., Chang, H., Geese, W. J., & Antonia, S. J. (2018). Tumor Mutational Burden and Efficacy of Nivolumab Monotherapy and in Combination with Ipilimumab in Small-Cell Lung Cancer. *Cancer Cell*, *33*(5), 853-861 e854. https://doi.org/10.1016/j.ccell.2018.04.001
Hellmann, M. D., Ciuleanu, T. E., Pluzanski, A., Lee, J. S., Otterson, G. A., Audigier-Valette, C., Minenza, E., Linardou, H., Burgers, S., Salman, P., Borghaei, H., Ramalingam, S. S., Brahmer, J., Reck, M., O'Byrne, K. J., Geese, W. J., Green, G., Chang, H., Szustakowski, J., . . . Paz-Ares, L. (2018). Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden. *N Engl J Med*, *378*(22), 2093-2104. https://doi.org/10.1056/NEJMoa1801946
Hellmann, M. D., Nathanson, T., Rizvi, H., Creelan, B. C., Sanchez-Vega, F., Ahuja, A., Ni, A., Novik, J. B., Mangarin, L. M. B., Abu-Akeel, M., Liu, C., Sauter, J. L., Rekhtman, N., Chang, E., Callahan, M. K., Chaft, J. E., Voss, M. H., Tenet, M., Li, X. M., . . . Wolchok, J. D. (2018). Genomic Features of Response to Combination Immunotherapy in Patients

with Advanced Non-Small-Cell Lung Cancer. *Cancer Cell*, *33*(5), 843-852 e844. https://doi.org/10.1016/j.ccell.2018.03.018

Illumina. (2024). *TruSight Oncology 500*. Retrieved October 28, 2021 from https://www.illumina.com/products/by-type/clinical-research-products/trusight-oncology-500.html?scid=2018-271doco175#productLongDescription

Klempner, S. J., Fabrizio, D., Bane, S., Reinhart, M., Peoples, T., Ali, S. M., Sokol, E. S., Frampton, G., Schrock, A. B., Anhorn, R., & Reddy, P. (2020). Tumor Mutational Burden as a Predictive Biomarker for Response to Immune Checkpoint Inhibitors: A Review of Current Evidence. *Oncologist*, *25*(1), e147-e159. https://doi.org/10.1634/theoncologist.2019-0244

Le, D. T., Uram, J. N., Wang, H., Bartlett, B. R., Kemberling, H., Eyring, A. D., Skora, A. D., Luber, B. S., Azad, N. S., Laheru, D., Biedrzycki, B., Donehower, R. C., Zaheer, A., Fisher, G. A., Crocenzi, T. S., Lee, J. J., Duffy, S. M., Goldberg, R. M., de la Chapelle, A., . . . Diaz, L. A., Jr. (2015). PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med*, *372*(26), 2509-2520. https://doi.org/10.1056/NEJMoa1500596

Luchini, C., Bibeau, F., Ligtenberg, M. J. L., Singh, N., Nottegar, A., Bosse, T., Miller, R., Riaz, N., Douillard, J. Y., Andre, F., & Scarpa, A. (2019). ESMO recommendations on microsatellite instability testing for immunotherapy in cancer, and its relationship with PD-1/PD-L1 expression and tumour mutational burden: a systematic review-based approach. *Annals of Oncology*, *30*(8), 1232-1243. https://doi.org/10.1093/annonc/mdz116

Marabelle, A., Fakih, M., Lopez, J., Shah, M., Shapira-Frommer, R., Nakagawa, K., Chung, H. C., Kindler, H. L., Lopez-Martin, J. A., Miller, W. H., Jr., Italiano, A., Kao, S., Piha-Paul, S. A., Delord, J. P., McWilliams, R. R., Fabrizio, D. A., Aurora-Garg, D., Xu, L., Jin, F., . . . Bang, Y. J. (2020). Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. *Lancet Oncol*, *21*(10), 1353-1365. https://doi.org/10.1016/S1470-2045(20)30445-9

Marabelle, A., Le, D. T., Ascierto, P. A., Di Giacomo, A. M., De Jesus-Acosta, A., Delord, J. P., Geva, R., Gottfried, M., Penel, N., Hansen, A. R., Piha-Paul, S. A., Doi, T., Gao, B., Chung, H. C., Lopez-Martin, J., Bang, Y. J., Frommer, R. S., Shah, M., Ghori, R., . . . Diaz, L. A., Jr. (2020). Efficacy of Pembrolizumab in Patients With Noncolorectal High Microsatellite Instability/Mismatch Repair-Deficient Cancer: Results From the Phase II KEYNOTE-158 Study. *J Clin Oncol*, *38*(1), 1-10. https://doi.org/10.1200/JCO.19.02105

Merino, D. M., McShane, L. M., Fabrizio, D., Funari, V., Chen, S. J., White, J. R., Wenz, P., Baden, J., Barrett, J. C., Chaudhary, R., Chen, L., Chen, W. S., Cheng, J. H., Cyanam, D., Dickey, J. S., Gupta, V., Hellmann, M., Helman, E., Li, Y., . . . Allen, J. (2020). Establishing guidelines to harmonize tumor mutational burden (TMB): in silico assessment of variation in TMB quantification across diagnostic platforms: phase I of the Friends of

Cancer Research TMB Harmonization Project. *J Immunother Cancer*, *8*(1). https://doi.org/10.1136/jitc-2019-000147

Mosele, F., Remon, J., Mateo, J., Westphalen, C. B., Barlesi, F., Lolkema, M. P., Normanno, N., Scarpa, A., Robson, M., Meric-Bernstam, F., Wagle, N., Stenzinger, A., Bonastre, J., Bayle, A., Michiels, S., Bieche, I., Rouleau, E., Jezdic, S., Douillard, J. Y., . . . Andre, F. (2020). Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. *Ann Oncol*, *31*(11), 1491-1505. https://doi.org/10.1016/j.annonc.2020.07.014

MSKCC. (2024). *MSK-IMPACT: A Targeted Test for Mutations in Both Rare and Common Cancers*. Retrieved October 20, 2021 from https://www.mskcc.org/msk-impact

NCCN. (2024a, August 20, 2024). *NCCN Clinical Practice Guidelines in Ampullary Adenocarcinoma Version 1.2025 — August 20, 2024*. https://www.nccn.org/professionals/physician_gls/pdf/ampullary.pdf

NCCN. (2024b, April 30, 2024). *NCCN Clinical Practice Guidelines in Oncology - Pancreatic Adenocarcinoma Version 2.2024* https://www.nccn.org/professionals/physician_gls/pdf/pancreatic.pdf

NCCN. (2024c). *NCCN Clinical Practice Guidelines in Oncology - Penile Cancer Version 1.2025 — November 1, 2024*. Retrieved November 05, 2022 from https://www.nccn.org/professionals/physician_gls/pdf/penile.pdf

NCCN. (2024d, September 24, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Biliary Tract Cancers Version 3.2024 — September 24, 2024*. https://www.nccn.org/professionals/physician_gls/pdf/btc.pdf

NCCN. (2024e, August 20, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Bone Cancer Version 1.2025 — August 20, 2024*. Retrieved November 05, 2022 from https://www.nccn.org/professionals/physician_gls/PDF/bone.pdf

NCCN. (2024f, October 15, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Breast Cancer Version 5.2024 —October 15, 2024*. Retrieved November 5, 2022 from https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf

NCCN. (2024g, September 24, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Cervical Cancer Version 3.2024* Retrieved October 12, 2021 from https://www.nccn.org/professionals/physician_gls/pdf/cervical.pdf

NCCN. (2024h, August 22, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Colon Cancer Version 5.2024* Retrieved November 5, 2022 from https://www.nccn.org/professionals/physician_gls/pdf/colon.pdf

NCCN. (2024i, July 30, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Esophageal and Esophagogastric Junction Cancers Version Version 4.2024* Retrieved November 5, 2022 from https://www.nccn.org/professionals/physician_gls/pdf/esophageal.pdf

NCCN. (2024j, August 12, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Gastric Cancer Version 4.2024* Retrieved November 5, 2022 from https://www.nccn.org/professionals/physician_gls/pdf/gastric.pdf
NCCN. (2024k, August 2, 2023). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Neuroendocrine and Adrenal Tumors Version 2.2024 — August 1, 2024*. Retrieved November 05, 2022 from https://www.nccn.org/professionals/physician_gls/pdf/neuroendocrine.pdf
NCCN. (2024l, October 15, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Non-Small Cell Lung Cancer Version 11.2024*. https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf
NCCN. (2024m, September 11, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Occult Primary (Cancer of Unknown Primary [CUP]) Version 2.2025 — September 11, 2024*. Retrieved November 5, 2022 from https://www.nccn.org/professionals/physician_gls/pdf/occult.pdf
NCCN. (2024n, July 15, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Ovarian Cancer, Including Fallopian Tube Cancer and Primary Peritoneal Cancer Version 3.2024*. Retrieved November 05, 2022 from https://www.nccn.org/professionals/physician_gls/pdf/ovarian.pdf
NCCN. (2024o, May 17, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Prostate Cancer Version 4.2024*. Retrieved November 05, 2022 from https://www.nccn.org/professionals/physician_gls/pdf/prostate.pdf
NCCN. (2024p, August 22, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Rectal Cancer Version 4.2024* Retrieved November 05, 2022 from https://www.nccn.org/professionals/physician_gls/pdf/rectal.pdf
NCCN. (2024q, September 13, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Small Bowel Adenocarcinoma Version 5.2024* Retrieved November 05, 2022 from https://www.nccn.org/professionals/physician_gls/pdf/small_bowel.pdf
NCCN. (2024r, October 29, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Testicular Cancer Version 2.2024 — October 29, 2024*. Retrieved November 05, 2022 from https://www.nccn.org/professionals/physician_gls/pdf/testicular.pdf
NCCN. (2024s, August 19, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Thyroid Carcinoma Version 4.2024 — August 19, 2024*. Retrieved November 5, 2022 from https://www.nccn.org/professionals/physician_gls/pdf/thyroid.pdf
NCCN. (2024t, September 20, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Uterine Neoplasms Version 3.2024 — September 20, 2024*. Retrieved October 12, 2021 from https://www.nccn.org/professionals/physician_gls/pdf/uterine.pdf

NCCN. (2024u, May 1, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Vulvar Cancer Version 4.2024 — May 1, 2024*. Retrieved November 05, 2022 from https://www.nccn.org/professionals/physician_gls/pdf/vulvar.pdf
NCCN. (2024v, March 4, 2024). *NCCN Clinical Practice Guidelines in Oncology Anal Carcinoma NCCN Evidence Blocks TM Version 1.2024 — March 4, 2024*. https://www.nccn.org/professionals/physician_gls/pdf/anal_blocks.pdf
NCCN. (2024w, October 24, 2024). *NCCN Clinical Practice Guidelines in Oncology Head and Neck Cancers Version 5.2024*. https://www.nccn.org/professionals/physician_gls/pdf/head-and-neck.pdf
NCCN. (2024x, September 24, 2024). *NCCN Clinical Practice Guidelines in Oncology in Hepatocellular Carcinoma Version 3.2024 — September 24, 2024*. https://www.nccn.org/professionals/physician_gls/pdf/hcc.pdf
NCCN. (2024y, February 26, 2024). *NCCN Clinical Practice Guidelines in Oncology in Pediatric Central Nervous System Cancers Version 1.2024 — February 26, 2024*. https://www.nccn.org/professionals/physician_gls/pdf/ped_cns.pdf
NCCN. (2024z, September 27, 2024). *NCCN Clinical Practice Guidelines in Oncology in Soft Tissue Sarcoma Version 3.2024*. https://www.nccn.org/professionals/physician_gls/pdf/sarcoma.pdf
NCI. (n.d.). *MSI-H cancer*. https://www.cancer.gov/publications/dictionaries/cancer-terms/def/msi-h-cancer
Nojadeh, J. N., Behrouz Sharif, S., & Sakhinia, E. (2018). Microsatellite instability in colorectal cancer. *EXCLI J*, *17*, 159-168. https://doi.org/10.17179/excli2017-948
Overman, M. J., McDermott, R., Leach, J. L., Lonardi, S., Lenz, H. J., Morse, M. A., Desai, J., Hill, A., Axelson, M., Moss, R. A., Goldberg, M. V., Cao, Z. A., Ledeine, J. M., Maglinte, G. A., Kopetz, S., & Andre, T. (2017). Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol*, *18*(9), 1182-1191. https://doi.org/10.1016/S1470-2045(17)30422-9
Qiagen. (2024). *QIAseq Tumor Mutational Burden Panels*. Retrieved October 28, 2021 from https://www.qiagen.com/us/products/next-generation-sequencing/dna-sequencing/targeted-dna-panels/qiaseq-tumor-mutational-burden-panels/
Quy, P. N., Kanai, M., Fukuyama, K., Kou, T., Kondo, T., Yamamoto, Y., Matsubara, J., Hiroshima, A., Mochizuki, H., Sakuma, T., Kamada, M., Nakatsui, M., Eso, Y., Seno, H., Masui, T., Takaori, K., Minamiguchi, S., Matsumoto, S., & Muto, M. (2019). Association Between Preanalytical Factors and Tumor Mutational Burden Estimated by Next-Generation Sequencing-Based Multiplex Gene Panel Assay. *Oncologist*, *24*(12), e1401-e1408. https://doi.org/10.1634/theoncologist.2018-0587
Ready, N., Hellmann, M. D., Awad, M. M., Otterson, G. A., Gutierrez, M., Gainor, J. F., Borghaei, H., Jolivet, J., Horn, L., Mates, M., Brahmer, J., Rabinowitz, I., Reddy, P. S., Chesney, J., Orcutt, J., Spigel, D. R., Reck, M., O'Byrne, K. J., Paz-Ares, L., . . .

Ramalingam, S. S. (2019). First-Line Nivolumab Plus Ipilimumab in Advanced Non-Small-Cell Lung Cancer (CheckMate 568): Outcomes by Programmed Death Ligand 1 and Tumor Mutational Burden as Biomarkers. *J Clin Oncol*, *37*(12), 992-1000. https://doi.org/10.1200/JCO.18.01042

Ritterhouse, L. L. (2019). Tumor mutational burden. *Cancer Cytopathol*, *127*(12), 735-736. https://doi.org/10.1002/cncy.22174

Rizvi, N. A., Hellmann, M. D., Snyder, A., Kvistborg, P., Makarov, V., Havel, J. J., Lee, W., Yuan, J., Wong, P., Ho, T. S., Miller, M. L., Rekhtman, N., Moreira, A. L., Ibrahim, F., Bruggeman, C., Gasmi, B., Zappasodi, R., Maeda, Y., Sander, C., . . . Chan, T. A. (2015). Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science*, *348*(6230), 124-128. https://doi.org/10.1126/science.aaa1348

Samstein, R. M., Lee, C. H., Shoushtari, A. N., Hellmann, M. D., Shen, R., Janjigian, Y. Y., Barron, D. A., Zehir, A., Jordan, E. J., Omuro, A., Kaley, T. J., Kendall, S. M., Motzer, R. J., Hakimi, A. A., Voss, M. H., Russo, P., Rosenberg, J., Iyer, G., Bochner, B. H., . . . Morris, L. G. T. (2019). Tumor mutational load predicts survival after immunotherapy across multiple cancer types. *Nat Genet*, *51*(2), 202-206. https://doi.org/10.1038/s41588-018-0312-8

Si, H., Kuziora, M., Quinn, K. J., Helman, E., Ye, J., Liu, F., Scheuring, U., Peters, S., Rizvi, N. A., Brohawn, P. Z., Ranade, K., Higgs, B. W., Banks, K. C., Chand, V. K., & Raja, R. (2021). A Blood-based Assay for Assessment of Tumor Mutational Burden in First-line Metastatic NSCLC Treatment: Results from the MYSTIC Study. *Clin Cancer Res*, *27*(6), 1631-1640. https://doi.org/10.1158/1078-0432.CCR-20-3771

Singal, G., Miller, P. G., Agarwala, V., Li, G., Kaushik, G., Backenroth, D., Gossai, A., Frampton, G. M., Torres, A. Z., Lehnert, E. M., Bourque, D., O'Connell, C., Bowser, B., Caron, T., Baydur, E., Seidl-Rathkopf, K., Ivanov, I., Alpha-Cobb, G., Guria, A., . . . Miller, V. A. (2019). Association of Patient Characteristics and Tumor Genomics With Clinical Outcomes Among Patients With Non-Small Cell Lung Cancer Using a Clinicogenomic Database. *JAMA*, *321*(14), 1391-1399. https://doi.org/10.1001/jama.2019.3241

Snyder, A., Makarov, V., Merghoub, T., Yuan, J., Zaretsky, J. M., Desrichard, A., Walsh, L. A., Postow, M. A., Wong, P., Ho, T. S., Hollmann, T. J., Bruggeman, C., Kannan, K., Li, Y., Elipenahli, C., Liu, C., Harbison, C. T., Wang, L., Ribas, A., . . . Chan, T. A. (2014). Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med*, *371*(23), 2189-2199. https://doi.org/10.1056/NEJMoa1406498

Stahler, A., Stintzing, S., von Einem, J. C., Westphalen, C. B., Heinrich, K., Kramer, N., Michl, M., Modest, D. P., von Weikersthal, L. F., Decker, T., Kiani, A., Heintges, T., Kahl, C., Kullmann, F., Scheithauer, W., Moehler, M., Kaiser, F., Kirchner, T., Jung, A., & Heinemann, V. (2020). Single-nucleotide variants, tumour mutational burden and microsatellite instability in patients with metastatic colorectal cancer: Next-

generation sequencing results of the FIRE-3 trial. *Eur J Cancer*, *137*, 250-259. https://doi.org/10.1016/j.ejca.2020.07.003

Van Allen, E. M., Miao, D., Schilling, B., Shukla, S. A., Blank, C., Zimmer, L., Sucker, A., Hillen, U., Foppen, M. H. G., Goldinger, S. M., Utikal, J., Hassel, J. C., Weide, B., Kaehler, K. C., Loquai, C., Mohr, P., Gutzmer, R., Dummer, R., Gabriel, S., . . . Garraway, L. A. (2015). Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science*, *350*(6257), 207-211. https://doi.org/10.1126/science.aad0095

Wei, B., Kang, J., Kibukawa, M., Arreaza, G., Maguire, M., Chen, L., Qiu, P., Lang, L., Aurora-Garg, D., Cristescu, R., & Levitan, D. (2022). Evaluation of the TruSight Oncology 500 Assay for Routine Clinical Testing of Tumor Mutational Burden and Clinical Utility for Predicting Response to Pembrolizumab. *J Mol Diagn*. https://doi.org/10.1016/j.jmoldx.2022.01.008

Willis, C., Fiander, M., Tran, D., Korytowsky, B., Thomas, J. M., Calderon, F., Zyczynski, T. M., Brixner, D., & Stenehjem, D. D. (2019). Tumor mutational burden in lung cancer: a systematic literature review. *Oncotarget*, *10*(61), 6604-6622. https://doi.org/10.18632/oncotarget.27287

Willis, J., Lefterova, M. I., Artyomenko, A., Kasi, P. M., Nakamura, Y., Mody, K., Catenacci, D. V. T., Fakih, M., Barbacioru, C., Zhao, J., Sikora, M., Fairclough, S. R., Lee, H., Kim, K.-M., Kim, S. T., Kim, J., Gavino, D., Benavides, M., Peled, N., . . . Odegaard, J. I. (2019). Validation of Microsatellite Instability Detection Using a Comprehensive Plasma-Based Genotyping Panel. *Clinical Cancer Research*. https://doi.org/10.1158/1078-0432.CCR-19-1324

Woodhouse, R., Li, M., Hughes, J., Delfosse, D., Skoletsky, J., Ma, P., Meng, W., Dewal, N., Milbury, C., Clark, T., Donahue, A., Stover, D., Kennedy, M., Dacpano-Komansky, J., Burns, C., Vietz, C., Alexander, B., Hegde, P., & Dennis, L. (2020). Clinical and analytical validation of FoundationOne Liquid CDx, a novel 324-Gene cfDNA-based comprehensive genomic profiling assay for cancers of solid tumor origin. *PLoS One*, *15*(9), e0237802. https://doi.org/10.1371/journal.pone.0237802

Wu, H. X., Wang, Z. X., Zhao, Q., Wang, F., & Xu, R. H. (2019). Designing gene panels for tumor mutational burden estimation: the need to shift from 'correlation' to 'accuracy'. *J Immunother Cancer*, *7*(1), 206. https://doi.org/10.1186/s40425-019-0681-2

Yarchoan, M., Albacker, L. A., Hopkins, A. C., Montesion, M., Murugesan, K., Vithayathil, T. T., Zaidi, N., Azad, N. S., Laheru, D. A., Frampton, G. M., & Jaffee, E. M. (2019). PD-L1 expression and tumor mutational burden are independent biomarkers in most cancers. *JCI Insight*, *4*(6). https://doi.org/10.1172/jci.insight.126908

Yarchoan, M., Hopkins, A., & Jaffee, E. M. (2017). Tumor Mutational Burden and Response Rate to PD-1 Inhibition. *N Engl J Med*, *377*(25), 2500-2501. https://doi.org/10.1056/NEJMc1713444

Zhao, P., Li, L., Jiang, X., & Li, Q. (2019). Mismatch repair deficiency/microsatellite instability-high as a predictor for anti-PD-1/PD-L1 immunotherapy efficacy. *J Hematol Oncol*, *12*(1), 54. https://doi.org/10.1186/s13045-019-0738-1

Minimal Residual Disease (MRD)

Policy Number: AHS –M2175 – Minimal Residual Disease (MRD)	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Minimal residual disease, also called measurable residual disease or MRD, refers to the subclinical levels of residual diseases, such as acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), and multiple myeloma (MM) (Horton & Steuber, 2023; Rajkumar, 2023; Stock & Estrov, 2022a, 2022b). MRD is a postdiagnosis, prognostic indicator that can be used for risk stratification and to guide therapeutic options when used alongside other clinical and molecular data (Schuurhuis et al., 2018). Many different techniques have been developed to detect residual disease. However, PCR-based techniques, multicolor flow cytometry, and deep sequencing-based MRD generally provide the best sensitivity, specificity, reproducibility, and applicability compared to other techniques such as fluorescence in situ hybridization (FISH), Southern blotting, or cell culture (Stock & Estrov, 2022b).

II. Related Policies

Policy Number	Policy Title
AHS-F2019	Flow Cytometry
AHS-G2054	Liquid Biopsy
AHS-M2182	Genomic Testing for Hematopoietic Neoplasms

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with multiple myeloma (MM), chronic lymphocytic leukemia (CLL), or small lymphocytic lymphoma (SLL), minimal residual disease (MRD) testing by

multiparameter flow cytometry **or** next-generation sequencing (NGS) **MEETS COVERAGE CRITERIA**.

2) For individuals with acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL), MRD testing by multiparameter flow cytometry, PCR-based techniques, **or** NGS **MEETS COVERAGE CRITERIA**.

3) For individuals with a diagnosis of human papilloma virus (HPV)-positive oropharyngeal squamous cell carcinoma (OPSCC) who are being monitored for disease recurrence (at least three months after completion of initial definitive therapy), blood-based detection of tumor tissue modified viral (TTMV)-HPV DNA fragments (i.e., NavDx®) **MEETS COVERAGE CRITERIA** once every three months.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

4) For all situations not addressed above (e.g., MRD testing for all other solid tumors), MRD testing by multiparameter flow cytometry, PCR, or NGS **DOES NOT MEET COVERAGE CRITERIA**.

IV. Table of Terminology

Term	Definition
ABL	*Abelson tyrosine kinase gene*
AL	Acute leukemia
ALL	Acute lymphoblastic leukemia
ALL	Pediatric acute lymphoblastic leukemia
AML	Acute myeloid leukemia
AML1	*Acute myeloid leukemia 1 protein gene*
ASCO	American Society of Clinical Oncology
ASCT	Autologous Stem Cell Transplantation
ASH	American Society of Hematology
B-ALL	B-cell acute lymphoblastic leukemia
BCL1	*B-cell lymphoma 1 gene*
BCL2	*B-cell lymphoma 2 gene*
BCP	B-cell precursor
BCR	Break point cluster gene
BM	Bone marrow
BMA	Bone marrow aspirate

BMR	Bone marrow relapse
BRAF	Proto-oncogene B-Raf/v-Raf murine sarcoma viral oncogene homolog B
CA	Cytogenetic abnormalities
CAP	College of American Pathologists
CAR	Post-chimeric antigen receptor
CBF	Core binding factor
CBFB-MYH11	*Cord binding factor subunit beta-myosin 11 fusion gene*
CCO	Cancer Care Ontario
CD19	Cluster of differentiation 19
CD20	Cluster of differentiation 20
CD43	Cluster of differentiation 43
CD49b	Cluster of differentiation 49b
CD5	Cluster of differentiation 5
CD81	Cluster of differentiation 81
2-CldA	Cladribine
CLIA	Clinical Laboratory Improvement Amendments
CLL	Chronic lymphocytic leukemia
CMS	Centers for Medicare and Medicaid Services
CR	Complete response/complete remission
CT	Computed Tomography
ctDNA	Circulating tumor deoxyribonucleic acid
CV	Coefficients of variance
DCF	Pentostatin
DFCI	Dana Farber Cancer Institute
DFS	Disease Free Survival
DJ	Diversity and joining segment rearrangement
DNA	Deoxyribonucleic acid
E2A	Transcription factor E2-alpha gene
EFS	Event free survival
ELN	European LeukemiaNet
EMN	European Myeloma Network
EOCT	End of combination therapy
ERIC	European Research Initiative on chronic lymphocytic leukemia
ESMO	European Society for Medical Oncology
EWS	Ewing sarcoma

FC	Flow cytometry
FCR	Fludarabine cyclophosphamide and rituximab
FDA	Food and Drug Administration
FISH	Fluorescence in situ hybridization
FUM3	Follow up month 3
gDNA	Genomic deoxyribonucleic acid
HPV	Human papillomavirus
HR-NB	High-risk neuroblastoma
HSCT	Haematopoietic stem cell transplantation
HTS	High-throughput sequencing
IgH	Immunoglobulin heavy chain
IGH	*Immunoglobulin heavy chain gene*
IgK	Immunoglobulin K
IgL	Immunoglobulin L
IHC	Immunohistochemistry
IMWG	International Myeloma Working Group
iwCLL	International Workshop on Chronic Lymphocytic Leukemia
J	Joining
KIT	KIT proto-oncogene receptor tyrosine kinase
LDTs	Laboratory developed tests
MFC/mFC	Multicolor flow cytometry
MFC	Mutliparameter flow cytometry
MM	Multiple myeloma
mpFC	Multi parametic flow cytometry
MRD	Minimal residual disease
MRD	Measurable residual disease
NCCN	National Comprehensive Cancer Network
NDMM	Newly diagnosed multiple myeloma
NGF	Next-generation flow cytometry
NGS	Next generation sequencing
NGS-MRD	Next-generation sequencing-minimal residual disease
non-TBI	Non-total body irradiation
NPV	Negative Predictive Value
NPM1mut	*Nucleophosmin 1 gene mutant allele*
OS	Overall survival
OPSCC	Oropharyngeal squamous cell carcinoma

PBX1	*Pre-B-cell leukemia transcription factor 1 gene*
PCR	Polymerase chain reaction
PET-CT	Positron emission tomography -computed tomography
PFS	Progression-free survival
PML-RARA	*Promyelocytic leukemia-retinoic acid receptor alpha fusion gene*
PPV	Positive predictive value
QALYs	Quality adjusted life years
RQ-PCR/RT-qPCR	Real-time quantitative polymerase chain reaction
RRMM	Relapsed refractory multiple myeloma
RT-PCR	Reverse transcriptase polymerase chain reaction
RUNX1-RUNX1T1	*Runt-related transcription factor 1- RUNX1 partner transcriptional co-repressor 1 gene*
sCR	Stringent complete remission
SR	Standard risk
T-ALL	T-cell acute lymphocytic leukemia
TBI	Total body irradiation
TCR	T cell receptor
TEL	*Translocation-Ets-leukemia virus gene*
TRG	*T cell receptor gamma locus gene*
TTMV	tumor tissue modified viral
uMRD	Undetectable minimal residual disease
VDJ	Variable-diversity-joining
VGPR	Very good partial response
WGS	Whole genome sequencing

V. Scientific Background

The goal of treating cancer has traditionally been "complete remission" (or response), defined as "absence of visible tumor" based on techniques, such as imaging and histological examination of tissue (Luskin et al., 2018). However, some cancer cells may remain undetected due to a lack of sensitivity of conventional methods, leading to relapse. This subclinical amount of cancer cells is referred to as minimal residual disease or MRD (Bai et al., 2018). While many techniques have been developed to determine MRD, multicolor flow cytometry and PCR-based, including next generation sequencing (NGS), MRD techniques are the most commonly used (Rai & Stilgenbauer, 2024; Stock & Estrov, 2022b).

Multicolor flow cytometry (MFC), also known as multiparameter flow cytometry, can be used to identify MRD by measuring for the aberrant expression of antigens on cancer cells. MFC uses lasers of different colors to simultaneously determine specific immunophenotypic features of the cells within a sample. "Classic flow cytometry techniques using four to six colors have limited sensitivity and specificity for MRD detection. Current flow cytometry techniques use six to eight colors to assess MRD with a sensitivity which is approximately 10^{-4}, or about 0.5 to 1 log lower than that of polymerase chain reaction" (PCR) (Stock & Estrov, 2022b).

Polymerase chain reaction-based MRD techniques, including NGS, amplify sequences of DNA unique to the cancerous cell. These techniques have amazing sensitivity. In fact, real-time quantitative PCR can be used to detect a single cancerous cell from 10^4 – 10^5 cells (Brüggemann et al., 2006; Stock & Estrov, 2022b). The targets of amplification can include T cell receptor (TCR) gene rearrangements, the immunoglobulin heavy chain (IgH), or even fusion-gene transcripts (Del Giudice et al., 2019; Stock & Estrov, 2022b; van der Velden et al., 2003). Reverse transcriptase PCR-based MRD can also be used to detect cancer-related transcripts, including *E2A/PBX1, TEL/AML1,* and *BCR/ABL* (Lee et al., 2003; Madzo et al., 2003; Stock & Estrov, 2022b).

Proprietary Testing

ClonoSEQ (Adaptive Biotechnologies, Seattle, WA) is a commercially available NGS-based assay intended to assess MRD in certain types of cancer, such as MM and ALL. This test identifies rearrangements in certain receptor gene sequences, representing the level of MRD in a patient. This test typically uses genomic DNA extracted from bone marrow but may use circulating tumor DNA (ctDNA) (Herrera et al., 2016). After testing, a report is provided which includes each nucleotide sequence identified for tracking residual disease, the amount of each identified marker (per million cells), and whether MRD is determined to be present in the sample (Adaptive Biotechnologies, 2024).

Signatera™ is an MRD assay that uses ctDNA to attempt to inform the likelihood of cancer relapse earlier than standard of care tools. Whole-exome sequencing of an individual's tumor tissue is first performed, allowing the identification of clonal somatic mutations which are expected to be present in all cells for the individual's specific tumor. The test is customized to an individual through selection of 16 clonal, single nucleotide variants (SNVs). After customization, a blood sample may be obtained from the individual, from which the 16 SNVs may be amplified and detected. An individual is considered MRD-positive when at least two SNVs from the set of 16 are detected. According to the test makers, "a positive Signatera™ result predicts relapse with overall positive predictive value more than 98%" (Natera, 2024).

NavDx is a blood test developed by Naveris that detects trace amounts of HPV-associated tumor DNA in the bloodstream, specifically targeting residual disease or early recurrence of HPV-driven oropharyngeal cancers. It detects and analyzes the tumor tissue modified viral (TTMV)-HPV DNA fragments released by dying cancer cells to provide a TTMV-Score. The test indicates cancer activity before it is visible through imaging or other diagnostics (NavDx, 2024).

Analytical Validity

The EuroFlow Consortium has reported on the analytical validity of the use of an 8-color mFC for MRD. Theunissen et al. (2017) reported on the use of this methodology for B-cell precursor (BCP) ALL in a multicenter study (319 patients). Using samples containing more than four million cells, they note concordant results in 93% of samples, and "[m]ost discordances were clarified upon high-throughput sequencing of antigen-receptor rearrangements and blind multicenter reanalysis of flow cytometric data, resulting in an unprecedented concordance of 98% (97% for samples with MRD < 0.01%). In conclusion, the fully standardized EuroFlow BCP-ALL MRD strategy is applicable in >98% of patients with sensitivities at least similar to RQ-PCR ($\leq 10^{-5}$), if sufficient cells (>4 × 10^6, preferably more) are evaluated" (Theunissen et al., 2017). Another study reports the use of next-generation flow cytometry (NGF) using an "optimized 2-tube 8-color antibody panel" in five cycles to further increase the sensitivity. The authors report "a higher sensitivity for NGF-MRD vs conventional 8-color flow-MRD -MRD-positive rate of 47 vs 34% (P=0.003). Thus, 25% of patients classified as MRD-negative by conventional 8-color flow were MRD-positive by NGF, translating into a significantly longer progression-free survival for MRD-negative vs MRD-positive CR [complete response] patients by NGF (75% progression-free survival not reached vs 7 months; P=0.02)." Another study using a single-tube 10-fluorochrome analysis NGF method of MRD in myeloma reports a five-fold increase over the target minimum of 5 X 10^6 white blood cells per acquisition (Royston et al., 2016).

The FDA included an assessment of the analytical validity of ClonoSEQ in their approval summary of a *de novo* request evaluation. A total of 23 patients with MM, 21 patients with ALL, and 22 patients with other lymphoid malignancies were included. The study tested three different volumes of DNA: 500ng, 2µg, and 20 µg. Six MRD levels were tested for each sample, corresponding to the following amounts of malignant cells: 2.14, 6.13, 21.44, 61.26, 214.40, and 612.56. The authors found the coefficients of variance (%CV) to range from 72% at 2.14 cells to 21% at 612.56 cells. The authors noted that this precision trend was predictable, as ClonoSEQ is dependent on the number of cells evaluated instead of the actual MRD frequency. Regarding DNA extraction reproducibility, all samples were found to pass the "pre-established acceptance criteria of ± 30% MRD frequency." Regarding precision of the

nucleotide/base cells, the authors created a set of "baseline calibrating clonotype nucleotide sequences." From this set, replicates of each sample used to create the calibration sequence were created and the disagreement rate was identified. Out of 442.5 million nucleotides, ClonoSEQ was found to have a disagreement rate of 3.5 parts per million. The FDA notes a Phred Score of >30 is considered a "high-quality base call for NGS applications"; ClonoSEQ had a Phred Score of 44.5. When compared to multiparametic flow cytometry (mpFC), both ClonoSEQ and mpFC were tested at five dilutions (from $5x10^{-7}$ to $1x10^{-2}$)s and both techniques were found to be of similar accuracy at frequencies above 1×10^{-4} (FDA, 2018).

In 2020, the FDA made a substantially equivalent decision on ClonoSEQ, providing in their summary an assessment with further analytical validity. Here, they looked at patients with CLL, measuring MRD levels from gDNA samples extracted from either bone marrow (22 patients) or blood samples (15 patients). The study tested six MRD levels in three different volumes of DNA: 500ng, 2µg, and 20 µg. In bone marrow samples, precision ranged from 59% CV at 2.14 cells to 20% CV at 612.56 cells. In blood samples, precision ranged from 53% CV at 3.10 cells to 19% CV at 765.70 cells. The authors note that "like BMA [bone marrow aspirate], the precision of the clonoSEQ assay in CLL blood is largely dependent on the number of malignant cells that are being evaluated by the assay." From these data and those presented in the 2018 *de novo* approval document, the indications for use were developed. The authors report that "the clonoSEQ Assay measures minimal residual disease (MRD) to monitor changes in burden of disease during and after treatment. The test is indicated for use by qualified healthcare professionals in accordance with professional guidelines for clinical decision-making and in conjunction with other clinicopathological features" (FDA, 2020).

Othman Al-Sawaf et al. (2020) analyzed the clonal growth patterns of patients treated with venetoclax-obinutuzumab therapy within the CLL14 trial. In this case, MRD was analyzed using NGS via the adaptive clonoSEQ assay with cutoffs of 10^{-4}, 10^{-5}, and 10^{-6}; "the limit-of-quantification of the clonoSEQ assay is less than 10^{-6}." A total of 432 patients with untreated CLL were either treated with chlorambucil or venetoclax, in combination with 8binutuzumab for the first six cycles. Using samples from peripheral blood (PB) collected every three to six months until nine years from last patient enrollment, the researchers found that two months post-treatment completion, among patients treated with venetoclax and obinutuzmab, 40% had uMRD levels $<10^{-6}$, 26% had uMRD levels that were $\geq 10^{-6}$ and $<10^{-5}$, 8% had uMRD levels $\geq 10^{-5}$ and $<10^{-4}$, 5% had uMRD levels $\geq 10^{-4}$ and $<10^{-2}$, and 3% had uMRD levels $\geq 10^{-2}$. In comparison, among patients treated with chlorambucil and obinutuzmab, 7% had uMRD levels $<10^{-6}$, 13% had uMRD levels $\geq 10^{-6}$ and $<10^{-5}$, 14% had uMRD levels $\geq 10^{-5}$ and $<10^{-4}$, 21% had uMRD levels $\geq 10^{-4}$ and $<10^{-2}$, and 26% had uMRD levels $\geq 10^{-2}$. Furthermore, "In a

PFS landmark analysis after [end of treatment], patients in the [venetoclax-obinutuzumab] arm with MRD levels ≤ 10^{-5} had a 2-year PFS after [end of treatment] of approximately 93%, while patients with detectable MRD >10^{-2} had a 2-year PFS of [approximately] 37%." The average growth rate among patients treated with venetoclax-obinutuzumab was lower compared to the contrasting arm, and thus had a larger MRD doubling time. Because the clonal growth rate was lower, the study, using NGS, indicated "more effective MRD eradication and clonal growth modulation" (Othman Al-Sawaf et al., 2020; O. Al-Sawaf et al., 2020).

Nguyen Hoang et al. (2024) studied the analytical validity and clinical utilization of K-4CARE™, a comprehensive genomic profiling assay that integrates ctDNA tracking for residual cancer surveillance. K-4CARE™ is proposed to be a more time-efficient and tissue-efficient method than single or small gene panel testing. The assay tests for 473 cancer-relevant genes with a total length of 1.7 Mb. The authors rested the assay with 155 samples from 10 cancer types. "For detection of somatic SNVs and Indels, gene fusion and amplification, the assay had sensitivity of >99%, 94% and >99% respectively, and specificity of >99%." Furthermore, "when CGP-informed mutations were used to personalize ctDNA tracking, the detection rate of ctDNA in liquid biopsy was 79%, and clinical utility in cancer surveillance was demonstrated in 2 case studies." The authors concluded that the K-4CARE™ assay is "comprehensive and reliable" as a biomarker test for targeted therapy and immunotherapy. The authors also state that "integration of ctDNA tracking helps clinicians to further monitor treatment response and ultimately provide well-rounded care to cancer patients" (Nguyen Hoang et al., 2024).

Gunning et al. (2023) investigated the DNA stability and the ability to detect TTMV-HPV DNA analytes from patient samples stored in Streck collection tubes over a seven day interval. The ESR1 gene was chosen as the analyte for DNA stability analysis; DNA recovery was reported as the average, minimum, and maximum detected ESR1 per mL of plasma isolated. TTMV-HPV analyte detection was accomplished by measuring the TTMV-HPV DNA percent positivity rate, tracked daily. Percent positivity for TTMV-HPV DNA remained consistently between 26.2% - 32.5% from days one to seven. The average ESR1 values trended upward and more than doubled between days 1-6, which may be due to an increase in cellular lysis and DNA release within the sample over time. The LODs (limit of detection) for TTMV-HPV16, 18, 31, 33 and 35 DNA were reported to be: 0.56, 1.31, 0.63, 1.10 and 0.57 copies per microliter (copies/µL); LOQs (limit of quantitation) were reported as <1.20, 3.56, 4.11, 4.00, and 3.50 copies/ µL. The analytical specificity of this study was reported to be between 0 and 0.32 copies/µL for the five high-risk HPV subtypes. The authors investigated "the ability of the NavDx test to obtain a response that is directly proportional to the concentration of analyte standard." It was determined that all five HPV serotypes demonstrated linearity down

to the limit of quantitation. The coefficients of determination (R2) equaled one for all serotypes at all timepoints. Intra-assay and inter-assay precision were investigated through determination of % CVs (coefficients of variation) of the measured concentrations of each HPV serotype:

Dilution *	%CVs of Mean Effective Concentration (Copies/µL)									
	Intra-Assay					Inter-Assay				
	TTMV-HPV16	TTMV-HPV18	TTMV-HPV31	TTMV-HPV33	TTMV-HPV35	TTMV-HPV16	TTMV-HPV18	TTMV-HPV31	TTMV-HPV33	TTMV-HPV35
1:1	2.1	2.7	2.2	3.0	1.4	2.9	2.4	1.9	2.5	1.9
1:4	2.1	2.1	1.0	1.5	3.5	3.3	3.6	2.6	4.3	3.8
1:20	3.3	2.7	3.0	5.4	1.2	4.7	2.9	6.3	4.9	3.0
1:40	2.0	6.2	8.0	11.5	1.1	4.6	5.4	7.5	8.4	6.4
1:100	12.2	10.1	9.0	5.3	7.1	7.5	11.9	10.8	10.8	5.8
1:200	13.3	13.8	7.2	13.9	4.7	9.0	20.0	16.5	13.1	11.2

Table 4. Intra-assay precision (repeatability) and inter-assay precision (within-lab on differing days), reflected by percent relative standard deviations of effective concentrations of the five TTMV-HPV DNA types measured at six concentrations in triplicate.

Table borrowed from Gunning et al. (2023).

Clinical Utility and Validity

The FDA *de novo* approval document for ClonoSEQ contains two clinical validation studies. The first study for MM in ClonoSEQ's *de novo* approval document (DFCI 10-106) included 323 patients. The authors intended "to assess the ability of clonoSEQ to predict progression-free survival (PFS) and disease-free survival (DFS)." At the time of first MRD measurement, ClonoSEQ was found to be predictive of PFS at the MRD threshold of 10^{-5}. Each 10-fold increase in MRD level was associated with a 70% increase in "event" rate across all MRD values (FDA, 2018).

A second clinical validation study described in the FDA's *de novo* approval document was for ALL (AALL0232, AALL0331). A total of 273 samples were included (210 MRD $\leq 10^{-4}$, [negative], 63 MRD > 10^{-4} [positive]). The authors report that clonoSEQ MRD-negativity status was found to predict event-free survival (EFS) at all ages. MRD-positivity status was also associated with a 2.74-fold higher event risk compared to MRD-negativity status. Across all MRD values, a 10-fold increase in clonoSEQ MRD measurement was associated with a 50% increase in event rate and MRD-negative patients were found to have longer EFS compared to patients with higher frequencies of malignancies (FDA, 2018).

The FDA's 2020 substantially equivalent approval document for ClonoSEQ analyzed two separate studies to "support that MRD as estimated with the clonoSEQ Assay is prognostic of patient outcomes in CLL…" The first study was for CLL (NCT02242942) and included 337 patients to evaluate the ability of ClonoSEQ to predict PFS. Samples were collected three months or later following treatment (FUM3) and MRD positivity

was defined as >1 x 10^{-5} [malignant cells]. Patients found to be MRD-positive had an "event risk" 6.64 times higher than the MRD-negative cohort. A 10-fold increase in MRD was also associated with a 2.35-fold increase in event risk. The authors also analyzed the results for other confounding factors and found "that the MRD level at FUM3 is a stronger predictor of PFS than age, sex, geographic region, Binet stage, or treatment arm of the clinical trial. Together, these results demonstrate the clinical validity of MRD measurement in CLL." This study also found that "patients with clonoSEQ MRD ≤ 10^{-6} or between 10^{-6} and 10^{-5} had longer PFS, followed by patients with MRD between 10^{-5} and 10^{-4} and patients with MRD ≥ 10^{-4} (log-rank P = 4.902 x 10-31, Figure 11). These data demonstrate that patients with MRD ≤ 10^{-5} have better outcomes than patients with MRD > 10^{-5}, and that increasing MRD levels above 10^{-5} are associated with an increased risk of progression within the follow-up time of this study" (FDA, 2020).

The second clinical validation described in FDA's 2020 substantially equivalent approval document was also for CLL (NCT00759798). This study was a "phase 2 clinical trial that evaluated six cycles of fludarabine, cyclophosphamide, and rituximab (FCR) in 111 front-line chronic lymphocytic leukemia (CLL) patients with clonoSEQ ID samples and a corresponding 137 clonoSEQ MRD samples also evaluated by 4-color flow cytometry at an MRD threshold of 10-4 (NCT00759798) and with pertinent co-variate data. Within this cohort of 111 patients with flow MRD results, bone marrow was available for 75 patients and blood was available for 62 patients, of which 26 patients provided both blood and bone marrow. Due to some missing clinical covariates, three patients that provided bone marrow only, were excluded from analyses requiring these covariates. There was an association between PFS and continuous clonoSEQ MRD measurement in both blood and bone marrow, after end of treatment, where PFS is defined as the time from start of treatment until death, disease progression, or last time of disease assessment (p = 9.66 x 10^{-4} for blood, p = 2.13 x 10^{-4} for bone marrow). Additionally, patients who were MRD negative at a threshold ≤ 10^{-5} had superior progression-free survival compared to patients with MRD > 10^{-5} (p = .02 for blood and p = 8.17 x 10^{-5} for bone marrow… Taken together these results support the use of the clonoSEQ assay in CLL patients" (FDA, 2020).

Hay et al. (2019) evaluated the impact of MRD negativity status on relapse rates of ALL patients that post-chimeric antigen receptor [CAR] T-cell therapy. Per flow cytometry, 45 of 53 patients achieved an MRD-negative status. At a median follow-up of 30.9 months, the authors found that EFS and overall survival (OS) were significantly better in patients achieving MRD-negativity than patients that did not (median EFS: 7.6 months vs 0.8 months; median OS: 20 months vs five months). The authors also identified that the cytometric absence of the *IGH* index malignant clone was associated with better EFS (Hay et al., 2019).

Herrera et al. (2016) evaluated "whether the presence of ctDNA [circulating tumor DNA, measured with next-generation sequencing] was associated with outcome after allogeneic haematopoietic stem cell transplantation (HSCT) in lymphoma patients." A total of 88 patients were included from a "phase 3 clinical trial of reduced-intensity conditioning HSCT in lymphoma." Patients with detectable ctDNA three months after HSCT were found to have inferior progression-free survival compared to patients without detectable ctDNA (58% vs 84%, 2-year PFS rate). Detectable ctDNA was confered a 10.8-times higher risk of relapse/progression and a 3.9-times higher risk of progression/death compared to the non-detectable ctDNA group. The authors concluded that "detectable ctDNA is associated with an increased risk of relapse/progression, but further validation studies are necessary to confirm these findings and determine the clinical utility of NGS-based minimal residual disease monitoring in lymphoma patients after HSCT" (Herrera et al., 2016).

Perrot et al. (2018) examined the prognostic value of MRD (measured with NGS) in MM cases. A total of 127 patients achieved MRD negativity (defined as "the absence of tumor plasma cell within 1 000 000 bone marrow cells ($<10^{-6}$) at least once during maintenance therapy. At the start of therapy, MRD was found to be a strong prognostic factor for both progression-free survival as well as overall survival (hazard ratio = .22 and .24 respectively). From a previous cohort, the authors identified 233 patients labeled as MRD-negative, of which 120 were confirmed as MRD-negative with NGS (52%)" (Perrot et al., 2018).

Friend et al. (2020) investigated the impact of NGS-MRD in predicting relapse in ALL patients. Total body irradiation (TBI)-based regimens were the standard of care for ALL patients requiring allogeneic HSCT, but this procedure has numerous harmful side effects. The authors hypothesized that identifying MRD-negative patients may help some individuals avoid exposure to this radiation. The authors examined outcomes of 57 patients that received TBI and non-TBI regimens and found that relapse rates were similar for both methods of treatment. However, NGS-MRD positivity prior to treatment was "highly" predictive of relapse (for up to three years post-transplant). Based on their data, the authors suggested "that the decision to use either a TBI or non-TBI regimens in ALL should depend on NGS-MRD status, with conditioning regimens based on TBI reserved for patients that cannot achieve NGS-MRD negativity prior to allogeneic HSCT" (Friend et al., 2020).

Thörn et al. (2011) performed a comparative analysis of MFC and real-time quantitative polymerase chain reaction (RT-qPCR)-based MRD in pediatric ALL. The study, consisting of 726 follow-up samples from 228 children, used an MRD threshold of 0.1% and reported at day 29 a 84% concordance between the two different methods. For B-cell precursor ALL, the authors note that MFC was better at discriminating higher risk

of bone marrow relapse (BMR), whereas RT-qPCR performed better for T-ALL. Regardless, the authors state, "MRD levels of ≥0.1%, detected by either method at day 29, could not predict isolated extramedullary relapse." They conclude that "both methods are valuable clinical tools for identifying childhood ALL cases with increased risk of BMR" (Thörn et al., 2011).

Wood et al. (2018) compared high-throughput sequencing (HTS) of *IGH* and *TRG* genes to flow cytometry (FC) to evaluate measurable residual disease detection at the end of induction chemotherapy in pediatric patients with newly diagnosed B-ALL [B-cell acute lymphoblastic leukemia]. A total of 619 paired pre-treatment and end of induction bone marrow samples were included. At an MRD threshold of 0.01%, both HTS and FC showed similar event-free survival (EFS) and OS for both MRD-positive and MRD-negative patients. However, HTS identified 55 more patients as "MRD-positive" compared to FC. These "discrepant" patients were found to have worse outcomes than FC MRD-negative patients. HTS was also found to identify 19.9% of "standard risk" (SR) without MRD at any detectable level with excellent EFS and OS (98.1% and 100% respectively). The authors suggested that "the higher analytic sensitivity and lower false-negative rate of HTS improves upon FC for MRD detection in pediatric B-ALL by identifying a novel subset of patients at end of induction who are essentially cured using current chemotherapy and identifying MRD at 0.01% in up to one-third of patients who are missed at the same threshold by FC" (Wood et al., 2018).

Rawstron et al. (2016) conducted a parallel analysis of MRD using both ClonoSEQ and multiparameter flow cytometry in CLL as part of the European Research Initiative on CLL (ERIC) study. The MFC approach used within the ERIC study is validated to the level of 10^{-5} and consists of six different markers—CD5, CD19, CD20, CD43, CD49b, and CD81. The ERIC study reports that the ClonoSEQ method "provides good linearity to a detection limit of one in a million (10^{-6})." The authors also note, "a parallel analysis of high-throughput sequencing using the ClonoSEQ assay showed good concordance with flow cytometry results at the 0.010% (10^{-4}) level, the MRD threshold defined in the 2008 International Workshop on CLL guidelines… The combination of both technologies would permit a highly sensitive approach to MRD detection while providing a reproducible and broadly accessible method to quantify residual disease and optimize treatment in CLL" (Rawstron et al., 2016).

Thompson et al. (2019) evaluated 62 patients with CLL that were considered negative for MRD by flow cytometry (sensitivity of 10^{-4}). Using ClonoSEQ, the authors found that 72.6% of these MRD-negative patients were MRD-positive by ClonoSEQ (a discordant result). Only 27.4% of patients were found to be negative by both methods. The authors also found that patients that were negative by both methods were found to have superior PFS compared to patients that were only negative by flow cytometry,

thereby suggesting that ClonoSEQ was a superior prognostic discriminator (Thompson et al., 2019).

Z. Wang et al. (2019) published a study on the applicability of multiparameter (multicolor) flow cytometry (MFC) for detecting MRD to predict relapse in patients with AML after allogeneic transplantation. The researchers determined MFC and MRD status using real-time quantitative polymerase chain reaction (RT-qPCR) from 158 bone marrow samples from 44 different individuals and compared the statuses between the two. They noted that "Strong concordance was found between MFC-based and RT-qPCR-based MRD status (κ = 0.868)." Moreover, for individuals in complete remission (CR), "the positive MRD status detected using MFC was correlated with a worse prognosis [HRs (*P* values) for relapse, event-free survival, and overall survival: 4.83 (<0.001), 2.23 (0.003), and 1.79 (0.049), respectively]; the prognosis was similar to patients with an active disease before HSCT [hematopoietic stem cell transplantation]" (Z. Wang et al., 2019).

Carlson et al. (2019) published a cost-effectiveness study of NGS-based MRD testing during maintenance treatment for MM. The authors compared use of MRD testing to no MRD testing. A Markov model with six health states was developed; "MRD positive or MRD negative, on or off treatment, relapsed, or dead." From there, the authors compared yearly NGS-MRD to no MRD testing over a lifetime horizon. Overall, the authors found that "MRD testing saved $1,156,600 over patients remaining lifetime." Health outcomes were found to slightly favor MRD testing (0.01 quality-adjusted life years [QALYs]) compared to no testing. The authors concluded that "NGS MRD testing is cost saving, with potential QALY gains due to avoidance of [treatment-related adverse events] compared with no testing for MM patients on maintenance therapy" (Carlson et al., 2019).

Medina et al. (2020) evaluated MRD three months after transplantation in 106 myeloma patients, noting that "detecting persistent minimal residual disease (MRD) allows the identification of patients with an increased risk of relapse and death." In this study, they compared the results of NGS with NGF, where they noted that "correlation between NGS and NGF was high (R2 = 0.905). The three-year PFS rates by NGS and NGF were longer for undetectable vs. positive patients (NGS: 88.7% vs. 56.6%; NGF: 91.4% vs. 50%; p < 0.001 for both comparisons), which resulted in a three-year overall survival (OS) advantage (NGS: 96.2% vs. 77.3%; NGF: 96.6% vs. 74.9%, p < 0.01 for both comparisons). In the Cox regression model, NGS and NGF negativity had similar results but favoring the latter in PFS (HR: 0.20, 95% CI: 0.09-0.45, p < 0.001) and OS (HR: 0.21, 95% CI: 0.06-0.75, p = 0.02). All these results reinforce the role of MRD detection by different strategies in patient prognosis and highlight the use of MRD as an endpoint for multiple myeloma treatment" (Medina et al., 2020).

Goicoechea et al. (2021) examined MRD as a possible endpoint marker in MM. They note that while patients with MM that carry standard- or high-risk cytogenetic abnormalities (CA) are achieving similar CR rates, high-risk patients have an inferior PFS. They note that this "questions the legitimacy of CR as a treatment endpoint..." Using NGF cytometry to evaluate MRD in MM patients, they compared standard- vs high-risk CAs (n=300 and 90, respectively) and identified mechanisms that determine MRD resistance in both patient subgroups (n=40). In patients achieving undetectable MRD with either standard- or high-risk CAs, the 36-month PFS rates were higher than 90%. In comparison, patients with persistent MRD had a median PFS of about three (standard-risk CA) and two (high-risk CA) years. They found that "further use of NGF to isolate MRD, followed by whole-exome sequencing of paired diagnostic and MRD tumor cells, revealed greater clonal selection in patients with standard-risk CAs, higher genomic instability with acquisition of new mutations in high-risk MM, and no unifying genetic event driving MRD resistance." Ultimately, their results support "undetectable MRD as a treatment endpoint for patients with MM who have high-risk CAs and proposes characterizing MRD clones to understand and overcome MRD resistance" (Goicoechea et al., 2021).

Subhash et al. (2022) investigated the "feasibility of using whole-genome sequencing (WGS) data to design tumour-specific polymerase chain reaction (PCR)-based MRD tests (WGS-MRD)" for children with cancers with high-risk of relapse, like ALL, high-risk neuroblastoma (HR-NB), and Ewing sarcoma (EWS). The significance of this study was that pediatric solid tumor MRD DNA-based assays currently remains experimental, and remains focused on mRNA, methylated DNA, or microRNA. Through their experimentation, the researchers found that "sensitive WGS-MRD assays were generated for each patient and allowed quantification of 1 tumour cell per 10^{-4} (0.01%)-10^{-5} (0.001%) mononuclear cells." They also found that WGS-MRD and Ig/TCR-MRD assays were concordant in ALL, and the WGS-MRD assays "showed good concordance between quantitative PCR and droplet digital PCR formats." The WGS-MRD assay clinical samples also correlated with disease course and was found to be more sensitive than RNA-based MRD assays. This demonstrated how WGS could aid the development of MRD assays for pediatric cancers as it has been done in adults (Subhash et al., 2022).

Kater et al. (2019) utilized the MURANO study to investigate the effects of a fixed duration treatment with venetoclax and ritixumab on MRD. The study itself demonstrated "significant progression-free survival (PFS) benefit for fixed-duration venetoclax-rituximab compared with bendamustine-rituximab in relapsed/refractory chronic lymphocytic leukemia." However, among patients who received venetoclax-rituximab, there was a "higher rate of PB [peripheral blood] undetectable MRD (uMRD; less than 10^{-4}) at EOCT [end of combination therapy] (62% v 13%) with superiority

sustained through month 24 (end of therapy)," which predicted longer PFS. At the end of the therapy treatment period, 70% of patients remained in uMRD and 98% without disease progression. This demonstrated the correlation between uMRD and PFS using PB MRD in the setting of venetoclax-combination treatment (Kater et al., 2019).

Kim et al. (2022) investigated the clinical utility of NGF-based MRD assessment in a heterogeneous population of patients with MM at the Samsung Medical Center in Korea and found that "sCR samples showed a lower MRD-positive rate (25%) than CR (43%) and VGPR (53%) samples, although the difference was not significant (P=0.051)." When evaluating survival analysis based on clinical response and MRD, "PFS in VGPR patients was lower than that in sCR/CR patients (P<0.001) …whereas PFS in VGPR patients was lower than that in sCR/CR patients (P<0.001)." Cytogenetic risk survival analysis yielded similar results: "There was no significant difference in PFS between patients with high-risk and standard-risk cytogenetics (P=0.222)" and "Further analysis according to MRD status also revealed no significant difference in PFS in patients with standard-risk cytogenetics (P=0.246)," though it is worth noting that "among patients with high-risk cytogenetics, MRD-positive patients showed lower PFS than MRD-negative patients (P=0.016)." Overall, the authors concluded that "Sustained MRD negativity was only observed in patients with sustained sCR, and their PFS was superior to that of patients who were not MRD-negative (P=0.035)," though by their own admission the study was limited by the sample size (n=12 over 18 months) (Kim et al., 2022).

There is a growing pool of literature investigating the use of ctDNA-based MRD detection in the clinical context of solid tumors. Some studies suggest that inclusion of MRD assessment may offer some improvement over standard of care; this has been reported for gastrointestinal malignancies (Zhang et al., 2021), skin cancer (Eroglu et al., 2023; Khaddour et al., 2022), lung cancer (Zhong et al., 2023), and especially colorectal cancer, where several reports demonstrate potential benefits of ctDNA measurement, which include response monitoring, prognosis, post-surgical surveillance, chemotherapeutic management, and informing recurrence risk (Hofste et al., 2023; Sato et al., 2023; Tie et al., 2019; Tie et al., 2016; Y. Wang et al., 2019).

Signatera™ has been clinically validated across several cancer types for recurrence monitoring. In the context of colorectal cancer, Loupakis et al. (2021) demonstrated that a two-timepoint analysis of ctDNA (a baseline measure plus last follow-up time point–either the time of radiologic progression or last evidence of radiologic disease's absence) improved the sensitivity of the assay. For patients who were ctDNA positive at both timepoints, the authors reported that the assay demonstrated a sensitivity of 91.4%, specificity of 93.3%, and positive predictive value (PPV) of 96.7%. Reinert et al. (2019) also investigated the validity of ctDNA for MRD detection for individuals with

stages I-III colorectal cancer and reported promising metrics; the test demonstrated a sensitivity of 88%, specificity of 98%, PPV of 93.3%, and negative predictive value (NPV) of 96.7%. However, Fakih et al. (2022) recently determined that Signatera™ may not "provide advantages as a surveillance strategy compared with standard imaging combined with CEA levels when performed per National Comprehensive Cancer Network guidelines," after a cohort study failed to demonstrate a sufficient ability (sensitivity) of the test to detect disease recurrence in individuals with resected colorectal cancer.

Coombes et al. (2019) described the ability of ctDNA to detect disease recurrence ahead of clinical or radiological approaches for individuals with breast cancer with a sensitivity of 89%, specificity of 100%, PPV of 100%, and NPV of 94%. Christensen et al. (2019) found that ctDNA accurately identified individuals diagnosed with advanced bladder cancer who eventually relapsed (sensitivity of 100% and specificity of 98%). For individuals with non-small cell lung cancer, Abbosh et al. (2017) reported that Signatera™ detected relapse with 93% sensitivity and 90% specificity.

While evidence supporting MRD analysis for solid tumors continues to emerge, a consensus on the clinical validity and utility of this approach has not yet been reached. Sullivan et al. (2023) cite several limitations of ctDNA-based approaches to the management of gastrointestinal cancer; O'Sullivan et al. (2023) argue that the utility of this technology in the context of non-small cell lung cancer is still investigational; Moding et al. (2021) cite a lack of prospective clinical trials confirming clinical utility of ctDNA MRD assessment in general; and, Jacome and Johnson (2023) argue that there is not yet consensus on how to apply the results of ctDNA-based testing in the management of colorectal cancer.

Faulkner et al. (2023) conducted a systematic review and meta-analysis to study the utility of ctDNA for detecting MRD in colorectal cancer. The review included 37 studies involving 3002 patients. The authors calculated hazard ratios and PFS based on ctDNA detection. Overall, there was a "poorer" PFS associated with ctDNA detection at the first liquid biopsy post-surgery, with a hazard ratio of 6.92. Further, "this effect was also seen in subgroup analysis by disease extent, adjuvant chemotherapy and assay type." The authors conclude that "ctDNA detection post-surgery is associated with a greater propensity to disease relapse and is an independent indicator of poor prognosis," but note that "prior to incorporation into clinical practice, consensus around timing of measurements and assay methodology are critical" (Faulkner et al., 2023).

Berger et al. (2022) evaluated the use of circulating tumor tissue modified viral (TTMV)-HPV DNA as a biomarker for detecting recurrence in patients with HPV-driven oropharyngeal squamous cell carcinoma (OPSCC). The authors conducted a

retrospective analysis of 1,076 patients from 108 U.S sites who were at least three months post-treatment and had undergone TTMV-HPV DNA testing (NavDx) during routine surveillance between February 2020 and June 2021. The results determined that 80 out of 1,076 (7.4%) tested positive for TTMV-HPV DNA. Among these, 21 patients had known recurrences at the time of the first positive test, while 59 did not, and 93% of the latter group subsequently developed confirmed recurrences. The overall positive predictive value for the TTMV-HPV DNA test was 95%, and the negative predictive value was also 95%. These findings suggest that TTMV-HPV DNA testing can serve as an effective surveillance tool, often identifying recurrences earlier than traditional clinical evaluations and imaging techniques, and may influence future surveillance strategies for HPV-related cancers (Berger et al., 2022).

VI. Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

The NCCN has published several relevant guidelines on management of MRD in hematologic malignancies.

Multiple Myeloma (MM)

For MM, MRD is considered an "important" prognostic factor. The NCCN recommends measuring MRD during follow-up/surveillance for symptomatic MM patients after response to primary therapy "as indicated for prognostication." Next-generation flow and next-generation sequencing (or both) are recommended for methodology and a sensitivity of 1 in 10^5 (or better) is recommended for accuracy. The NCCN recommends to "consider baseline clone identification and storage of aspirate sample for future minimal residual disease (MRD) testing by NGS." MRD is a required criterion listed within the IMWG MM response criteria. The NCCN notes that "for MRD there is no need for two consecutive assessments, but information on MRD after each treatment state is recommended (eg, after induction, high-dose therapy/Autologous stem cell transplant (ASCT), consolidation, maintenance). MRD tests should be initiated only at the time of suspected complete response." Sustained MRD-negative status is only confirmed when taken a minimum of one year apart, but "subsequent evaluations can be used to further specify the duration of negativity (eg, MRD-negative at 5 years)." The NCCN also notes that MRD is being used in post-hematopoietic stem cell transplantation treatment assessments (NCCN, 2024e).

Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma

The NCCN remarks that "undetectable MRD in the peripheral blood at the end of fixed duration treatment is an important predictor of efficacy." The NCCN recommends

performing MRD assessment with an assay at a sensitivity of 10^{-4} "according to the standardized European Research Initiative on CLL (ERIC) method or standardized NGS method." NCCN also stated that "allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) and six-color flow cytometry (MRD flow) are the two validated methods used for the detection of MRD at the level of 10^{-4} to 10^{-5}. NGS-based assays have been shown to be more sensitive, thus allowing for the detection of MRD at the level of 10^{-6}" (NCCN, 2024c).

Acute Myeloid Leukemia (AML)

The NCCN recommends measuring MRD "upon completion of initial induction" and "before allogenic HCT." The NCCN also states, "Additional time points should be guided by the regimen used." NGS-based assays to detect mutated genes are not routinely used in AML, as the sensitivity of PCR-based assays and flow cytometry is superior to what is achieved by NGS. The NCCN states that "if using flow cytometry to assess MRD, it is recommended that a specific MRD assay is utilized, but, most importantly, that it is interpreted by an experienced hematopathologist" since there are differences between "diagnostic threshold assessments and MRD assessments." They also note that "some evidence suggest MRD testing may be more prognostic than *KIT* mutation status in CBF-AML, but this determination depends on the method used to assess MRD and the trend of detectable MRD." The NCCN further states that "Based on the techniques, the optimal sample for MRD assessment is either peripheral blood (NPM1 PCR-based techniques) or an early, dedicated pull of the BM aspirate (i.e., other PCR, flow cytometry, NGS). The quality of the sample is of paramount importance to have reliable evaluation" (NCCN, 2024b).

Acute Lymphoblastic Leukemia (ALL)/Pediatric Acute Lymphoblastic Leukemia

The NCCN states that MRD quantification is an "essential component of patient evaluation over the course of sequential ALL therapy," noting the prognostic significance of MRD. Techniques used to assess for MRD include: flow cytometry assays, real-time quantitative PCR assays, reverse transcriptase quantitative PCR assays, and NGS-based assays. NGS is recognized as one of the most sensitive methods at detection levels of 10^{-6}, as can some PCRs. An entire section within the ALL guidelines is devoted to MRD assessment. They note the timing of MRD assessment to be as follows:

- Upon completion of initial induction.
- End of consolidation.
- Additional time points should be guided by the regimen used and risk features.
- Serial monitoring frequency may be increased in patients with molecular relapse or persistent low-level disease burden.

- For some techniques, a baseline sample (i.e., prior to treatment) is needed to characterize the leukemic clone for subsequent MRD assessment.

The guidelines also recommend "For patients with negative MRD by flow cytometry but positive MRD by an FDA-approved NGS assay, consider repeat testing before consolidation is started to confirm MRD status" (NCCN, 2024a). Overall, MRD has a strong correlation with risks for relapse and is considered to have a high prognostic value. MRD has a role in identifying optimal treatments for patients, both adult and pediatric, with ALL (NCCN, 2024a, 2024f).

Hairy Cell Leukemia

The NCCN writes that, for a complete response (CR) with or without MRD, "if CR is achieved, an IHC [immunohistochemistry] assessment of the percentage of MRD will be useful to stratify patients based on level of CR (with or without evidence of MRD)". The NCCN notes that "it has been suggested that MRD monitoring as a component of response assessment should be incorporated in all clinical trials for relapsed HCL" but clarifies that "MRD assessment is not recommended (outside of clinical trials) as part of response evaluation" (NCCN, 2024d).

International Myeloma Working Group (IMWG)

The IMWG recommends assessing MRD response at a sensitivity of $1/10^5$ nucleated cells or better (Kumar et al., 2016).

European Myeloma Network (EMN)

Regarding NGS in assessment of MRD in MM, the EMN writes that "Results from next-generation sequencing are highly concordant with flow-based MRD detection, highly reproducible and reach a sensitivity of 10^{-6}" and that the primary restraints for NGS are "a lack of standardization and limited commercial availability" (Caers et al., 2018).

American Society of Clinical Oncology (ASCO) and Cancer Care Ontario (CCO)

These joint guidelines focus on treatment of MM. Their MRD-related recommendations are listed below:

- "There is insufficient evidence to make modifications to maintenance therapy based on depth of response, including MRD status"
- "MRD-negative status has been associated with improved outcomes, but it should not be used to guide treatment goals outside the context of a clinical trial"

- "There is insufficient evidence to support change in type and length of therapy based on depth of response as measured by conventional IMWG approaches or MRD"
- "There are not enough data to recommend risk-based versus response-based duration of treatment (such as MRD)" (Mikhael et al., 2019).

The ASCO also fully endorsed the "Initial Diagnostic Work-Up of Acute Leukemia" released jointly by the College of American Pathologists and the American Society of Hematology in 2018 (de Haas et al., 2018).

European LeukemiaNet (ELN) MRD Working Party

The ELN states, "Measurable residual disease (MRD; previously termed minimal residual disease) is an independent, postdiagnosis, prognostic indicator in acute myeloid leukemia (AML) that is important for risk stratification and treatment planning, in conjunction with other well-established clinical, cytogenetic, and molecular data assessed at diagnosis." The ELN remarks that quantitative PCR is applicable to approximately 40% of AML patients with "1 or more suitable abnormalities." However, NGS for MRD assessment may provide assessment to an additional 40%-50% of AML patients, as NGS can "theoretically, be applied to all leukemia-specific genetic aberrations." The ELN recommends a sensitivity of at least 1 / 103 cells, and states that NGS platforms will be used after careful validation (Schuurhuis et al., 2018).

International Workshop on Chronic Lymphocytic Leukemia (iwCLL)

The iwCLL published guidelines on CLL in 2018. In it, they consider MRD assessment to be a necessary component in identifying complete remission of CLL. The iwCLL also writes that eradication of leukemia is a "desired end point." They go on to state: "Use of sensitive multicolor flow cytometry, PCR, or next-generation sequencing can detect MRD in many patients who achieved a complete clinical response… Six-color flow cytometry (MRD flow), allele-specific oligonucleotide PCR, or high-throughput sequencing using the ClonoSEQ assay are reliably sensitive down to a level of <1 CLL cell in 10 000 leukocytes" (Hallek et al., 2018).

College of American Pathologists (CAP) and the American Society of Hematology (ASH)

This CAP/ASH joint guideline was published in 2017 and focuses on Initial Diagnostic Workup of Acute Leukemia (AL). The guideline strongly recommends that "For patients with suspected or confirmed AL, the pathologist or treating clinician should ensure that flow cytometry analysis or molecular characterization is comprehensive enough to

allow subsequent detection of MRD." The guideline also notes that MRD is a "powerful" predictor of adverse outcome in patients with AL (Arber et al., 2017).

This guideline was endorsed by the American Society of Clinical Oncology in 2018 (Arber et al., 2017).

European Society for Medical Oncology (ESMO)

Chronic Lymphocytic Leukaemia

The ESMO notes that "detection of MRD by multicolour flow cytometry or RT-PCR has a strong prognostic impact following CIT73,74 as well as venetoclax plus CD20-antibody combinations. A total of 75 patients with undetectable MRD after therapy show a longer response duration and survival. Additional clinical consequences of MRD positivity after therapy with respect to treatment escalation remain unclear… Therefore, MRD assessment is not generally recommended for monitoring after therapy outside clinical studies. This may change soon, as increasing efforts are made to determine whether therapy with targeted agents could be discontinued on the basis of MRD status" (B. Eichhorst et al., 2021). The guidelines are also endorsed by the European Hematology Association (Barbara Eichhorst et al., 2021).

Acute Lymphoblastic Leukaemia

The ESMO writes that "Quantification of MRD is a major and well-established risk factor and should be obtained whenever possible for all patients also outside of clinical trials…If MRD is measured by flow cytometry, a good MRD response is often defined as less than 10^{-3} , although MRD levels less than 10^{-4} can be achieved with the 8–12 colour flow cytometers" (Hoelzer et al., 2016).

Multiple Myeloma

The ESMO states, "One of the most significant improvements in the response criteria is the introduction of minimal residual disease (MRD) both in the bone marrow (BM) [using either next-generation sequencing or next-generation flow cytometry (NGF)] and outside the BM [using positron emission tomography-computed tomography (PET-CT); imaging MRD]. MRD negativity in the BM in patients who have achieved conventional complete response (CR) consistently correlates with prolonged progression-free survival (PFS) and overall survival (OS) in both newly diagnosed MM (NDMM) and relapsed/refractory MM (RRMM) patients." ESMO also notes that "MRD has been found to be a surrogate endpoint for PFS in patients receiving first-line treatment. Therefore, MRD may be used as an endpoint to accelerate drug development. The use of MRD to drive treatment decisions is under investigation" (Dimopoulos et al., 2021).

Acute Myeloid Leukaemia

The ESMO includes MRD status as part of the treatment algorithm for AML. They state, "Morphological enumeration of the blast percentage should be refined by immunophenotypic or molecular MRD assessment in patients with <10% blasts. ELN recommendations on MRD assessment in AML specify its clinical use and technical requirements. It is recommended to assess MRD by reverse transcriptase polymerase chain reaction (RT-PCR) for patients positive for *NPM1*^{mut}*, RUNX1-RUNX1T1, CBFB-MYH11* or *PML-RARA* fusion genes; ~40% of all AML patients. In the remaining patients, MRD should be assessed by MFC, which relies on antigens aberrantly expressed by leukaemic cells that can be found in >90% of AML patients. Many clinical studies have shown the strong prognostic impact of MRD, as measured by MFC, with levels 0.1% defined as positive" (Heuser et al., 2020).

Hairy Cell Leukaemia

Concerning hairy cell leukemia, ESMO notes, "Recently, monoclonal antibodies that detect the mutated BRAF protein have been developed and shown to be useful for the diagnosis and detection of minimal residual disease (MRD)." Within the section on response evaluation, ESMO states, "Immunophenotypic analysis of peripheral blood or bone marrow is not required but is useful to detect MRD… The eradication of MRD is generally not recommended in routine clinical practice. Assessment of response should be performed 4 – 6 months after treatment with 2-CldA and after 8 – 9 courses of DCF. Relapse is defined as any deterioration in blood counts related to the detection of hairy cells in peripheral blood and/or bone marrow" (Robak et al., 2015).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

On August 5, 2020, the FDA approved ClonoSEQ, marketed by Adaptive Biotechnologies, as a substantially equivalent DNA-based test for minimal residual disease. It is "an in vitro diagnostic device that identifies and quantifies specific nucleic

acid sequences isolated from human specimens to estimate the percentage of cells that harbor the specific sequence pathology test." In its Decision Summary, the FDA states that the "The clonoSEQ Assay is an in vitro diagnostic that uses multiplex polymerase chain reaction (PCR) and next-generation sequencing (NGS) to identify and quantify rearranged IgH (VDJ), IgH (DJ), IgK and IgL receptor gene sequences, as well as translocated BCL1/IgH (J) and BCL2/IgH (J) sequences in DNA extracted from bone marrow from patients with B-cell acute lymphoblastic leukemia (ALL) or multiple myeloma (MM), and blood or bone marrow from patients with chronic lymphocytic leukemia (CLL). The clonoSEQ Assay measures MRD to monitor changes in burden of disease during and after treatment. The test is indicated for use by qualified healthcare professionals in accordance with professional guidelines for clinical decision-making and in conjunction with other clinicopathological-p features" (FDA, 2020).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81479	Unlisted molecular pathology procedure
88184	Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; first marker
88185	Flow cytometry, cell surface, cytoplasmic, or nuclear marker, technical component only; each additional marker (List separately in addition to code for first marker)
0171U	Targeted genomic sequence analysis panel, acute myeloid leukemia, myelodysplastic syndrome, and myeloproliferative neoplasms, DNA analysis, 23 genes, interrogation for sequence variants, rearrangements, and minimal residual disease, reported as presence/absence Proprietary test: MyMRD® NGS Panel Lab/Manufacturer: Laboratory for Personalized Molecular Medicine
0306U	Oncology (minimal residual disease [MRD]), next-generation targeted sequencing analysis, cell-free DNA, initial (baseline) assessment to determine a patient-specific panel for future comparisons to evaluate for MRD Proprietary test: Invitae PCM Tissue Profiling and MRD Baseline Assay Lab/Manufacturer: Invitae Corporation

0307U	Oncology (minimal residual disease [MRD]), next-generation targeted sequencing analysis of a patient-specific panel, cell-free DNA, subsequent assessment with comparison to previously analyzed patient specimens to evaluate for MRD Proprietary test: Invitae PCM MRD Monitoring Lab/Manufacturer: Invitae Corporation
0340U	Oncology (pan-cancer), analysis of minimal residual disease (MRD) from plasma, with assays personalized to each patient based on prior next-generation sequencing of the patient's tumor and germline DNA, reported as absence or presence of MRD, with disease-burden correlation, if appropriate Protietary test: Signatera™ Lab/Manufacturer: Natera, Inc
0356U	Oncology (oropharyngeal or anal), evaluation of 17 DNA biomarkers using droplet digital PCR (ddPCR), cell-free DNA, algorithm reported as a prognostic risk score for cancer recurrence Proprietary test: NavDx® Lab/Manufacturer: Naveris, Inc
0364U	Oncology (hematolymphoid neoplasm), genomic sequence analysis using multiplex (PCR) and next-generation sequencing with algorithm, quantification of dominant clonal sequence(s), reported as presence or absence of minimal residual disease (MRD) with quantitation of disease burden, when appropriate Proprietary test: clonoSEQ® Assay Lab/Manufacturer: Adaptive Biotechnologies
0422U	Oncology (pan-solid tumor), analysis of DNA biomarker response to anti-cancer therapy using cell-free circulating DNA, biomarker comparison to a previous baseline pre-treatment cell-free circulating DNA analysis using next-generation sequencing, algorithm reported as a quantitative change from baseline, including specific alterations, if appropriate. Proprietary test: Guardant360 Response™ Lab/Manufacturer: Guardant Health, Inc,
0467U	Oncology (bladder), DNA, nextgeneration sequencing (NGS) of 60 genes and whole genome aneuploidy, urine, algorithms reported as minimal residual disease (MRD) status positive or negative and quantitative disease burden Proprietary test: UroAmp MRD Lab/Manufacturer: Convergent Genomics, Inc

0470U	Oncology (oropharyngeal), detection of minimal residual disease by next-generation sequencing (NGS) based quantitative evaluation of 8 DNA targets, cell-free HPV 16 and 18 DNA from plasma Proprietary test: HPV-SEQ Test Lab/Manufacturer: Sysmex Inostics, Inc

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Abbosh, C., Birkbak, N. J., Wilson, G. A., Jamal-Hanjani, M., Constantin, T., Salari, R., Le Quesne, J., Moore, D. A., Veeriah, S., Rosenthal, R., Marafioti, T., Kirkizlar, E., Watkins, T. B. K., McGranahan, N., Ward, S., Martinson, L., Riley, J., Fraioli, F., Al Bakir, M., . . . Swanton, C. (2017). Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. *Nature*, *545*(7655), 446-451. https://doi.org/10.1038/nature22364

Adaptive Biotechnologies. (2024). *ClonoSEQ MRD Monitoring*. Retrieved 07/10/2020 from https://www.clonoseq.com/

Al-Sawaf, O., Zhang, C., Robrecht, S., Wilson, C., Tandon, M., Ching, T., Fink, A.-M., Ritgen, M., Tausch, E., Kreuzer, K.-A., Schary, W., Wendtner, C.-M., Eichhorst, B., Stilgenbauer, S., Jiang, Y., Hallek, M., & Fischer, K. (2020). Clonal Dynamics after Venetoclax-Obinutuzumab Therapy: Novel Insights from the Randomized, Phase 3 CLL14 Trial. *Blood*, *136*(Supplement 1), 22-23. https://doi.org/10.1182/blood-2020-136977

Al-Sawaf, O., Zhang, C., Tandon, M., Sinha, A., Fink, A. M., Robrecht, S., Samoylova, O., Liberati, A. M., Pinilla-Ibarz, J., Opat, S., Sivcheva, L., Le Dû, K., Fogliatto, L. M., Niemann, C. U., Weinkove, R., Robinson, S., Kipps, T. J., Tausch, E., Schary, W., . . . Fischer, K. (2020). Venetoclax plus obinutuzumab versus chlorambucil plus obinutuzumab for previously untreated chronic lymphocytic leukaemia (CLL14): follow-up results from a multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol*, *21*(9), 1188-1200. https://doi.org/10.1016/s1470-2045(20)30443-5

Arber, D. A., Borowitz, M. J., Cessna, M., Etzell, J., Foucar, K., Hasserjian, R. P., Rizzo, J. D., Theil, K., Wang, S. A., Smith, A. T., Rumble, R. B., Thomas, N. E., & Vardiman, J. W. (2017). Initial Diagnostic Workup of Acute Leukemia: Guideline From the College of American Pathologists and the American Society of Hematology. *Arch Pathol Lab Med*, *141*(10), 1342-1393. https://doi.org/10.5858/arpa.2016-0504-CP

Bai, Y., Orfao, A., & Chim, C. S. (2018). Molecular detection of minimal residual disease in multiple myeloma. *Br J Haematol*, *181*(1), 11-26. https://doi.org/10.1111/bjh.15075

Berger, B. M., Hanna, G. J., Posner, M. R., Genden, E. M., Lautersztain, J., Naber, S. P., Del Vecchio Fitz, C., & Kuperwasser, C. (2022). Detection of Occult Recurrence Using Circulating Tumor Tissue Modified Viral HPV DNA among Patients Treated for HPV-

Driven Oropharyngeal Carcinoma. *Clinical Cancer Research*, *28*(19), 4292-4301. https://doi.org/10.1158/1078-0432.ccr-22-0562

Brüggemann, M., Raff, T., Flohr, T., Gökbuget, N., Nakao, M., Droese, J., Lüschen, S., Pott, C., Ritgen, M., Scheuring, U., Horst, H. A., Thiel, E., Hoelzer, D., Bartram, C. R., & Kneba, M. (2006). Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukemia. *Blood*, *107*(3), 1116-1123. https://doi.org/10.1182/blood-2005-07-2708

Caers, J., Garderet, L., Kortum, K. M., O'Dwyer, M. E., van de Donk, N., Binder, M., Dold, S. M., Gay, F., Corre, J., Beguin, Y., Ludwig, H., Larocca, A., Driessen, C., Dimopoulos, M. A., Boccadoro, M., Gramatzki, M., Zweegman, S., Einsele, H., Cavo, M., . . . Engelhardt, M. (2018). European Myeloma Network recommendations on tools for the diagnosis and monitoring of multiple myeloma: what to use and when. *Haematologica*, *103*(11), 1772-1784. https://doi.org/10.3324/haematol.2018.189159

Carlson, J. J., Eckert, B., & Zimmerman, M. (2019). Cost-effectiveness of next-generation sequencing minimal residual disease testing during maintenance treatment for multiple myeloma. *Journal of Clinical Oncology*, *37*(15_suppl), e19529-e19529. https://doi.org/10.1200/JCO.2019.37.15_suppl.e19529

Christensen, E., Birkenkamp-Demtroder, K., Sethi, H., Shchegrova, S., Salari, R., Nordentoft, I., Wu, H. T., Knudsen, M., Lamy, P., Lindskrog, S. V., Taber, A., Balcioglu, M., Vang, S., Assaf, Z., Sharma, S., Tin, A. S., Srinivasan, R., Hafez, D., Reinert, T., . . . Dyrskjot, L. (2019). Early Detection of Metastatic Relapse and Monitoring of Therapeutic Efficacy by Ultra-Deep Sequencing of Plasma Cell-Free DNA in Patients With Urothelial Bladder Carcinoma. *J Clin Oncol*, *37*(18), 1547-1557. https://doi.org/10.1200/JCO.18.02052

Coombes, R. C., Page, K., Salari, R., Hastings, R. K., Armstrong, A., Ahmed, S., Ali, S., Cleator, S., Kenny, L., Stebbing, J., Rutherford, M., Sethi, H., Boydell, A., Swenerton, R., Fernandez-Garcia, D., Gleason, K. L. T., Goddard, K., Guttery, D. S., Assaf, Z. J., . . . Shaw, J. A. (2019). Personalized Detection of Circulating Tumor DNA Antedates Breast Cancer Metastatic Recurrence. *Clin Cancer Res*, *25*(14), 4255-4263. https://doi.org/10.1158/1078-0432.CCR-18-3663

de Haas, V., Ismaila, N., Advani, A., Arber, D. A., Dabney, R. S., Patel-Donnelly, D., Kitlas, E., Pieters, R., Pui, C.-H., Sweet, K., & Zhang, L. (2018). Initial Diagnostic Work-Up of Acute Leukemia: ASCO Clinical Practice Guideline Endorsement of the College of American Pathologists and American Society of Hematology Guideline. *Journal of Clinical Oncology*, *37*(3), 239-253. https://doi.org/10.1200/JCO.18.01468

Del Giudice, I., Raponi, S., Della Starza, I., De Propris, M. S., Cavalli, M., De Novi, L. A., Cappelli, L. V., Ilari, C., Cafforio, L., Guarini, A., & Foà, R. (2019). Minimal Residual Disease in Chronic Lymphocytic Leukemia: A New Goal? *Front Oncol*, *9*, 689. https://doi.org/10.3389/fonc.2019.00689

Dimopoulos, M. A., Moreau, P., Terpos, E., Mateos, M. V., Zweegman, S., Cook, G., Delforge, M., Hajek, R., Schjesvold, F., Cavo, M., Goldschmidt, H., Facon, T., Einsele, H., Boccadoro, M., San-Miguel, J., Sonneveld, P., Mey, U., guidelines@ehaweb.org, E. H. A. G. C. E. a., & clinicalguidelines@esmo.org, E. G. C. E. a. (2021). Multiple myeloma: EHA-ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up(dagger). *Ann Oncol*, *32*(3), 309-322. https://doi.org/10.1016/j.annonc.2020.11.014

Eichhorst, B., Ghia, P., & on behalf of the, E. H. A. G. C. (2021). EHA Endorsement of ESMO Clinical Practice Guidelines for Diagnosis, Treatment, and Follow-up of Chronic Lymphocytic Leukemia. *HemaSphere*, *5*(1). https://doi.org/10.1097/HS9.0000000000000520

Eichhorst, B., Robak, T., Montserrat, E., Ghia, P., Niemann, C. U., Kater, A. P., Gregor, M., Cymbalista, F., Buske, C., Hillmen, P., Hallek, M., Mey, U., & clinicalguidelines@esmo.org, E. G. C. E. a. (2021). Chronic lymphocytic leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, *32*(1), 23-33. https://doi.org/10.1016/j.annonc.2020.09.019

Eroglu, Z., Krinshpun, S., Kalashnikova, E., Sudhaman, S., Ozturk Topcu, T., Nichols, M., Martin, J., Bui, K. M., Palsuledesai, C. C., Malhotra, M., Olshan, P., Markowitz, J., Khushalani, N. I., Tarhini, A. A., Messina, J. L., & Aleshin, A. (2023). Circulating tumor DNA-based molecular residual disease detection for treatment monitoring in advanced melanoma patients. *Cancer*, *129*(11), 1723-1734. https://doi.org/10.1002/cncr.34716

Fakih, M., Sandhu, J., Wang, C., Kim, J., Chen, Y. J., Lai, L., Melstrom, K., & Kaiser, A. (2022). Evaluation of Comparative Surveillance Strategies of Circulating Tumor DNA, Imaging, and Carcinoembryonic Antigen Levels in Patients With Resected Colorectal Cancer. *JAMA Netw Open*, *5*(3), e221093. https://doi.org/10.1001/jamanetworkopen.2022.1093

Faulkner, L. G., Howells, L. M., Pepper, C., Shaw, J. A., & Thomas, A. L. (2023). The utility of ctDNA in detecting minimal residual disease following curative surgery in colorectal cancer: a systematic review and meta-analysis. *British journal of cancer*, *128*(2), 297-309.

FDA. (2018, 09/28/2020). *EVALUATION OF AUTOMATIC CLASS II DESIGNATION FOR clonoSEQ® ASSAY DECISION SUMMARY*. Retrieved 07/13/2020 from https://www.accessdata.fda.gov/cdrh_docs/reviews/DEN170080.pdf

FDA. (2020). *510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY*. Retrieved 08/02/2021 from https://www.accessdata.fda.gov/cdrh_docs/reviews/K200009.pdf

Friend, B. D., Bailey-Olson, M., Melton, A., Shimano, K. A., Kharbanda, S., Higham, C., Winestone, L. E., Huang, J., Stieglitz, E., & Dvorak, C. C. (2020). The impact of total body irradiation-based regimens on outcomes in children and young adults with acute lymphoblastic leukemia undergoing allogeneic hematopoietic stem cell

transplantation. *Pediatr Blood Cancer*, *67*(2), e28079. https://doi.org/10.1002/pbc.28079

Goicoechea, I., Puig, N., Cedena, M. T., Burgos, L., Cordon, L., Vidriales, M. B., Flores-Montero, J., Gutierrez, N. C., Calasanz, M. J., Ramos, M. M., Lara-Astiaso, D., Vilas-Zornoza, A., Alignani, D., Rodriguez, I., Sarvide, S., Alameda, D., Garces, J. J., Rodriguez, S., Fresquet, V., . . . Paiva, B. (2021). Deep MRD profiling defines outcome and unveils different modes of treatment resistance in standard- and high-risk myeloma. *Blood*, *137*(1), 49-60. https://doi.org/10.1182/blood.2020006731

Gunning, A., Kumar, S., Williams, C. K., Berger, B. M., Naber, S. P., Gupta, P. B., Del Vecchio Fitz, C., & Kuperwasser, C. (2023). Analytical Validation of NavDx, a cfDNA-Based Fragmentomic Profiling Assay for HPV-Driven Cancers. *Diagnostics*, *13*(4), 725. https://www.mdpi.com/2075-4418/13/4/725

Hallek, M., Cheson, B. D., Catovsky, D., Caligaris-Cappio, F., Dighiero, G., Dohner, H., Hillmen, P., Keating, M., Montserrat, E., Chiorazzi, N., Stilgenbauer, S., Rai, K. R., Byrd, J. C., Eichhorst, B., O'Brien, S., Robak, T., Seymour, J. F., & Kipps, T. J. (2018). iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. *Blood*, *131*(25), 2745-2760. https://doi.org/10.1182/blood-2017-09-806398

Hay, K. A., Gauthier, J., Hirayama, A. V., Voutsinas, J. M., Wu, Q., Li, D., Gooley, T. A., Cherian, S., Chen, X., Pender, B. S., Hawkins, R. M., Vakil, A., Steinmetz, R. N., Schoch, G., Chapuis, A. G., Till, B. G., Kiem, H. P., Ramos, J. D., Shadman, M., . . . Turtle, C. J. (2019). Factors associated with durable EFS in adult B-cell ALL patients achieving MRD-negative CR after CD19 CAR T-cell therapy. *Blood*, *133*(15), 1652-1663. https://doi.org/10.1182/blood-2018-11-883710

Herrera, A. F., Kim, H. T., Kong, K. A., Faham, M., Sun, H., Sohani, A. R., Alyea, E. P., Carlton, V. E., Chen, Y. B., Cutler, C. S., Ho, V. T., Koreth, J., Kotwaliwale, C., Nikiforow, S., Ritz, J., Rodig, S. J., Soiffer, R. J., Antin, J. H., & Armand, P. (2016). Next-generation sequencing-based detection of circulating tumour DNA After allogeneic stem cell transplantation for lymphoma. *Br J Haematol*, *175*(5), 841-850. https://doi.org/10.1111/bjh.14311

Heuser, M., Ofran, Y., Boissel, N., Brunet Mauri, S., Craddock, C., Janssen, J., Wierzbowska, A., & Buske, C. (2020). Acute myeloid leukaemia in adult patients: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. https://doi.org/10.1016/j.annonc.2020.02.018

Hoelzer, D., Bassan, R., Dombret, H., Fielding, A., Ribera, J. M., & Buske, C. (2016). Acute lymphoblastic leukaemia in adult patients: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, *27*(suppl 5), v69-v82. https://doi.org/10.1093/annonc/mdw025

Hofste, L. S. M., Geerlings, M. J., Kamping, E. J., Kouwenhoven, N. D. H., von Rhein, D., Jansen, E. A. M., Garms, L. M., Nagtegaal, I. D., van der Post, R. S., de Wilt, J. H. W.,

Klarenbeek, B. R., & Ligtenberg, M. J. L. (2023). Clinical Validity of Tumor-Informed Circulating Tumor DNA Analysis in Patients Undergoing Surgery of Colorectal Metastases. *Dis Colon Rectum*, *66*(6), 796-804. https://doi.org/10.1097/DCR.0000000000002443

Horton, T. M., & Steuber, C. P. (2023, December 19). *Risk group stratification and prognosis for acute lymphoblastic leukemia/lymphoblastic lymphoma in children and adolescents*. https://www.uptodate.com/contents/risk-group-stratification-and-prognosis-for-acute-lymphoblastic-leukemia-lymphoblastic-lymphoma-in-children-and-adolescents

Jacome, A. A., & Johnson, B. (2023). Minimal Residual Disease in Colorectal Cancer: Are We Finding the Needle in a Haystack? *Cells*, *12*(7). https://doi.org/10.3390/cells12071068

Kater, A. P., Seymour, J. F., Hillmen, P., Eichhorst, B., Langerak, A. W., Owen, C., Verdugo, M., Wu, J., Punnoose, E. A., Jiang, Y., Wang, J., Boyer, M., Humphrey, K., Mobasher, M., & Kipps, T. J. (2019). Fixed Duration of Venetoclax-Rituximab in Relapsed/Refractory Chronic Lymphocytic Leukemia Eradicates Minimal Residual Disease and Prolongs Survival: Post-Treatment Follow-Up of the MURANO Phase III Study. *J Clin Oncol*, *37*(4), 269-277. https://doi.org/10.1200/jco.18.01580

Khaddour, K., Zhou, A., Butt, O. H., Budde, G., Malashevich, A. K., & Ansstas, G. (2022). Case report: Real-world experience using a personalized cancer-specific circulating tumor DNA assay in different metastatic melanoma scenarios. *Front Oncol*, *12*, 978996. https://doi.org/10.3389/fonc.2022.978996

Kim, H. Y., Yoo, I. Y., Lim, D. J., Kim, H. J., Kim, S. H., Yoon, S. E., Kim, S. J., Cho, D., & Kim, K. (2022). Clinical Utility of Next-Generation Flow-Based Minimal Residual Disease Assessment in Patients with Multiple Myeloma. *Ann Lab Med*, *42*(5), 558-565. https://doi.org/10.3343/alm.2022.42.5.558

Kumar, S., Paiva, B., Anderson, K. C., Durie, B., Landgren, O., Moreau, P., Munshi, N., Lonial, S., Blade, J., Mateos, M. V., Dimopoulos, M., Kastritis, E., Boccadoro, M., Orlowski, R., Goldschmidt, H., Spencer, A., Hou, J., Chng, W. J., Usmani, S. Z., . . . Avet-Loiseau, H. (2016). International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. *Lancet Oncol*, *17*(8), e328-e346. https://doi.org/10.1016/s1470-2045(16)30206-6

Lee, S., Kim, D. W., Cho, B., Kim, Y. J., Kim, Y. L., Hwang, J. Y., Park, Y. H., Shin, H. J., Park, C. Y., Min, W. S., Kim, H. K., & Kim, C. C. (2003). Risk factors for adults with Philadelphia-chromosome-positive acute lymphoblastic leukaemia in remission treated with allogeneic bone marrow transplantation: the potential of real-time quantitative reverse-transcription polymerase chain reaction. *Br J Haematol*, *120*(1), 145-153. https://doi.org/10.1046/j.1365-2141.2003.03988.x

Loupakis, F., Sharma, S., Derouazi, M., Murgioni, S., Biason, P., Rizzato, M. D., Rasola, C., Renner, D., Shchegrova, S., Koyen Malashevich, A., Malhotra, M., Sethi, H.,

Zimmermann, B. G., Aleshin, A., Moshkevich, S., Billings, P. R., Sedgwick, J. D., Schirripa, M., Munari, G., . . . Fassan, M. (2021). Detection of Molecular Residual Disease Using Personalized Circulating Tumor DNA Assay in Patients With Colorectal Cancer Undergoing Resection of Metastases. *JCO Precis Oncol*, *5*. https://doi.org/10.1200/PO.21.00101

Luskin, M. R., Murakami, M. A., Manalis, S. R., & Weinstock, D. M. (2018). Targeting minimal residual disease: a path to cure? *Nat Rev Cancer*, *18*(4), 255-263. https://doi.org/10.1038/nrc.2017.125

Madzo, J., Zuna, J., Muzíková, K., Kalinová, M., Krejcí, O., Hrusák, O., Otová, B., Starý, J., & Trka, J. (2003). Slower molecular response to treatment predicts poor outcome in patients with TEL/AML1 positive acute lymphoblastic leukemia: prospective real-time quantitative reverse transcriptase-polymerase chain reaction study. *Cancer*, *97*(1), 105-113. https://doi.org/10.1002/cncr.11043

Medina, A., Puig, N., Flores-Montero, J., Jimenez, C., Sarasquete, M. E., Garcia-Alvarez, M., Prieto-Conde, I., Chillon, C., Alcoceba, M., Gutierrez, N. C., Oriol, A., Rosinol, L., Blade, J., Gironella, M., Hernandez, M. T., Gonzalez-Calle, V., Cedena, M. T., Paiva, B., San-Miguel, J. F., . . . Garcia-Sanz, R. (2020). Comparison of next-generation sequencing (NGS) and next-generation flow (NGF) for minimal residual disease (MRD) assessment in multiple myeloma. *Blood Cancer J*, *10*(10), 108. https://doi.org/10.1038/s41408-020-00377-0

Mikhael, J., Ismaila, N., Cheung, M. C., Costello, C., Dhodapkar, M. V., Kumar, S., Lacy, M., Lipe, B., Little, R. F., Nikonova, A., Omel, J., Peswani, N., Prica, A., Raje, N., Seth, R., Vesole, D. H., Walker, I., Whitley, A., Wildes, T. M., . . . Martin, T. (2019). Treatment of Multiple Myeloma: ASCO and CCO Joint Clinical Practice Guideline. *Journal of Clinical Oncology*, *37*(14), 1228-1263. https://doi.org/10.1200/JCO.18.02096

Moding, E. J., Nabet, B. Y., Alizadeh, A. A., & Diehn, M. (2021). Detecting Liquid Remnants of Solid Tumors: Circulating Tumor DNA Minimal Residual Disease. *Cancer Discov*, *11*(12), 2968-2986. https://doi.org/10.1158/2159-8290.CD-21-0634

Natera. (2024). *Signatera™ Transforming the management of cancer with personalized testing*. https://www.natera.com/oncology/signatera-advanced-cancer-detection/

NavDx. (2024). *Get Answers to Patients' Frequently Asked Questions (FAQs) Regarding NavDx®*. https://navdx.com/patient-faqs/

NCCN. (2024a, July 19, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) Acute Lymphoblastic Leukemia Version 2.2024*. https://www.nccn.org/professionals/physician_gls/pdf/all.pdf

NCCN. (2024b, May 17, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) Acute Myeloid Leukemia Version 3.2024*. https://www.nccn.org/professionals/physician_gls/pdf/aml.pdf

NCCN. (2024c, October 1, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) Chronic Lymphocytic Leukemia / Small Lymphocytic Leukemia Version 1.2025*. https://www.nccn.org/professionals/physician_gls/pdf/cll.pdf

NCCN. (2024d, September 26, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) Hairy Cell Leukemia Version 1.2025*. https://www.nccn.org/professionals/physician_gls/pdf/hairy_cell.pdf

NCCN. (2024e, September 17, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) Multiple Myeloma Version 1.2025*. https://www.nccn.org/professionals/physician_gls/pdf/myeloma.pdf

NCCN. (2024f, August 28, 2024). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines) Pediatric Acute Lymphoblastic Leukemia Version 1.2025*. https://www.nccn.org/professionals/physician_gls/pdf/ped_all.pdf

Nguyen Hoang, T. P., Nguyen, T. A., Tran, N. H. B., Nguyen Hoang, V. A., Thi Dao, H. T., Tran, V. U., Nguyen, Y. N., Nguyen, A. T., Nguyen Thi, C. T., Do Thi, T. T., Nguyen, D. S., Nguyen, H. N., Giang, H., & Tu, L. N. (2024). Analytical validation and clinical utilization of K-4CARE™: a comprehensive genomic profiling assay with personalized MRD detection. *Front Mol Biosci*, *11*, 1334808. https://doi.org/10.3389/fmolb.2024.1334808

O'Sullivan, H. M., Feber, A., & Popat, S. (2023). Minimal Residual Disease Monitoring in Radically Treated Non-Small Cell Lung Cancer: Challenges and Future Directions. *Onco Targets Ther*, *16*, 249-259. https://doi.org/10.2147/OTT.S322242

Perrot, A., Lauwers-Cances, V., Corre, J., Robillard, N., Hulin, C., Chretien, M. L., Dejoie, T., Maheo, S., Stoppa, A. M., Pegourie, B., Karlin, L., Garderet, L., Arnulf, B., Doyen, C., Meuleman, N., Royer, B., Eveillard, J. R., Benboubker, L., Dib, M., . . . Munshi, N. (2018). Minimal residual disease negativity using deep sequencing is a major prognostic factor in multiple myeloma. *Blood*, *132*(23), 2456-2464. https://doi.org/10.1182/blood-2018-06-858613

Rai, K. R., & Stilgenbauer, S. (2024, February 13). *Evaluating response to treatment of chronic lymphocytic leukemia*. https://www.uptodate.com/contents/evaluating-response-to-treatment-of-chronic-lymphocytic-leukemia

Rajkumar, S. V. (2023, September 25, 2023). *Multiple myeloma: Evaluating response to treatment*. https://www.uptodate.com/contents/multiple-myeloma-evaluating-response-to-treatment

Rawstron, A. C., Fazi, C., Agathangelidis, A., Villamor, N., Letestu, R., Nomdedeu, J., Palacio, C., Stehlikova, O., Kreuzer, K. A., Liptrot, S., O'Brien, D., de Tute, R. M., Marinov, I., Hauwel, M., Spacek, M., Dobber, J., Kater, A. P., Gambell, P., Soosapilla, A., . . . Ghia, P. (2016). A complementary role of multiparameter flow cytometry and high-throughput sequencing for minimal residual disease detection in chronic lymphocytic leukemia: an European Research Initiative on CLL study. *Leukemia*, *30*(4), 929-936. https://doi.org/10.1038/leu.2015.313

Reinert, T., Henriksen, T. V., Christensen, E., Sharma, S., Salari, R., Sethi, H., Knudsen, M., Nordentoft, I., Wu, H. T., Tin, A. S., Heilskov Rasmussen, M., Vang, S., Shchegrova, S., Frydendahl Boll Johansen, A., Srinivasan, R., Assaf, Z., Balcioglu, M., Olson, A., Dashner, S., . . . Lindbjerg Andersen, C. (2019). Analysis of Plasma Cell-Free DNA by Ultradeep Sequencing in Patients With Stages I to III Colorectal Cancer. *JAMA Oncol*, *5*(8), 1124-1131. https://doi.org/10.1001/jamaoncol.2019.0528

Robak, T., Matutes, E., Catovsky, D., Zinzani, P. L., & Buske, C. (2015). Hairy cell leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, *26 Suppl 5*, v100-107. https://doi.org/10.1093/annonc/mdv200

Royston, D. J., Gao, Q., Nguyen, N., Maslak, P., Dogan, A., & Roshal, M. (2016). Single-Tube 10-Fluorochrome Analysis for Efficient Flow Cytometric Evaluation of Minimal Residual Disease in Plasma Cell Myeloma. *Am J Clin Pathol*, *146*(1), 41-49. https://doi.org/10.1093/ajcp/aqw052

Sato, S., Nakamura, Y., Oki, E., & Yoshino, T. (2023). Molecular Residual Disease-guided Adjuvant Treatment in Resected Colorectal Cancer: Focus on CIRCULATE-Japan. *Clin Colorectal Cancer*, *22*(1), 53-58. https://doi.org/10.1016/j.clcc.2022.12.001

Schuurhuis, G. J., Heuser, M., Freeman, S., Bene, M. C., Buccisano, F., Cloos, J., Grimwade, D., Haferlach, T., Hills, R. K., Hourigan, C. S., Jorgensen, J. L., Kern, W., Lacombe, F., Maurillo, L., Preudhomme, C., van der Reijden, B. A., Thiede, C., Venditti, A., Vyas, P., . . . Ossenkoppele, G. J. (2018). Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. *Blood*, *131*(12), 1275-1291. https://doi.org/10.1182/blood-2017-09-801498

Stock, W., & Estrov, Z. (2022a, June 16, 2022). *Clinical use of measurable residual disease detection in acute lymphoblastic leukemia*. https://www.uptodate.com/contents/clinical-use-of-measurable-residual-disease-detection-in-acute-lymphoblastic-leukemia

Stock, W., & Estrov, Z. (2022b, March 7, 2022). *Detection of measurable residual disease in acute lymphoblastic leukemia*. https://www.uptodate.com/contents/detection-of-measurable-residual-disease-in-acute-lymphoblastic-leukemia

Subhash, V. V., Huang, L., Kamili, A., Wong, M., Chen, D., Venn, N. C., Atkinson, C., Mayoh, C., Venkat, P., Tyrrell, V., Marshall, G. M., Cowley, M. J., Ekert, P. G., Norris, M. D., Haber, M., Henderson, M. J., Sutton, R., Fletcher, J. I., & Trahair, T. N. (2022). Whole-genome sequencing facilitates patient-specific quantitative PCR-based minimal residual disease monitoring in acute lymphoblastic leukaemia, neuroblastoma and Ewing sarcoma. *Br J Cancer*, *126*(3), 482-491. https://doi.org/10.1038/s41416-021-01538-z

Sullivan, B. G., Dayyani, F., & Senthil, M. (2023). ASO Author Reflections: Challenges of Circulating Tumor DNA in the Management of Gastrointestinal Peritoneal Carcinomatosis. *Ann Surg Oncol*, *30*(1), 285-286. https://doi.org/10.1245/s10434-022-12543-8

Theunissen, P., Mejstrikova, E., Sedek, L., van der Sluijs-Gelling, A. J., Gaipa, G., Bartels, M., Sobral da Costa, E., Kotrová, M., Novakova, M., Sonneveld, E., Buracchi, C., Bonaccorso, P., Oliveira, E., Te Marvelde, J. G., Szczepanski, T., Lhermitte, L., Hrusak, O., Lecrevisse, Q., Grigore, G. E., . . . van der Velden, V. H. (2017). Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. *Blood*, *129*(3), 347-357. https://doi.org/10.1182/blood-2016-07-726307

Thompson, P. A., Srivastava, J., Peterson, C., Strati, P., Jorgensen, J. L., Hether, T., Keating, M. J., O'Brien, S. M., Ferrajoli, A., Burger, J. A., Estrov, Z., Jain, N., & Wierda, W. G. (2019). Minimal residual disease undetectable by next-generation sequencing predicts improved outcome in CLL after chemoimmunotherapy. *Blood*, *134*(22), 1951-1959. https://doi.org/10.1182/blood.2019001077

Thörn, I., Forestier, E., Botling, J., Thuresson, B., Wasslavik, C., Björklund, E., Li, A., Lindström-Eriksson, E., Malec, M., Grönlund, E., Torikka, K., Heldrup, J., Abrahamsson, J., Behrendtz, M., Söderhäll, S., Jacobsson, S., Olofsson, T., Porwit, A., Lönnerholm, G., . . . Sundström, C. (2011). Minimal residual disease assessment in childhood acute lymphoblastic leukaemia: a Swedish multi-centre study comparing real-time polymerase chain reaction and multicolour flow cytometry. *Br J Haematol*, *152*(6), 743-753. https://doi.org/10.1111/j.1365-2141.2010.08456.x

Tie, J., Cohen, J. D., Wang, Y., Christie, M., Simons, K., Lee, M., Wong, R., Kosmider, S., Ananda, S., McKendrick, J., Lee, B., Cho, J. H., Faragher, I., Jones, I. T., Ptak, J., Schaeffer, M. J., Silliman, N., Dobbyn, L., Li, L., . . . Gibbs, P. (2019). Circulating Tumor DNA Analyses as Markers of Recurrence Risk and Benefit of Adjuvant Therapy for Stage III Colon Cancer. *JAMA Oncol*, *5*(12), 1710-1717. https://doi.org/10.1001/jamaoncol.2019.3616

Tie, J., Wang, Y., Tomasetti, C., Li, L., Springer, S., Kinde, I., Silliman, N., Tacey, M., Wong, H. L., Christie, M., Kosmider, S., Skinner, I., Wong, R., Steel, M., Tran, B., Desai, J., Jones, I., Haydon, A., Hayes, T., . . . Gibbs, P. (2016). Circulating tumor DNA analysis detects minimal residual disease and predicts recurrence in patients with stage II colon cancer. *Sci Transl Med*, *8*(346), 346ra392. https://doi.org/10.1126/scitranslmed.aaf6219

van der Velden, V. H. J., Hochhaus, A., Cazzaniga, G., Szczepanski, T., Gabert, J., & van Dongen, J. J. M. (2003). Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches, and laboratory aspects. *Leukemia*, *17*(6), 1013-1034. https://doi.org/10.1038/sj.leu.2402922

Wang, Y., Li, L., Cohen, J. D., Kinde, I., Ptak, J., Popoli, M., Schaefer, J., Silliman, N., Dobbyn, L., Tie, J., Gibbs, P., Tomasetti, C., Kinzler, K. W., Papadopoulos, N., Vogelstein, B., & Olsson, L. (2019). Prognostic Potential of Circulating Tumor DNA Measurement in Postoperative Surveillance of Nonmetastatic Colorectal Cancer. *JAMA Oncol*, *5*(8), 1118-1123. https://doi.org/10.1001/jamaoncol.2019.0512

Wang, Z., Guo, M., Zhang, Y., Xu, S., Cheng, H., Wu, J., Zhang, W., Hu, X., Yang, J., Wang, J., & Tang, G. (2019). The applicability of multiparameter flow cytometry for the detection of minimal residual disease using different-from-normal panels to predict relapse in patients with acute myeloid leukemia after allogeneic transplantation. *Int J Lab Hematol*, *41*(5), 607-614. https://doi.org/10.1111/ijlh.13070

Wood, B., Wu, D., Crossley, B., Dai, Y., Williamson, D., Gawad, C., Borowitz, M. J., Devidas, M., Maloney, K. W., Larsen, E., Winick, N., Raetz, E., Carroll, W. L., Hunger, S. P., Loh, M. L., Robins, H., & Kirsch, I. (2018). Measurable residual disease detection by high-throughput sequencing improves risk stratification for pediatric B-ALL. *Blood*, *131*(12), 1350-1359. https://doi.org/10.1182/blood-2017-09-806521

Zhang, S., Brazel, D., Kumar, P., Schafer, L. N., Eidenschink, B., Senthil, M., & Dayyani, F. (2021). Utility of tumor-informed circulating tumor DNA in the clinical management of gastrointestinal malignancies. *J Gastrointest Oncol*, *12*(6), 2643-2652. https://doi.org/10.21037/jgo-21-484

Zhong, R., Gao, R., Fu, W., Li, C., Huo, Z., Gao, Y., Lu, Y., Li, F., Ge, F., Tu, H., You, Z., He, J., & Liang, W. (2023). Accuracy of minimal residual disease detection by circulating tumor DNA profiling in lung cancer: a meta-analysis. *BMC Med*, *21*(1), 180. https://doi.org/10.1186/s12916-023-02849-z

Molecular Analysis for Gliomas

Policy Number: AHS – M2139 – Molecular Analysis for Gliomas	Prior Policy Name and Number, as applicable: • AHS – M2139 – Analysis of MGMT Promoter Methylation for Malignant Gliomas
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Glioma refers to tumors resulting from metaplastic transformation of glial tissue of the nervous system. Tumors have historically been classified by the retained histologic features of the three types of glial cells: astrocytes, oligodendrocytes, and ependymal cells. Tumors of each type can vary widely in aggressiveness, response to treatment, and prognosis (Louis et al., 2023).

Molecular genetic features were added to histopathologic appearance in the current WHO classification to yield more biologically homogeneous and narrowly defined diagnostic entities for greater diagnostic accuracy, improved patient management, more accurate determinations of prognosis, and better treatment response (Louis et al., 2016; Louis et al., 2021).

II. Related Policies

Policy Number	Policy Title
N/A	

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For the prognosis of gliomas, the following tests **MEET COVERAGE CRITERIA:**
   a) Array-based genomic copy number testing or fluorescence in situ hybridization (FISH) for the co-deletion of 1p and 19q.
   b) *ATRX* mutation testing via gene sequencing **or** loss of ATRX protein expression via immunohistochemistry.
   c) *BRAF* fusion and mutation testing, including *BRAF* V600E common variant.
   d) *IDH1* and *IDH2* testing.
   e) *MGMT* promoter methylation testing.
   f) *TERT* promotor mutation testing.
2) For the prognosis of diffuse midline gliomas, the following tests **MEET COVERAGE CRITERIA:**
   a) *H3-3A* and *HIST1H3B* gene sequencing.
   b) *H3-3A* mutation testing by immunohistochemistry using an H3-3A K27M histone antibody.
3) For the prognosis of ependymomas, *ZFTA* fusion testing using either RNA sequencing analysis (RNA-Seq) or FISH **MEETS COVERAGE CRITERIA**.
4) *ATRX* mutation co-testing using **both** immunohistochemistry and gene sequencing **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
AG	Anaplastic glioma
ATRX	*Alpha-Thalassemia/Mental Retardation Syndrome X-Linked* gene
BRAF	*B-Raf proto-oncogene*
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare and Medicaid
CNA	Copy number alterations
CNS	Central nervous system

CSF	Cerebrospinal fluid
ddPCR	Digital droplet polymerase chain reaction
DIPG	Diffuse intrinsic pontine gliomas
EANO	European Association of Neuro-Oncology
ELISA	Enzyme-linked immunoassay
ESMO	European Society for Medical Oncology
FISH	Fluorescence in situ hybridization
H3-3A	*H3.3 histone A* gene
H3F3A	Previous gene name of *H3.3 histone A*
H3FA	*H3 clustered histone 1* gene
IDH1	*Isocitrate Dehydrogenase 1* gene
IDH2	*Isocitrate Dehydrogenase 2* gene
LDTs	Laboratory-developed tests
MGMT	*O-6-Methylguanine-Dna Methyltransferase* gene
NCCN	National Comprehensive Cancer Network
NICE	National Institute for Health and Care Excellence
NFkB	Nuclear factor kappa B
NGS	Next-generation sequencing
NICE	National Institute for Health and Care Excellence
PCR	Polymerase chain reaction
PFA	Posterior fossa ependymoma group A
PFB	Posterior fossa ependymoma group B
RELA	*RELA proto-oncogene, NF-kB subunit*
RNA-Seq	Ribonucleic acid sequencing analysis
SNVs	Single nucleotide variants
sPD-L1	Soluble programmed cell death ligand 1
TERT	*Telomerase reverse transcriptase*
TMB	Tumor mutational burden
TP53	*Tumor protein 53*
WHO	World Health Organization
YAP1	*Yes1 associated transcriptional regulator* gene
ZFTA	*Zinc finger translocation associated* gene

V. Scientific Background

According to the American Cancer Society, an estimated 25,400 adults in the United States were diagnosed with malignant tumors of the brain and spinal cord in 2023.

Additionally, The American Cancer Society reported that there was an estimated 18,760 deaths from brain and spinal cord tumors in 2023. (American Cancer Society, 2024).

Studies over the past two decades have clarified the genetic basis of tumorigenesis in the common, and some rarer, brain tumor entities (Louis et al., 2016), and identified clinically relevant molecular genetic characterizations that complement standard histologic analysis providing additional diagnostic and prognostic information to improve diagnostic accuracy, influence treatment selection, and improve survival. Molecular and/or genetic characterization do not replace standard histologic assessment, but rather serve as a complimentary approach (NCCN, 2023).

Isocitrate dehydrogenase (IDH1/2) mutations

Metabolic enzymes, IDH one and two, oxidize isocitrate to alpha-ketoglutarate, and are important in the mitigation of cellular oxidative damage (Horbinski, 2013b). Mutations in genes encoding these enzymes leads to the aberrant production of D-2 hydroxyglutarate (Dang et al., 2009), an oncometabolite that causes epigenetic modifications in affected cells (Horbinski, 2013b).

The *IDH* mutations are a defining feature of WHO grade II and III astrocytomas and oligodendrogliomas (Louis et al., 2016). Their presence distinguishes lower grade gliomas from primary glioblastomas, which are *IDH* wild type. *IDH* mutations are commonly associated with O-6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation and are also associated with a relatively favorable prognosis (Brat et al., 2015; Eckel-Passow et al., 2015).

O-6-methylguanine-DNA methyltransferase (MGMT) methylation

The DNA repair enzyme, MGMT, reverses the DNA damage caused by alkylating agents, resulting in tumor resistance to temozolomide and nitrosourea-based chemotherapy. Methylation of the *MGMT* promoter silences *MGMT,* making the tumor more sensitive to treatment with alkylating agents (Esteller et al., 2000; Gusyatiner & Hegi, 2018).

The *MGMT* promoter methylation is strongly associated with *IDH* mutation and genome-wide epigenetic change (Eckel-Passow et al., 2015); it is also associated with longer survival in patients with glioblastoma who receive alkylating agents (Hegi et al., 2005; Zhao et al., 2016). *MGMT* promoter methylation is particularly useful in treatment decisions for elderly patients with high grade gliomas (Malmstrom et al., 2012; Wick et al., 2012; Wick et al., 2014).

Codeletion of chromosomes 1p and 19q

The codeletion of 1p and 19q represents an unbalanced translocation (1;19) (q10;p10) leading to the whole arm deletion of chromosome 1p and chromosome 19q (Jenkins et al., 2006). Codeletion of 1p and 19q is a defining feature of oligodendroglial tumors, is strongly associated with oligodendroglial histology, and helps to confirm the oligodendroglial character of tumors with equivocal or mixed histologic features (Brat et al., 2015; Burger et al., 2001; Eckel-Passow et al., 2015). Combined loss involving chromosomes 1p and 19q is significantly associated with both favorable therapeutic response and longer recurrence-free survival after chemotherapy (Cairncross et al., 1998).

Alpha-thalassemia/mental retardation syndrome X-linked (ATRX) mutations

Mutations in the chromatin regulator gene, *ATRX*, enable alternative lengthening of telomeres (Abedalthagafi et al., 2013). ATRX is a switch/sucrose helicase that assists with H3.3 chromatin deposition in telomeric regions. Disruption of this gene leads to the alternative lengthening of telomeres stated above and is thought to represent an early event in gliomagenesis (Batchelor & Louis, 2023).

The *ATRX* mutations in glioma are strongly associated with *IDH* and *TP53* mutations and are nearly always mutually exclusive with 1p19q codeletion (Reuss et al., 2015). ATRX deficiency, coupled with *IDH* mutation, is typical of astrocytoma (Brat et al., 2015).

Tumor protein 53 (TP53) mutation

Tumor protein 53 is essential for regulating cell division and preventing tumor formation (Parikh et al., 2014). Missense mutations in the *TP53* gene are present in the clear majority of IDH-mutant astrocytomas (Brat et al., 2015). Immunopositivity for mutant p53 is not entirely sensitive or specific for a *TP53* mutation, however, and loss of *ATRX* expression may be a more reliable marker of astrocytic differentiation (Louis et al., 2023).

Telomerase reverse transcriptase (TERT) mutations

Telomerase reverse transcriptase encodes the catalytic active site of telomerase, the enzyme responsible for maintaining telomere length in dividing cells. *TERT* mutations in the noncoding promoter region cause increased expression of the TERT protein and are one of the major mechanisms of telomerase activation in gliomas (Arita et al., 2013). *TERT* mutations are strongly associated with 1p19q codeletion and are found in most glioblastomas. A *TERT* mutation in combination with an *IDH* mutation and 1p19q codeletion is characteristic of oligodendroglioma. The absence of a *TERT* mutation, coupled with an *IDH* mutation, designates astrocytoma (Eckel-Passow et al., 2015). In

terms of survival, mutation in the *TERT* promoter is generally unfavorable in the absence of *IDH* mutation and favorable in the presence of *IDH* mutation and 1p/19q codeletion. *TERT* promoter mutation is associated with an older age of the patient at presentation, regardless of whether *IDH* mutation is present (Eckel-Passow et al., 2015).

Histone (H3FA) mutations

A lysine to methionine substitution in the *H3F3A* gene (*H3K27M*) is the most common histone mutation in brain tumors and inhibits the trimethylation of H3.3 histone (Sturm et al., 2012), arresting cells in a primitive state refractory to differentiation induction (Weinberg et al., 2017). *G34R/G34V* mutations in the *H3F3A* gene are more common in cortical gliomas in children (Schwartzentruber et al., 2012). *H3FA* mutations can be useful in the diagnosis of infiltrative glioma (Sturm et al., 2012). The *H3K27M* mutation is an adverse prognostic marker in children and adults (Meyronet et al., 2017). The *G34* mutation does not appear to have any prognostic significance once the diagnosis of a glioblastoma has been established (Sturm et al., 2012).

A similar mutation to *H3K27M* may also occur in the *HIST1H3B/C* gene, which encodes the histone H3.1 variant. However, the mutation at *HIST1H3B/C* is about one third as common as *H3F3A* and often confers a better prognosis than its *H3F3A* counterpart (Louis et al., 2023).

B-Raf proto-oncogene (BRAF) mutations

The serine-threonine protein kinase, BRAF, is involved in cell survival, proliferation, and differentiation (Davies et al., 2002). Activating mutations in *BRAF*, most often V600E, have been discovered in most pediatric and some adult gliomas (Chappe et al., 2013; Horbinski, 2013a), including approximately 80% of pleomorphic xanthoastrocytomas, 20% of gangliogliomas, 10% of pilocytic astrocytomas, and occasionally diffuse gliomas (Chi et al., 2013). Tandem duplication of chromosome 7q34 resulting in an activating fusion of the *BRAF* and *KIAA1549* genes occur in 60-80% of pilocytic astrocytoma (Jones et al., 2008).

The presence of a *BRAF* fusion is reliable evidence that the tumor is a pilocytic astrocytoma and predicts a better clinical outcome (Hawkins et al., 2011). A *BRAF* mutation is more complicated, as it can occur in a variety of tumors and requires integration with histology. Tumors with *BRAF* mutations may respond to BRAF inhibitors; however, in pediatric gliomas, *BRAF* V600E indicates poor prognosis when treated with current adjuvant therapy, especially in combination with a *CDKN2A* mutation (Lassaletta et al., 2017).

v-rel avian reticuloendotheliosis viral oncogene homolog A (RELA, p65, NFKB3) fusion

Fusion between the *C11orf95* and *RELA* genes defines approximately 70 percent of all childhood supratentorial ependymomas (Louis et al., 2023). These fusions are associated with increased NF-kappa-B (NFkB) signaling and poor outcome (Malgulwar et al., 2018). Normally, NFkB is an inactive transcription factor in the cytoplasm. When its inhibitor degrades, it activates transcription of certain genes, *RELA* among them (NCBI, 2011). New research supports the hypothesis that the status of *RELA* fusion and p53 overexpression are significantly associated with the prognosis of supratentorial extraventricular ependymomas (Wang et al., 2019).

New Tests

Assessment of gliomas is incredibly difficult, and new methods of molecular analyses for gliomas are consistently being developed. For example, Miller et al. (2019) devised a liquid-biopsy based method to evaluate cerebrospinal fluid from 42 (of 85) patients. The genomic profile developed from the cerebrospinal fluid (CSF) samples closely matched established profiles, such as the characteristic 1p/19q codeletion and *IDH1/2* mutations. The authors stated that the ability to monitor the glioma genome in real time could be useful in management of this condition (Miller et al., 2019). Other researchers report that "A cerebrospinal fluid ct-DNA liquid biopsy approach may virtually support all the stages of glioma management, from facilitating molecular diagnosis when surgery is not feasible, to monitoring tumor response, identifying early recurrence, tracking longitudinal genomic evolution, providing a new molecular characterization at recurrence and allowing patient selection for targeted therapies" (Simonelli et al., 2020).

Clinical Utility and Validity

Nikiforova et al. (2016) validated GlioSeq, a commercial next generation sequencing (NGS) panel of 30 genes, in 54 patients with CNS tumors against fluorescence in-situ hybridization (FISH), Sanger sequencing, and reverse transcription polymerase chain reaction (PCR). GlioSeq correctly identified 71/71 (100%) genetic alterations known to be present by conventional techniques. The assay sensitivity was three to five percent for mutant alleles of single nucleotide variants (SNVs), and one to five percent for gene fusions. Likewise, Zacher et al. (2017) developed an NGS panel of 20 genes that allowed for molecular classification of 121 gliomas. The researchers conclude that gene panel NGS is a promising diagnostic technique that may facilitate integrated histological and molecular glioma classification.

Ramkissoon et al. (2017) used OncoPanel and OncoCopy to identify targetable alterations in tumors for the establishment of best practices in routine clinical pediatric oncology. They analyzed 117 samples by OncoPanel and 146 by OncoCopy; further, 60 tumors were subjected to both methodologies. OncoPanel revealed clinically relevant

alterations in 56% of patients (44 cancer mutations and 20 rearrangements), including *BRAF* alterations that directed the use of targeted inhibitors. Rearrangements in *MYB-QKI, MYBL1, BRAF*, and *FGFR1* were also detected. Furthermore, while copy number profiles differed across histologies, the combined use of OncoPanel and OncoCopy identified subgroup-specific alterations in 89% (17/19) of medulloblastomas.

Ryall et al. (2016) evaluated the prognostic impact of H3K27M and MAPK pathway aberrations in 64 gliomas (44 low grade, 22 high grade). Tumors are designated as low-grade if the cells are well differentiated, are less aggressive overall, and suggest a better prognosis for the patient. Five low grade gliomas contained the *H3F3A/HIST1H3B K27M* (*H3K27M*) mutation, and 11 high grade gliomas contained the *H3K27M* mutation. Survival analysis evaluated the median survival at 9.12 years for wildtype H3 patients compared to 1.02 years for patients with the *H3K27M* mutation. MAPK pathway mutations (through *BRAF* or *FGFR1* mutation) were associated with long-term survival in absence of *H3K27M* mutations. Further, H3K27M status and high-grade histology were found to be the most significant independent predictors of poor overall survival with hazard ratios of 6.945 and 7.721 respectively. MAPK pathway activation was a predictor of "favourable patient outcome," but dependent on other factors (Ryall et al., 2016).

Houdova Megova et al. (2017) evaluated the prognostic value of the *IDH1/2* mutation in glioblastomas. A total of 37 *IDH* mutations were examined and studied. The authors found that *IDH1* mutations were positively associated with *MGMT* methylation (odds ratio [OR]: 3.08), 1p/19q co-loss (OR: 8.85), and negatively associated with *EGFR* amplification. IDH-mutant patients had an overall survival of 25 months compared to only nine months for IDH-wildtype gliomas (Houdova Megova et al., 2017).

Johnson et al. (2017) performed comprehensive genomic profiling of 282 pediatric gliomas: 157 high-grade and 125 low-grade. The investigators used a 315 gene panel and calculated the tumor mutational burden (TMB). In low grade gliomas, *BRAF* was the most frequent mutation found (48%), followed by *FGFR* missense (17.6%), *NF1* loss of function (8.8%), and *TP53* (5.6%). Rearrangements were found in 35% of low-grade gliomas. In high-grade gliomas, *TP53* was the most frequent mutation found (49%), followed by *H3F3A* (37.6%), *ATRX* (24.2%), *NF1* (22.2%), and *PDGFRA* (21.7%). *H3F3A* mutations were found to be the K28M variant. Approximately six percent of the high-grade gliomas were found have a TMB of >20 mutations/Mb ("hypermutated") (Johnson et al., 2017).

Back et al. (2020) studied the pattern of failure in anaplastic glioma (AG) patients with an *IDH1/2* mutation. A total of 156 patients participated in the study, with data collected from 2008 to 2014; the median follow-up time was 5.1 years. Of all 156

patients, 75% were found to have an *IDH1 or IDH2* mutation. The authors concluded that "patients with *IDH*-mutated AG have improved outcomes"; however, this population also had a greater number of distant relapses approximately two years after intensity-modulated radiation therapy compared to individuals with *IDH* wild type mutations (Back et al., 2020).

Ji et al. (2021) studied the clinical utility of comprehensive genomic profiling to detect CNS tumors in children and young adults using the OncoKids next-generation sequencing panel, chromosomal microarray analysis, and germline testing. NGS was performed on 222 samples and CMA was performed on 146 of the 222 samples. The OncoKids NGS panel identified diagnostic biomarkers in 138/222 samples (62%), prognostic information in 49/222 cases (22%), and targetable genomic alterations in 41/222 samples (18%). Additionally, CMA revealed prognostic copy number alterations (CNA) in 101/146 cases (69%). Further, germline cancer predisposition testing was performed in 57 of 212 patients which identified 20 patients which a confirmed germline pathogenic/likely pathogenic variant of genes *TP53, NF1, SMARCB1, NF2, MSH6, PMS2*, and a patient with Klinefelter syndrome. Overall, the authors conclude that there is "significant clinical utility of integrating genomic profiling into routine clinical testing for pediatric and young adult patients with CNS tumors" (Ji et al., 2021).

Muralidharan et al. (2021) studied the diagnostic utility of a novel digital droplet PCR (ddPCR) assay for detection of two TERT promoter mutations (*C228T* and *C250T*) and monitoring of gliomas. In comparison with the gold-standard tumor tissue-based detection of *TERT* mutations, the ddPCR assay had an overall sensitivity of 62.5% and a specificity of 90%. Longitudinal monitoring of five patients demonstrated that the peripheral *TERT* mutant allele frequency reflects the clinical course of the disease. *TERT* mutant alleles decreased after surgical intervention and pharmacotherapy but increased with tumor progression. The authors conclude that the ddPCR assay has feasibility in "detecting circulating cfDNA TERT promoter mutations in patients with glioma with clinically relevant sensitivity and specificity" (Muralidharan et al., 2021).

Cabezas-Camarero et al. (2021) studied the levels of soluble PD-L1 (sPD-L1) in patients with gliomas according to histologic grade and IDH mutation status, evaluating its predicted role and dynamic changes in sPD-L1. Plasma samples were obtained prior to and after radiotherapy/chemotherapy and were evaluated using ELISA. The authors compared 12 healthy controls with 57 patients with grade II to IV gliomas. They found that sPD-L1 levels were numerically higher in glioma patients as compared to the healthy control group. The authors found that elevated sPD-L1 levels pre- and post-treatment associated with a worse prognosis in IDH-MUT gliomas. Dynamics of SPDL1 and other immune-biomarkers should still be explored in gliomas (Cabezas-Camarero et al., 2021).

Rios et al. (2022) studied the health and economic impacts of using tumor molecular testing for guiding treatments for pediatric patients with low grade glioma. With their microsimulation for modeling health and cost outcomes for 100,000 simulated patients, they found that there was a statistically significant increase in life expectancy in those who received molecular testing for the BRAF mutation (40.08 vs 39.01 in those who did not receiving testing), and an increase of 0.38 quality-adjusted life-years (QALY) and $1384 reduction in costs due to likely avoidance of adverse events associated with radiation, like stroke and other neoplastic transformations. This study ultimately demonstrates that in this subset of patients, there could be long-term benefits in the treatment plans derived for childhood cancers, including the increased timely use of *BRAF*-specific biologic agents versus standard treatment with radiation (Rios et al., 2022).

VI. Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

The NCCN published Clinical Practice Guidelines in Oncology (2023) for Central Nervous System Cancers which recommend:

IDH1 and *IDH2* mutation

Recommendation: *IDH* mutation testing is required for the workup of glioma.

"The most common *IDH1* mutation (R132H) is reliably screened by mutation specific immunohistochemistry (IHC), which is recommended for all glioma patients. If the R132H immunostain result is negative, in the appropriate clinical context, sequencing of *IDH1* and *IDH2* is highly recommended to detect less common *IDH1* and *IDH2* mutations. Prior to age 55 years, sequencing of *IDH1* and *IDH2* is required if the R132H immunostain result is negative, or if the glioma is only grade 2 or 3 histologically. Standard sequencing methods include Sanger sequencing, pyrosequencing, and next-generation sequencing (NGS), and should be performed on formalin fixed, paraffin embedded tissue" (NCCN, 2023).

MGMT promoter methylation

Recommendation: *MGMT* promoter methylation is an essential part of molecular diagnostics for all high-grade gliomas (grade 3 and 4). The NCCN also notes that "*MGMT* promoter methylation is strongly associated with *IDH* mutations and genome-wide epigenetic changes (G-CIMP phenotype)" (NCCN, 2023).

"There are multiple ways to test for *MGMT* promoter methylation, including methylation-specific polymerase chain reaction (PCR), methylation-specific high-resolution melting, pyrosequencing, and droplet-digital PCR" (NCCN, 2023).

"MGMT promoter methylation testing is particularly useful in treatment decisions for older adult patients with high-grade gliomas (grades 3–4) " (NCCN, 2023).

Codeletion of 1p and 19q

Recommendation: 1p/19q testing is an essential part of molecular diagnostics for oligodendroglioma.

"The codeletion of 1p and 19q is detectable by array-based genomic copy number testing (preferable), or fluorescence in situ hybridization (FISH) … IDH-mutated gliomas that do NOT show loss of *ATRX* (for example, by IHC) should be strongly considered for 1p/19q testing, even if not clearly oligodendroglial by histology. Conversely, *IDH1* wild-type gliomas do not contain true whole-arm 1p/19q codeletion. Therefore, 1p/19q testing is unnecessary if a glioma is definitely *IDH*-wild-type, and a glioma should not be regarded as 1p19q-codeleted without an accompanying *IDH* mutation, regardless of the test results" (NCCN, 2023).

ATRX mutation

"Recommendation: *ATRX* mutation testing is required for the workup of glioma."

"*ATRX* mutations can be detected by IHC for wild-type *ATRX* (loss of wild type expression) and/or sequencing. ATRX mutations in glioma are strongly associated with *IDH* mutations and are nearly always mutually exclusive with 1p/19q codeletion. *ATRX* deficiency, coupled with IDH mutation and *TP53* mutation, is typical of astrocytoma. A lack of *ATRX* immunostaining in glioblastoma should trigger *IDH1/2* sequencing if *IDH1* R132H immunostaining is negative, due to frequent co-occurrence of *ATRX* and *IDH* mutations" (NCCN, 2023).

TERT mutation

"Recommendation: *TERT* promoter mutation testing is required for the workup of gliomas."

"*TERT* promoter mutations are nearly always present in 1p/19q codeleted oligodendroglioma and are found in most glioblastomas. *TERT* promoter mutation, in combination with *IDH* mutation and 1p/19q codeletion, is characteristic of oligodendroglioma. Absence of *TERT* promoter mutation, coupled with the presence of mutant *IDH*, strongly suggests astrocytoma"(NCCN, 2023).

H3F3A and *HIST1H3B* mutation

"Recommendation: *H3-3A* and *HIST1H3B* mutation testing is recommended in the appropriate clinical context."

"Diffuse midline gliomas should be screened for *H3-3A* mutations, specifically the H3K27M mutation. While sequencing is the gold standard, H3K27M-specific IHC, paired with H3K27 trimethylation immunostaining, is a reasonable alternative, especially when tissue is scarce. In these gliomas, H3K27M immunopositivity should be associated with loss of histone trimethylation immunostaining."

"Although a K27M histone antibody is available, it is not 100% specific and interpretation can be difficult for non-experts. Therefore, screening by *H3F3A* and *HIST1H3B* sequencing is a viable alternative and preferred approach, especially since it will also detect mutations in G34" (NCCN, 2023).

"Diagnostic value: Histone mutations most commonly occur in pediatric midline gliomas (e.g., diffuse intrinsic pontine gliomas [DIPG]), although midline gliomas in adults can also contain histone modifications. Their presence can be considered solid evidence of an infiltrative glioma, which is often helpful in small biopsies of midline lesions that may not be fully diagnostic with light microscopy or do not fully resemble infiltrative gliomas."

"Prognostic value: The *K27M* gliomas typically do not have MGMT promoter methylation, and the mutation is an adverse prognostic marker in children and adults. The *G34* mutation does not appear to have any prognostic significance once the diagnosis of a glioblastoma has been established" (NCCN, 2023).

BRAF mutation

"Recommendation: *BRAF* fusion and/or mutation testing is recommended in the appropriate clinical context."

"*BRAF* V600E is best detected by sequencing, and *BRAF* fusions can be detected with RNA-Seq or other PCR-based breakpoint methods that capture the main 16-9, 15-9, and 16-11 breakpoints between *BRAF* and its main fusion partner, *KIAA1549*. FISH is too unreliable to detect *BRAF* fusions" (NCCN, 2023).

"The presence of a *BRAF* fusion is reliable evidence that the tumor is a [pilocytic astrocytoma], provided the histology is compatible. *BRAF* V600E is more complicated, as it can occur in a variety of tumors over all four WHO grades and requires integration with histology."

"Tumors with *BRAF* fusions tend to be indolent, with occasional recurrence but only rare progression to lethality. *BRAF* V600E tumors show a much greater range of outcomes and need to be considered in context with other mutations and clinicopathologic findings (e.g., CDKN2A/B deletion). *BRAF* V600E tumors may respond to BRAF inhibitors, such as vemurafenib, but comprehensive clinical trials are still ongoing" (NCCN, 2023).

ZFTA fusion

"Testing for *ZFTA* and *YAP1* fusions is recommended in the appropriate clinical context. Ependymomas arising in the supratentorium often contain activating fusions of *ZFTA*. This leads to increased NF-kappa-B signaling and more aggressive behavior. This event is more common in children than in adults, and occurs only in the supratentorium, not the posterior fossa or spine" (NCCN,2022).

"*ZFTA* fusion can be detected with RNA sequencing or a break-apart FISH probe set… Detection of *ZFTA* fusion is not required for the diagnosis of ependymoma, as this entity is still diagnosed by light microscopy."

"*ZFTA* fusion-positive ependymomas are now a distinct entity in the WHO classification of CNS tumors, as this subset of ependymomas tends to be far more aggressive than other supratentorial ependymomas, including those with *YAP1* fusions. PFA vs. PFB via methylation profiling is reasonable for posterior fossa ependymoma" (NCCN, 2023).

Finally, the NCCN states there are no identified targeted agents with demonstrated efficacy in glioblastoma (NCCN, 2023).

National Institute for Health and Care Excellence (NICE)

NICE recommends the following molecular markers for investigation of gliomas: *IDH1/2* mutations, *ATRX* mutations, 1p/19q co-deletion, histone H3.3 K27M, *BRAF* mutation, and *MGMT* promoter methylation (for prognosis). NICE also notes that testing *IDH* wild type gliomas for *TERT* promoter mutations may be considered (NICE, 2021).

European Society for Medical Oncology (ESMO)

The ESMO has published clinical practice guidelines for the diagnosis, treatment, and follow-up of high-grade gliomas. They state that *MGMT* promoter methylation status, *IDH1/2* mutation status, and 1p/19q codeletions are "commonly determined" for assessment of gliomas (ESMO, 2014).

World Health Organization (WHO)

In 2016 and 2021, the WHO published guidelines on the classification of central nervous system tumors. These WHO guidelines, for the first time, incorporated molecular testing in the diagnosis of gliomas and medulloblastomas. The following key points were given by the WHO regarding molecular testing:

- "*IDH1* R132H, which accounts for approximately 90% of *IDH* mutations, can be detected immunohistochemically. If this testing is negative, sequencing of *IDH1* and *IDH2* is necessary to ensure that no other *IDH* mutations are present.
- Given the importance of *IDH* mutational status in the diagnosis or gliomas, at a minimum, it will be important that most institutions have the capacity to both stain tumor specimens for *IDH1* R132H by immunohistochemistry and, ideally, sequence those tumors that are negative for both *IDH1* and *IDH2* mutations" (Wen & Huse, 2016).
- "Because of the growing importance of molecular information in CNS tumor classification, diagnoses and diagnostic reports need to combine different data types into a single, 'integrated' diagnosis. To display the full range of diagnostic information available, the use of layered (or tiered) diagnostic reports is strongly encouraged. Such reports feature an integrated diagnosis at the top, followed by layers that display histological, molecular, and other key types of information" (Louis et al., 2021).
- Certain tumors (Diffuse astrocytoma, MYB- or MYBL1-altered; Angiocentric glioma; Polymorphous low-grade neuroepithelial tumor of the young; and Diffuse low-grade glioma, MAPK pathway-altered) "require molecular characterization and the integration of histopathological and molecular information in a tiered diagnostic format as molecular work-up helps to characterize the lesion as one type or the other" (Louis et al., 2021).
- For other tumors such as Myxopapillary ependymoma and Subependymoma, "although these can be identified with methylome studies, molecular classification does not provide added clinicopathological utility for these two tumors" (Louis et al., 2021).
- "Several molecular biomarkers are also associated with classification and grading of meningiomas, including SMARCE1 (clear cell subtype), BAP1 (rhabdoid and papillary subtypes), and KLF4/TRAF7 (secretory subtype) mutations, TERT promoter mutation and/or homozygous deletion of CDKN2A/B62, H3K27me3 loss of nuclear expression (potentially worse prognosis), and methylome profiling (prognostic subtyping)" (Louis et al., 2021).

European Association of Neuro-Oncology (EANO)

In 2021, the EANO published guidelines regarding diagnosis and management of adult patients with diffuse gliomas. The following recommendations were made on molecular testing:

- "Patients with relevant germline variants or suspected hereditary cancer syndromes should receive genetic counselling and might subsequently be referred for molecular genetic testing.
- Immunohistochemistry for mutant *IDH1* R132H protein and nuclear expression of *ATRX* should be performed routinely in the diagnostic assessment of diffuse gliomas.
- If immunohistochemistry for *IDH1* R132H is negative, sequencing of *IDH1* codon 132 and *IDH2* codon 172 should be conducted in all WHO grade 2 and 3 diffuse astrocytic and oligodendroglial gliomas as well as in all glioblastomas of patients aged < 55 years to enable integrated diagnoses according to the WHO classification and to guide treatment decisions.
- 1p/19q codeletion status should be determined in all *IDH*-mutant gliomas with retained nuclear expression of *ATRX*.
- *MGMT* promoter methylation status should be determined in glioblastoma, notably in elderly or frail patients, to aid in decision-making for the use of temozolomide.
- *CDKN2A/B* homozygous deletions should be explored in *IDH*-mutant astrocytomas.
- Combined chromosome seven gain and chromosome 10 loss (+7/–10 signature), *EGFR* amplification and *TERT* promoter mutation should be tested in *IDH*-wild-type diffuse gliomas lacking microvascular proliferation and necrosis as histological features of WHO grade 4 to allow for a diagnosis of *IDH*-wild-type glioblastoma" (Weller et al., 2021).

In addition, EANO published a table to summarize the molecular markers used for the diagnosis and management of gliomas.

Table 1: Molecular Markers for the Diagnosis and Management of Gliomas (Weller et al., 2021)

Molecular Marker	**Diagnostic Roles**
IDH1 R132 or IDH2 R172 mutation	"Distinguishes diffuse gliomas with IDH mutation from IDH-wild-type glioblastomas and other IDH-wild-type gliomas
1p/19q codeletion	Distinguishes oligodendroglioma, IDH-mutant and 1p/19q-codeleted from astrocytoma, IDH-mutant

Loss of nuclear ATRX	Loss of nuclear ATRX in an IDH-mutant glioma is diagnostic for astrocytic lineage tumours
Histone H3 K27M mutation	Defining molecular feature of diffuse midline glioma, H3 K27M-mutant
Histone H3.3 G34R/V mutation	Defining molecular feature of diffuse hemispheric glioma, H3.3 G34-mutant
MGMT promoter methylation	None, but is a predictive biomarker of benefit from alkylating chemotherapy in patients with IDH-wild-type glioblastoma
Homozygous deletion of CDKN2A/CDKN2B	A marker of poor outcome and WHO grade 4 disease in IDH-mutant astrocytomas
EGFR amplification	EGFR amplification occurs in ~40–50% of glioblastoma, IDH wild type Molecular marker of glioblastoma, IDH wild type, WHO grade 4
TERT promotor mutation	TERT promoter mutation occurs in ~70% of glioblastoma, IDH wild type and >95% of oligodendroglioma, IDH-mutant and 1p/19q-codeleted Molecular marker of glioblastoma, IDH wild type, WHO grade 4
+7/–10 cytogenetic signature	Molecular marker of glioblastoma, IDH wild type, WHO grade 4
$BRAF^{V600E}$ mutation	Rare in adult diffuse gliomas but amenable to pharmacological intervention" (Weller et al., 2021).

College of American Pathologists

In 2022, The College of American Pathologists in Collaboration with the American Association of Neuropathologists, Association for Molecular Pathology, and Society for Neuro-Oncology published guidelines that suggests these following recommendations for molecular biomarker testing for the diagnosis of diffuse gliomas:

- "IDH mutational testing must be performed on all diffuse gliomas (DG) (Strong recommendation)"
- "ATRX status should be assessed in all IDH-mutant DG unless they show 1p/19q codeletion (Strong recommendation)"
- "TP53 status should be assessed in all IDH-mutant DGs unless they show 1p/19q codeletion (Conditional recommendation)"
- "1p/19q codeletion must be assessed in IDH-mutant DGs unless they show ATRX loss or *TP53* mutations (Strong recommendation)"

- "*CDKN2A/B* homozygous deletion testing should be performed on IDH-mutant astrocytomas (Conditional recommendation)"
- "*MGMT* promoter methylation testing should be performed on all glioblastoma (GBM), IDH-wild type (WT) (Strong recommendation)"
- "For IDH-mutant DG, *MGMT* promoter methylation testing may not be necessary (Conditional recommendation)"
- "*TERT* promoter mutation testing may be used to provide further support for the diagnosis of oligodendroglioma and IDH-WT GBM (Conditional recommendation)"
- "For histologic grade 2-3 DGs that are IDH-WT, testing should be performed for whole chromosome 7 gain/whole chromosome 10 loss, *EGFR* amplification, and *TERT* promoter mutation to establish the molecular diagnosis of GBM, IDH-WT, grade IV (Strong recommendation)"
- "H3 K27M testing must be performed in DGs that involve the midline in the appropriate clinical and pathologic setting (Strong recommendation)"
- "H3 G34 testing may be performed in pediatric and young adult patients with IDH-WT DG (Conditional recommendation)"
- "*BRAF* mutation testing (V600) may be performed in DGs that are IDH-WT and H3-WT (Conditional recommendation)"
- "*MYB/MYBL1* AND *FGFR1* testing may be performed in children and young adults with DGs that are histologic grade 2-3 and are IDH-WT and H3-WT (Conditional recommendation)" (Brat et al., 2022).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration

has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81120	IDH1 (isocitrate dehydrogenase 1 [NADP+], soluble) (eg, glioma), common variants (eg, R132H, R132C)
81121	IDH2 (isocitrate dehydrogenase 2 [NADP+], mitochondrial) (eg, glioma), common variants (eg, R140W, R172M)
81210	BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer, melanoma), gene analysis, V600 variant(s);
81287	MGMT (O-6-methylguanine-DNA methyltransferase) (eg, glioblastoma multiforme) promoter methylation analysis
81345	TERT (telomerase reverse transcriptase) (eg, thyroid carcinoma, glioblastoma multiforme) gene analysis, targeted sequence analysis (eg, promoter region)
81479	Unlisted molecular pathology procedure ***H3F3A gene sequencing*** ***RELA Testing*** ***H3F3A testing using a K27M histone antibody***
88341	Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)
88342	Immunohistochemistry or immunocytochemistry, per specimen; initial single antibody stain procedure
88374	Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), using computer-assisted technology, per specimen; each multiplex probe stain procedure
88377	Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), manual, per specimen; each multiplex probe stain procedure

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Abedalthagafi, M., Phillips, J. J., Kim, G. E., Mueller, S., Haas-Kogen, D. A., Marshall, R. E., Croul, S. E., Santi, M. R., Cheng, J., Zhou, S., Sullivan, L. M., Martinez-Lage, M., Judkins,

A. R., & Perry, A. (2013). The alternative lengthening of telomere phenotype is significantly associated with loss of ATRX expression in high-grade pediatric and adult astrocytomas: a multi-institutional study of 214 astrocytomas. *Mod Pathol*, *26*(11), 1425-1432. https://doi.org/10.1038/modpathol.2013.90

American Cancer Society. (2024). Key Statistics for Brain and Spinal Cord Tumors. https://www.cancer.org/cancer/types/brain-spinal-cord-tumors-adults/about/key-statistics.html

Arita, H., Narita, Y., Takami, H., Fukushima, S., Matsushita, Y., Yoshida, A., Miyakita, Y., Ohno, M., Shibui, S., & Ichimura, K. (2013). TERT promoter mutations rather than methylation are the main mechanism for TERT upregulation in adult gliomas. *Acta Neuropathol*, *126*(6), 939-941. https://doi.org/10.1007/s00401-013-1203-9

Back, M., Jayamanne, D., Brazier, D., Newey, A., Bailey, D., Schembri, G., Hsiao, E., Khasraw, M., Wong, M., Kastelan, M., Brown, C., & Wheeler, H. (2020). Pattern of failure in anaplastic glioma patients with an IDH1/2 mutation. *Strahlenther Onkol*, *196*(1), 31-39. https://doi.org/10.1007/s00066-019-01467-0 (Rezidivmuster bei Patienten mit anaplastischem Glioma mit einer IDH1/2-Mutation.)

Batchelor, T., & Louis, D. N. (2023, March 16). *Molecular pathogenesis of diffuse gliomas*. https://www.uptodate.com/contents/molecular-pathogenesis-of-diffuse-gliomas

Brat, D. J., Aldape, K., Bridge, J. A., Canoll, P., Colman, H., Hameed, M. R., Harris, B. T., Hattab, E. M., Huse, J. T., Jenkins, R. B., Lopez-Terrada, D. H., McDonald, W. C., Rodriguez, F. J., Souter, L. H., Colasacco, C., Thomas, N. E., Yount, M. H., van den Bent, M. J., & Perry, A. (2022). Molecular Biomarker Testing for the Diagnosis of Diffuse Gliomas. *Arch Pathol Lab Med*, *146*(5), 547-574. https://doi.org/10.5858/arpa.2021-0295-CP

Brat, D. J., Verhaak, R. G., Aldape, K. D., Yung, W. K., Salama, S. R., Cooper, L. A., Rheinbay, E., Miller, C. R., Vitucci, M., Morozova, O., Robertson, A. G., Noushmehr, H., Laird, P. W., Cherniack, A. D., Akbani, R., Huse, J. T., Ciriello, G., Poisson, L. M., Barnholtz-Sloan, J. S., . . . Radenbaugh, A. J. (2015). Comprehensive, Integrative Genomic Analysis of Diffuse Lower-Grade Gliomas. *N Engl J Med*, *372*(26), 2481-2498. https://doi.org/10.1056/NEJMoa1402121

Burger, P. C., Minn, A. Y., Smith, J. S., Borell, T. J., Jedlicka, A. E., Huntley, B. K., Goldthwaite, P. T., Jenkins, R. B., & Feuerstein, B. G. (2001). Losses of chromosomal arms 1p and 19q in the diagnosis of oligodendroglioma. A study of paraffin-embedded sections. *Mod Pathol*, *14*(9), 842-853. https://doi.org/10.1038/modpathol.3880400

Cabezas-Camarero, S., García-Barberán, V., Pérez-Alfayate, R., Lopez Cade, I., Tejerina-Peces, J., Casado-Fariñas, I., & Pérez-Segura, P. (2021). 34P Dynamic changes in plasma PD-L1 in patients with gliomas: Prognostic value and association with IDH status. *Annals of Oncology*. https://doi.org/10.1016/j.annonc.2021.10.050

Cairncross, J. G., Ueki, K., Zlatescu, M. C., Lisle, D. K., Finkelstein, D. M., Hammond, R. R., Silver, J. S., Stark, P. C., Macdonald, D. R., Ino, Y., Ramsay, D. A., & Louis, D. N. (1998). Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. *J Natl Cancer Inst*, *90*(19), 1473-1479. https://doi.org/10.1093/jnci/90.19.1473

Chappe, C., Padovani, L., Scavarda, D., Forest, F., Nanni-Metellus, I., Loundou, A., Mercurio, S., Fina, F., Lena, G., Colin, C., & Figarella-Branger, D. (2013). Dysembryoplastic neuroepithelial tumors share with pleomorphic xanthoastrocytomas and gangliogliomas BRAF(V600E) mutation and expression. *Brain Pathol*, *23*(5), 574-583. https://doi.org/10.1111/bpa.12048

Chi, A. S., Batchelor, T. T., Yang, D., Dias-Santagata, D., Borger, D. R., Ellisen, L. W., Iafrate, A. J., & Louis, D. N. (2013). BRAF V600E mutation identifies a subset of low-grade diffusely infiltrating gliomas in adults. *J Clin Oncol*, *31*(14), e233-236. https://doi.org/10.1200/jco.2012.46.0220

Dang, L., White, D. W., Gross, S., Bennett, B. D., Bittinger, M. A., Driggers, E. M., Fantin, V. R., Jang, H. G., Jin, S., Keenan, M. C., Marks, K. M., Prins, R. M., Ward, P. S., Yen, K. E., Liau, L. M., Rabinowitz, J. D., Cantley, L. C., Thompson, C. B., Vander Heiden, M. G., & Su, S. M. (2009). Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature*, *462*(7274), 739-744. https://doi.org/10.1038/nature08617

Davies, H., Bignell, G. R., Cox, C., Stephens, P., Edkins, S., Clegg, S., Teague, J., Woffendin, H., Garnett, M. J., Bottomley, W., Davis, N., Dicks, E., Ewing, R., Floyd, Y., Gray, K., Hall, S., Hawes, R., Hughes, J., Kosmidou, V., . . . Futreal, P. A. (2002). Mutations of the BRAF gene in human cancer. *Nature*, *417*(6892), 949-954. https://doi.org/10.1038/nature00766

Eckel-Passow, J. E., Lachance, D. H., Molinaro, A. M., Walsh, K. M., Decker, P. A., Sicotte, H., Pekmezci, M., Rice, T., Kosel, M. L., Smirnov, I. V., Sarkar, G., Caron, A. A., Kollmeyer, T. M., Praska, C. E., Chada, A. R., Halder, C., Hansen, H. M., McCoy, L. S., Bracci, P. M., . . . Jenkins, R. B. (2015). Glioma Groups Based on 1p/19q, IDH, and TERT Promoter Mutations in Tumors. *N Engl J Med*, *372*(26), 2499-2508. https://doi.org/10.1056/NEJMoa1407279

ESMO. (2014). High-grade glioma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. https://www.annalsofoncology.org/article/S0923-7534(19)34077-3/fulltext

Esteller, M., Garcia-Foncillas, J., Andion, E., Goodman, S. N., Hidalgo, O. F., Vanaclocha, V., Baylin, S. B., & Herman, J. G. (2000). Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *N Engl J Med*, *343*(19), 1350-1354. https://doi.org/10.1056/nejm200011093431901

Gusyatiner, O., & Hegi, M. E. (2018). Glioma epigenetics: From subclassification to novel treatment options. *Semin Cancer Biol*, *51*, 50-58. https://doi.org/10.1016/j.semcancer.2017.11.010

Hawkins, C., Walker, E., Mohamed, N., Zhang, C., Jacob, K., Shirinian, M., Alon, N., Kahn, D., Fried, I., Scheinemann, K., Tsangaris, E., Dirks, P., Tressler, R., Bouffet, E., Jabado, N., & Tabori, U. (2011). BRAF-KIAA1549 fusion predicts better clinical outcome in pediatric low-grade astrocytoma. *Clin Cancer Res*, *17*(14), 4790-4798. https://doi.org/10.1158/1078-0432.ccr-11-0034

Hegi, M. E., Diserens, A. C., Gorlia, T., Hamou, M. F., de Tribolet, N., Weller, M., Kros, J. M., Hainfellner, J. A., Mason, W., Mariani, L., Bromberg, J. E., Hau, P., Mirimanoff, R. O., Cairncross, J. G., Janzer, R. C., & Stupp, R. (2005). MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med*, *352*(10), 997-1003. https://doi.org/10.1056/NEJMoa043331

Horbinski, C. (2013a). To BRAF or not to BRAF: is that even a question anymore? *J Neuropathol Exp Neurol*, *72*(1), 2-7. https://doi.org/10.1097/NEN.0b013e318279f3db

Horbinski, C. (2013b). What do we know about IDH1/2 mutations so far, and how do we use it? *Acta Neuropathol*, *125*(5), 621-636. https://doi.org/10.1007/s00401-013-1106-9

Houdova Megova, M., Drabek, J., Dwight, Z., Trojanec, R., Koudelakova, V., Vrbkova, J., Kalita, O., Mlcochova, S., Rabcanova, M., & Hajduch, M. (2017). Isocitrate Dehydrogenase Mutations are Better Prognostic Marker than O6-methylguanine-DNA Methyltransferase Promoter Methylation in Glioblastomas - a Retrospective, Single-centre Molecular Genetics Study of Gliomas. *Klin Onkol*, *30*(5), 361-371. https://doi.org/10.14735/amko2017361 (Mutace isocitratdehydrogenazy jsou lepsi prognosticky marker nez metylace promotoru O6-metylguanin-DNA-metyltransferazy u glioblastomu - retrospektivni molekularne geneticka studie gliomu z jednoho centra.)

Jenkins, R. B., Blair, H., Ballman, K. V., Giannini, C., Arusell, R. M., Law, M., Flynn, H., Passe, S., Felten, S., Brown, P. D., Shaw, E. G., & Buckner, J. C. (2006). A t(1;19)(q10;p10) mediates the combined deletions of 1p and 19q and predicts a better prognosis of patients with oligodendroglioma. *Cancer Res*, *66*(20), 9852-9861. https://doi.org/10.1158/0008-5472.can-06-1796

Ji, J., Kaneva, K., Hiemenz, M. C., Dhall, G., Davidson, T. B., Erdreich-Epstein, A., Hawes, D., Hurth, K., Margol, A. S., Mathew, A. J., Robison, N. J., Schmidt, R. J., Tran, H. N., Judkins, A. R., Cotter, J. A., & Biegel, J. A. (2021). Clinical utility of comprehensive genomic profiling in central nervous system tumors of children and young adults. *Neuro-Oncology Advances*, *3*(1). https://doi.org/10.1093/noajnl/vdab037

Johnson, A., Severson, E., Gay, L., Vergilio, J. A., Elvin, J., Suh, J., Daniel, S., Covert, M., Frampton, G. M., Hsu, S., Lesser, G. J., Stogner-Underwood, K., Mott, R. T., Rush, S. Z., Stanke, J. J., Dahiya, S., Sun, J., Reddy, P., Chalmers, Z. R., . . . Ramkissoon, S. H. (2017). Comprehensive Genomic Profiling of 282 Pediatric Low- and High-Grade Gliomas Reveals Genomic Drivers, Tumor Mutational Burden, and Hypermutation Signatures. *Oncologist*, *22*(12), 1478-1490. https://doi.org/10.1634/theoncologist.2017-0242

Jones, D. T., Kocialkowski, S., Liu, L., Pearson, D. M., Backlund, L. M., Ichimura, K., & Collins, V. P. (2008). Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. *Cancer Res*, *68*(21), 8673-8677. https://doi.org/10.1158/0008-5472.can-08-2097

Lassaletta, A., Zapotocky, M., Mistry, M., Ramaswamy, V., Honnorat, M., Krishnatry, R., Guerreiro Stucklin, A., Zhukova, N., Arnoldo, A., Ryall, S., Ling, C., McKeown, T., Loukides, J., Cruz, O., de Torres, C., Ho, C. Y., Packer, R. J., Tatevossian, R., Qaddoumi, I., . . . Tabori, U. (2017). Therapeutic and Prognostic Implications of BRAF V600E in Pediatric Low-Grade Gliomas. *J Clin Oncol*, *35*(25), 2934-2941. https://doi.org/10.1200/jco.2016.71.8726

Louis, D., Perry, A., Reifenberger, G., von Deimling, A., Figarella-Branger, D., Cavenee, W. K., Ohgaki, H., Wiestler, O. D., Kleihues, P., & Ellison, D. W. (2016). The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol*, *131*(6), 803-820. https://doi.org/10.1007/s00401-016-1545-1

Louis, D., Schiff, D., & Batchelor, T. (2023, August 18). *Classification and pathologic diagnosis of gliomas, glioneuronal tumors, and neuronal tumors*. https://www.uptodate.com/contents/classification-and-pathologic-diagnosis-of-gliomas-glioneuronal-tumors-and-neuronal-tumors

Louis, D. N., Perry, A., Wesseling, P., Brat, D. J., Cree, I. A., Figarella-Branger, D., Hawkins, C., Ng, H. K., Pfister, S. M., Reifenberger, G., Soffietti, R., von Deimling, A., & Ellison, D. W. (2021). The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. *Neuro-Oncology*, *23*(8), 1231-1251. https://doi.org/10.1093/neuonc/noab106

Malgulwar, P. B., Nambirajan, A., Pathak, P., Faruq, M., Rajeshwari, M., Singh, M., Suri, V., Sarkar, C., & Sharma, M. C. (2018). C11orf95-RELA fusions and upregulated NF-KB signalling characterise a subset of aggressive supratentorial ependymomas that express L1CAM and nestin. *J Neurooncol*, *138*(1), 29-39. https://doi.org/10.1007/s11060-018-2767-y

Malmstrom, A., Gronberg, B. H., Marosi, C., Stupp, R., Frappaz, D., Schultz, H., Abacioglu, U., Tavelin, B., Lhermitte, B., Hegi, M. E., Rosell, J., & Henriksson, R. (2012). Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy in patients older than 60 years with glioblastoma: the Nordic randomised, phase 3 trial. *Lancet Oncol*, *13*(9), 916-926. https://doi.org/10.1016/s1470-2045(12)70265-6

Meyronet, D., Esteban-Mader, M., Bonnet, C., Joly, M. O., Uro-Coste, E., Amiel-Benouaich, A., Forest, F., Rousselot-Denis, C., Burel-Vandenbos, F., Bourg, V., Guyotat, J., Fenouil, T., Jouvet, A., Honnorat, J., & Ducray, F. (2017). Characteristics of H3 K27M-mutant gliomas in adults. *Neuro Oncol*, *19*(8), 1127-1134. https://doi.org/10.1093/neuonc/now274

Miller, A. M., Shah, R. H., Pentsova, E. I., Pourmaleki, M., Briggs, S., Distefano, N., Zheng, Y., Skakodub, A., Mehta, S. A., Campos, C., Hsieh, W.-Y., Selcuklu, S. D., Ling, L., Meng, F., Jing, X., Samoila, A., Bale, T. A., Tsui, D. W. Y., Grommes, C., . . . Mellinghoff, I. K. (2019). Tracking tumour evolution in glioma through liquid biopsies of cerebrospinal fluid.

Muralidharan, K., Yekula, A., Small, J. L., Rosh, Z. S., Kang, K. M., Wang, L., Lau, S., Zhang, H., Lee, H., Bettegowda, C., Chicoine, M. R., Kalkanis, S. N., Shankar, G. M., Nahed, B. V., Curry, W. T., Jones, P. S., Cahill, D. P., Balaj, L., & Carter, B. S. (2021). TERT Promoter Mutation Analysis for Blood-Based Diagnosis and Monitoring of Gliomas. *Clinical Cancer Research*, *27*(1), 169-178. https://doi.org/10.1158/1078-0432.Ccr-20-3083

NCBI. (2011). RELA - RELA proto-oncogene, NF-kB subunit. https://www.ncbi.nlm.nih.gov/gene/5970/ortholog/?scope=117570

NCCN. (2023, March 24). *NCCN Clinical Practice Guidelines in Oncology; Central Nervous System Cancers v1.2023*. National Comprehensive Cancer Network. https://www.nccn.org/professionals/physician_gls/pdf/cns.pdf

NICE. (2021). Brain tumours (primary) and brain metastases in adults. https://www.nice.org.uk/guidance/ng99/chapter/Recommendations

Nikiforova, M. N., Wald, A. I., Melan, M. A., Roy, S., Zhong, S., Hamilton, R. L., Lieberman, F. S., Drappatz, J., Amankulor, N. M., Pollack, I. F., Nikiforov, Y. E., & Horbinski, C. (2016). Targeted next-generation sequencing panel (GlioSeq) provides comprehensive genetic profiling of central nervous system tumors. *Neuro Oncol*, *18*(3), 379-387. https://doi.org/10.1093/neuonc/nov289

Parikh, N., Hilsenbeck, S., Creighton, C. J., Dayaram, T., Shuck, R., Shinbrot, E., Xi, L., Gibbs, R. A., Wheeler, D. A., & Donehower, L. A. (2014). Effects of TP53 Mutational Status on Gene Expression Patterns Across Ten Human Cancer Types. *J Pathol*, *232*(5), 522-533. https://doi.org/10.1002/path.4321

Ramkissoon, S. H., Bandopadhayay, P., Hwang, J., Ramkissoon, L. A., Greenwald, N. F., Schumacher, S. E., O'Rourke, R., Pinches, N., Ho, P., Malkin, H., Sinai, C., Filbin, M., Plant, A., Bi, W. L., Chang, M. S., Yang, E., Wright, K. D., Manley, P. E., Ducar, M., . . . Ligon, K. L. (2017). Clinical targeted exome-based sequencing in combination with genome-wide copy number profiling: precision medicine analysis of 203 pediatric brain tumors. *Neuro Oncol*, *19*(7), 986-996. https://doi.org/10.1093/neuonc/now294

Reuss, D. E., Sahm, F., Schrimpf, D., Wiestler, B., Capper, D., Koelsche, C., Schweizer, L., Korshunov, A., Jones, D. T., Hovestadt, V., Mittelbronn, M., Schittenhelm, J., Herold-Mende, C., Unterberg, A., Platten, M., Weller, M., Wick, W., Pfister, S. M., & von Deimling, A. (2015). ATRX and IDH1-R132H immunohistochemistry with subsequent copy number analysis and IDH sequencing as a basis for an "integrated" diagnostic approach for adult astrocytoma, oligodendroglioma and glioblastoma. *Acta Neuropathol*, *129*(1), 133-146. https://doi.org/10.1007/s00401-014-1370-3

Rios, J. D., Velummailum, R., Bennett, J., Nobre, L., Tsang, D. S., Bouffet, E., Hawkins, C., Tabori, U., Denburg, A., & Pechlivanoglou, P. (2022). Clinical and economic impact of molecular testing for BRAF fusion in pediatric low-grade Glioma. *BMC Pediatr*, *22*(1), 13. https://doi.org/10.1186/s12887-021-03069-1

Ryall, S., Krishnatry, R., Arnoldo, A., Buczkowicz, P., Mistry, M., Siddaway, R., Ling, C., Pajovic, S., Yu, M., Rubin, J. B., Hukin, J., Steinbok, P., Bartels, U., Bouffet, E., Tabori, U., & Hawkins, C. (2016). Targeted detection of genetic alterations reveal the prognostic impact of H3K27M and MAPK pathway aberrations in paediatric thalamic glioma. *Acta Neuropathol Commun*, *4*(1), 93. https://doi.org/10.1186/s40478-016-0353-0

Schwartzentruber, J., Korshunov, A., Liu, X. Y., Jones, D. T., Pfaff, E., Jacob, K., Sturm, D., Fontebasso, A. M., Quang, D. A., Tonjes, M., Hovestadt, V., Albrecht, S., Kool, M., Nantel, A., Konermann, C., Lindroth, A., Jager, N., Rausch, T., Ryzhova, M., . . . Jabado, N. (2012). Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature*, *482*(7384), 226-231. https://doi.org/10.1038/nature10833

Simonelli, M., Dipasquale, A., Orzan, F., Lorenzi, E., Persico, P., Navarria, P., Pessina, F., Nibali, M. C., Bello, L., Santoro, A., & Boccaccio, C. (2020). Cerebrospinal fluid tumor DNA for liquid biopsy in glioma patients' management: Close to the clinic? *Crit Rev Oncol Hematol*, *146*, 102879. https://doi.org/10.1016/j.critrevonc.2020.102879

Sturm, D., Witt, H., Hovestadt, V., Khuong-Quang, D. A., Jones, D. T., Konermann, C., Pfaff, E., Tonjes, M., Sill, M., Bender, S., Kool, M., Zapatka, M., Becker, N., Zucknick, M., Hielscher, T., Liu, X. Y., Fontebasso, A. M., Ryzhova, M., Albrecht, S., . . . Pfister, S. M. (2012). Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. *Cancer Cell*, *22*(4), 425-437. https://doi.org/10.1016/j.ccr.2012.08.024

Wang, L., Liu, L., Li, H., Wang, P., Hu, Z., Wei, Y., Zhang, M., Wen, W., Li, Z., Liu, L., Zhao, L., Lu, D., & Teng, L. (2019). RELA Fusion in Supratentorial Extraventricular Ependymomas: A Morphologic, Immunohistochemical, and Molecular Study of 43 Cases. *Am J Surg Pathol*, *43*(12), 1674-1681. https://doi.org/10.1097/pas.0000000000001342

Weinberg, D. N., Allis, C. D., & Lu, C. (2017). Oncogenic Mechanisms of Histone H3 Mutations. *Cold Spring Harb Perspect Med*, *7*(1). https://doi.org/10.1101/cshperspect.a026443

Weller, M., van den Bent, M., Preusser, M., Le Rhun, E., Tonn, J. C., Minniti, G., Bendszus, M., Balana, C., Chinot, O., Dirven, L., French, P., Hegi, M. E., Jakola, A. S., Platten, M., Roth, P., Rudà, R., Short, S., Smits, M., Taphoorn, M. J. B., . . . Wick, W. (2021). EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. *Nature Reviews Clinical Oncology*, *18*(3), 170-186. https://doi.org/10.1038/s41571-020-00447-z

Wen, P. Y., & Huse, J. T. (2016). 2016 World Health Organization Classification of Central Nervous System Tumors. *Continuum (Minneap Minn)*, *23*(6, Neuro-oncology), 1531-1547. https://doi.org/10.1212/con.0000000000000536

Wick, W., Platten, M., Meisner, C., Felsberg, J., Tabatabai, G., Simon, M., Nikkhah, G., Papsdorf, K., Steinbach, J. P., Sabel, M., Combs, S. E., Vesper, J., Braun, C., Meixensberger, J., Ketter, R., Mayer-Steinacker, R., Reifenberger, G., & Weller, M. (2012). Temozolomide chemotherapy alone versus radiotherapy alone for malignant astrocytoma in the elderly: the NOA-08 randomised, phase 3 trial. *Lancet Oncol*, *13*(7), 707-715. https://doi.org/10.1016/s1470-2045(12)70164-x

Wick, W., Weller, M., van den Bent, M., Sanson, M., Weiler, M., von Deimling, A., Plass, C., Hegi, M., Platten, M., & Reifenberger, G. (2014). MGMT testing--the challenges for biomarker-based glioma treatment. *Nat Rev Neurol*, *10*(7), 372-385. https://doi.org/10.1038/nrneurol.2014.100

Zacher, A., Kaulich, K., Stepanow, S., Wolter, M., Kohrer, K., Felsberg, J., Malzkorn, B., & Reifenberger, G. (2017). Molecular Diagnostics of Gliomas Using Next Generation Sequencing of a Glioma-Tailored Gene Panel. *Brain Pathol*, *27*(2), 146-159. https://doi.org/10.1111/bpa.12367

Zhao, H., Wang, S., Song, C., Zha, Y., & Li, L. (2016). The prognostic value of MGMT promoter status by pyrosequencing assay for glioblastoma patients' survival: a meta-analysis. *World J Surg Oncol*, *14*(1), 261. https://doi.org/10.1186/s12957-016-1012-4

Molecular Diagnostics for Breast Cancer Prognosis

Policy Number: AHS – M2020 – Molecular Diagnostics for Breast Cancer Prognosis	Prior Policy Name and Number, as applicable: • Molecular Expression Testing for Breast Cancer Prognosis • Gene Expression Testing for Breast Cancer Prognosis
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Molecular diagnostics for breast cancer management is emerging as a useful tool to aid in disease prognosis or therapeutic efficacy on an individual level. Many of the currently available molecular tests are gene expression assays that measure the number of transcribed copies of specific mRNAs to assess the genes that are active in tumor tissue, which can be clinically useful for disease classification, diagnosis, and prognosis, as well as for tailoring treatment to underlying genetic determinants of pharmacologic response (Steiling & Christenson, 2023).

II. Related Policies

Policy Number	Policy Title
AHS-G2059	Epithelial Cell Cytology in Breast Cancer Risk Assessment
AHS-M2003	Genetic Testing for Breast, Ovarian, Pancreatic, and Prostate Cancers
AHS-M2126	Use Of Common Genetic Variants (Single Nucleotide Polymorphisms) To Predict Risk of Non-Familial Breast Cancer

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with newly diagnosed breast cancer, tumor testing for hormone receptor (estrogen receptor and progesterone receptor) expression and human

epidermal growth factor receptor 2 (HER2) overexpression **MEETS COVERAGE CRITERIA**.

2) For individuals who have been diagnosed with invasive breast cancer and who are being considered for adjuvant chemotherapy, use of the Oncotype DX 21-gene expression assay (see Note 1) **MEETS COVERAGE CRITERIA** when **all** of the following criteria are also met:
   a) Histology that is ductal/no special type (NST) (see Note 2), lobular, mixed or micropapillary.
   b) pT1, pT2, or pT3 cancer.
   c) Hormone receptor (HR) positive (either estrogen-receptor [ER] or progesterone-receptor [PR] positive) cancer.
   d) Human epidermal growth factor receptor two (HER2) negative cancer.
   e) Tumor is **one** of the following:
      i) pN0 tumor that is >0.5 cm in size.
      ii) pN1mi (≤2 mm axillary node metastases).
      iii) pN1 (1-3 positive nodes).

3) For individuals who have been diagnosed with pT1, pT2, or operable pT3, HR positive, HER2-negative, **and** node-negative or pN1 (1-3 positive nodes) breast cancer and who have been determined to be at high clinical risk per MINDACT categorization (see Note 3), use of the MammaPrint assay **MEETS COVERAGE CRITERIA**.

4) For individuals who have been diagnosed with T1 or T2, HR positive, HER2-negative, **and** node-negative or pN1 (1-3 positive nodes) breast cancer, use of the 12-gene (EndoPredict) assay **or** the 50-gene (Prosigna) assay **MEETS COVERAGE CRITERIA**.

5) For individuals who have been diagnosed with T1-T3, HR-positive, HER2-negative breast cancer, use of the Breast Cancer Index (BCI) test to determine the benefit of extended adjuvant endocrine therapy **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

6) In males, use of gene expression assays other than 21-gene RT-PCR-based assays **DO NOT MEET COVERAGE CRITERIA**.

7) Use of gene expression assays not mentioned above (e.g., Mammostrat, BluePrint®) **DOES NOT MEET COVERAGE CRITERIA**.

8) The use of Oncotype DX for ductal carcinoma in situ (DCIS) **DOES NOT MEET COVERAGE CRITERIA**.

9) For all other indications or in any other situation not mentioned above, use of gene expression assays **DOES NOT MEET COVERAGE CRITERIA**.

10) Use of protein profiling assays (e.g., DCISionRT®, BBDRisk Dx®) **DOES NOT MEET COVERAGE CRITERIA.**

NOTES:

Note 1: The assay should only be ordered on a tissue specimen obtained during surgical removal of the tumor and after subsequent pathology examination of the tumor has been completed and determined to meet the above criteria (i.e., the test should not be ordered on a preliminary core biopsy without surgical staging). For patients who have multiple primary tumors, a specimen from the tumor with the most aggressive histological characteristics should be submitted for testing. It is not necessary to conduct testing on each tumor; treatment is based on the most aggressive lesion.

Note 2: As per NCCN Clinical Practice Guidelines in Oncology (Breast Cancer), "According to WHO, carcinoma of no special type (NST) encompasses multiple patterns including medullary pattern, cancers with neuroendocrine expression, and other rare patterns" (NCCN, 2024).

Note 3: As per NCCN Clinical Practice Guidelines in Oncology (Breast Cancer), "High clinical risk in the MINDACT trial was defined for grade 1 tumors as >3 cm N0 or T2N1, for grade 2 tumors T2N0-1, and for grade 3 tumors T1c-2N0-1" (NCCN, 2024).

IV. Table of Terminology

Term	Definition
ABHD14A	*Abhydrolase domain containing 14A* gene
ACS	American Cancer Society
AI	Aromatase inhibitor
ASCO	American Society of Clinical Oncology
ATP6V0A4	*ATPase H+ transporting V0 subunit a4* gene
AUC	Areas under the curve
AZGP1	*Alpha-2-glycoprotein 1, zinc-binding* gene

BCI	Breast cancer index
BEND3	*BEN domain containing 3* gene
BIRC5	*Baculoviral IAP repeat containing 5* gene
BTG2	*BTG anti-proliferation factor 2* gene
BUB1B	*BUB1 mitotic checkpoint serine/threonine kinase B* gene
CDYL	*Chromodomain Y like* gene
CEF	Cyclophosphamide, epirubicin and fluorouracil
CENPA	*Centromere protein A*
CET	Chemoendocrine Therapy
CHAD	*Chondroadherin* gene
CHER-LOB	Chemotherapy, 4erceptin, and lapatinib in operable breast cancer
CMF	Cyclophosphamide, methotrexate and fluorouracil
CNB	Core needle biopsy
CTCs	Circulating tumour cells
CTS	Clinical treatment score
DCIS	Ductal carcinoma in situ
DHCR7	*7-dehydrocholesterol reductase* gene
DRFR	Distant recurrence free rate
EBC	Early HER2-negative breast cancer
EET	Extended Endocrine Therapy
EGAPP	Evaluation of Genomic Applications in Practice and Prevention
EGTM	European Group on Tumour Markers
ER	Estrogen receptor
ESMO	European Society for Medical Oncology
ET	Endocrine therapy
FDA	Food and Drug Association
FNAB	Fine-needle aspirate biopsy
HER2	Human epidermal growth factor receptor two
HER2DX	Model for predicting disease-free survival
HR	Hazard ratio
IDFS	Invasive disease-free survival
IDUA	*Alpha-L-iduronidase* gene
IHC4	Immunohistochemistry 4
IL6ST	*Interleukin 6 cytokine family signal transducer* gene
InvBE	Ipsilateral breast event
KIF18A	*Kinesin family member 18A* gene
LN	Lymph node
MFS	Metastasis free survival
MGI	Molecular grade index

MGP	*Matrix Gla protein* gene
MINDACT	Microarray in node negative disease may avoid chemotherapy
mRNAs	Messenger ribonucleic acid
MYL5	*Myosin light chain 5* gene
NaCT	Neoadjuvant chemotherapy
NCCN	National Comprehensive Cancer Network
NEK2	*NIMA related kinase 2* gene
NET	Neoadjuvant endocrine therapy
NICE	National Institute for Health and Care Excellence
NPI	Nottingham prognostic index
NST	No special type
PAI-1	Plasminogen Activator Inhibitor Type 1
PAM50	Prosigna assay looking at the activity of 50 genes
PMBC	Patients with pure mucinous breast cancer
PR	Progesterone Receptor
PREP	*Prolyl endopeptidase*
RACGAP1	*Rac GTPase activating protein 1* gene
RBBP8	*RB binding protein 8, endonuclease* gene
RCB	Residual cancer burden
ROR	Risk of recurrence
RRM2	*Ribonucleotide reductase regulatory subunit M2* gene
RS	Recurrence Score
RTN4IP1	*Reticulon 4 interacting protein 1* gene
RT-PCR	Reverse transcriptase/transcription polymerase chain reaction
S100PBP	*S100P binding protein* gene
STC2	*Stanniocalcin 2* gene
TAILORx	Trial assigning individualized options for treatment (Rx)
TotBE	Total breast event
TPRG1	*Tumor protein p63 regulated 1* gene
UBE2C	*Ubiquitin conjugating enzyme E2 C* gene
uPA	Urokinase plasminogen activator
WDR6	*WD repeat domain 6* gene

V. Scientific Background

Breast cancer is the most frequently diagnosed cancer and is the leading cause of cancer death globally in women. Approximately one in eight women will develop breast cancer in their lifetime (Siegel et al., 2019; Taghian & Merajver, 2024).

While adjuvant systemic therapy has reduced mortality from breast cancer (Darby et al., 2011; Davies et al., 2011; Forouzanfar et al., 2011; Peto et al., 2012), it is not without its risks and costs; therefore, reliable prognostic profiles for recurrence and clinically applicable predictive factors are of great value in the management of the disease (Foukakis & Bergh, 2022).

Several biology-based prognostic profiles have been developed, validated, and are in clinical use to predict breast cancer recurrence or response to chemotherapy. Among the most useful biomarkers for this purpose are the estrogen receptors (ER),progesterone receptors (PR), and the human epidermal growth factor receptor two (HER2). Tumors that express one or more of these proteins may have different sensitivities to certain cytotoxic chemotherapeutic agents, influencing the strategic management of the cancer. Moreover, many proprietary panels that aim to provide predictive and prognostic information about an individual's cancer are only suitable for use in tumors of a particular type (i.e., HER2-negative). Intensive research efforts are ongoing to refine the clinical utility and the indications for these predictive and prognostic profiles (Simon et al., 2009). In addition, as next generation sequencing of tumor genomes progresses, these profiles will be improved or replaced by the next generation of molecular profiles (Foukakis & Bergh, 2022).

Chen et al. (2018) examined the association of genomic methylation and the changes in the subsequent transcriptome. The authors observed how a chronic condition influenced epigenomic changes. A human volunteer provided peripheral blood samples over 36 months and the authors created 28 methylome datasets as well as 57 transcriptome datasets. During this period, the human volunteer experienced six viral infections and two elevated periods of fasting glucose and glycated hemoglobin A1c. The authors noted that the methylome changes correlated with the glucose level changes and that the gene expression levels varied greatly, often during the viral infections. The authors proposed that the DNA methylation was the primary gene expression mechanism for chronic conditions, as it was generally a stable epigenetic marker (Chen et al., 2018).

Rueda et al. (2019) presented a disease model that stratifies distinct stages and incorporated factors such as "locoregional recurrence, distant recurrence, breast-cancer-related death and death from other causes" (Rueda et al., 2019). This model integrated these features into an individual risk-of-recurrence prediction and was applied to 3240 patients, of which 1980 had molecular data. From this model, the authors identified four late-recurring "integrative subtypes, comprising about one quarter (26%) of tumours that are both positive for ER and negative for human epidermal growth factor receptor 2, each with characteristic tumour-driving alterations in genomic copy number and a high risk of recurrence (mean 47–62%) up to 20 years

after diagnosis." These four subtypes were enriched in copy number alterations, and these alterations were labeled the likely drivers of each subgroup. A triple negative subgroup, IntCluster 10, was found to be largely relapse-free after five years whereas the IntCluster4 ER negative subgroup was found to have a large risk of recurrence (Rueda et al., 2019).

Innovative ideas continue to spread as the field burgeons, not limiting its inspiration to etiology. Savci-Heijink et al. (2019) believe in the clinical value of identifying a gene expression profile in tumors that selects a subpopulation of patients that is more likely to develop visceral organ metastases due to their association with poor survival outcomes in breast cancers. The researchers analyzed the genetic profiles of 157 primary tumors from breast cancer patients who developed distant metastases and differentially expressed genes between the group of tumors with visceral metastasis and those without visceral metastases were noted. The results yielded fourteen differentially expressed genes (*WDR6, CDYL, ATP6V0A4, CHAD, IDUA, MYL5, PREP, RTN4IP1, BTG2, TPRG1, ABHD14A, KIF18A, S100PBP* and *BEND3*) between the tumors with visceral metastasis and those without visceral metastases. According to the data, five of the genes (*CDYL, ATP6V0A4, PREP, RTN4IP1* and *KIF18A*) were upregulated while the other genes were downregulated, creating a list of biomarkers and genetic signatures of interest that may result in earlier diagnosis and treatment (Savci-Heijink et al., 2019).

Proprietary Testing

OncoType DX

The Oncotype Dx 21-gene recurrence score (RS) is the best-validated prognostic assay and may identify patients who are most and least likely to derive benefit from adjuvant chemotherapy. The expression levels of 16 genes (plus five reference genes) are measured by quantitative reverse transcription polymerase chain reaction (RT-PCR). The sum of this calculation is known as the RS to optimize prediction of distant relapse despite tamoxifen therapy. At this time, it is indicated for individuals with node-negative, estrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negative breast cancer to determine the prognosis in patients recommended to proceed with at least a five-year course of endocrine therapy. The optimal RS cutoff for omission of chemotherapy remains unclear given that the different studies have used different cutoffs (Mamounas et al., 2010; Paik et al., 2004; Paik et al., 2006; Sparano et al., 2015). However, it may be reasonable not to administer adjuvant chemotherapy for patients with node-negative, ER-positive breast cancer and an RS of <15 (Foukakis & Bergh, 2022).

Sparano et al. (2018) performed a prospective trial to assess the utility of the recurrence score based on the 21 gene breast cancer assay to predict chemotherapy in patients who have a midrange score. "Of the 9719 eligible patients with follow-up information, 6711 (69%) had a midrange recurrence score of 11 to 25 and were randomly assigned to receive either chemoendocrine therapy or endocrine therapy alone. The trial was designed to show noninferiority of endocrine therapy alone for invasive disease-free survival (defined as freedom from invasive disease recurrence, second primary cancer, or death)." They found that "Endocrine therapy was noninferior to chemoendocrine therapy in the analysis of invasive disease-free survival (hazard ratio for invasive disease recurrence, second primary cancer, or death [endocrine vs. chemoendocrine therapy], 1.08; 95% confidence interval, 0.94 to 1.24; P=0.26). At nine years, the two treatment groups had similar rates of invasive disease-free survival (83.3% in the endocrine-therapy group and 84.3% in the chemoendocrine-therapy group), freedom from disease recurrence at a distant site (94.5% and 95.0%) or at a distant or local-regional site (92.2% and 92.9%), and overall survival (93.9% and 93.8%). The chemotherapy benefit for invasive disease-free survival varied with the combination of recurrence score and age (P=0.004), with some benefit of chemotherapy found in women 50 years of age or younger with a recurrence score of 16 to 25." They concluded that "Adjuvant endocrine therapy and chemoendocrine therapy had similar efficacy in women with hormone-receptor-positive, HER2-negative, axillary node-negative breast cancer who had a midrange 21-gene recurrence score, although some benefit of chemotherapy was found in some women 50 years of age or younger" (Sparano et al., 2018).

In a prospective, randomized trial of endocrine therapy (ET) versus chemoendocrine therapy (CET), Kalinsky et al. (2021) attempted to gather support for the potential prognostic and predictive role of the RS for CT benefit in postmenopausal patients with one to three lymph node-positive breast cancer using Oncotype Dx in the RxPonder Trial. In the study, women with a RS less than 25 were randomized to receive ET or CET in 1:1 randomization using 3 stratification factors—RS (0-13 vs.14-25), menopausal status, and axillary nodal dissection vs. sentinel node biopsy—with the end goal of ascertaining whether the effect of CT on invasive disease-free survival (IDFS) was a function of RS. Of 9,383 patients screened in the study, 5,083 patients were randomized based on having met eligibility criteria and 5,018 participated in the trial. With a median follow-up period of 5.1 years, the authors observed 447 IDFS events. It was reported that in a model with CT, RS, and menopausal status (no interaction term), higher continuous RS was associated with worse IDFS (hazard ratio HR 1.06, 2-sided p<0) and that and CT was associated with an improvement in IDFS (HR 0.81, p=0.026). Moreover, a distinction exists between postmenopausal and premenopausal patients in their reception of chemoendocrine versus endocrine

therapy based on their RS: in postmenopausal patients (n=3350), the HR for CET vs. ET was not significant (HR=0.97, p=0.82, indicating no benefit from CT, whereas in premenopausal patients (N=1665, 33%), the HR (0.54) was statistically significant (p=0.0004), suggesting CT benefit. In other words, postmenopausal patients in this situation may be spared adjuvant chemotherapy, while premenopausal patients have much to benefit from the same. Therefore, the authors concluded that "the current data show that adjuvant therapy can be de-escalated to ET alone in postmenopausal pts with a RS < 25 and 1-3 +LN" (Kalinsky et al., 2021).

Lashen et al. (2023) studied the performance of Oncotype DX compared to conventional clinicopathological parameters. The study included 420 estrogen receptor-positive/HER2-negative breast cancer patients in the United Kingdom. All patients included in the study had undergone Oncotype DX testing. The authors found a significant association between a high Oncotype DX recurrence score and grade 3 tumors, as well as a significant association between low Oncotype DX recurrence score and special tumor types. Additionally, there was an inverse association between Oncotype DX recurrence score and the levels of estrogen receptor and progesterone receptor expression, and a positive linear relationship between Oncotype DX recurrence score and Ki67 labelling index. The authors state that "some tumour features such as tumour grade, type, PR status and Ki67 index can be used as surrogate markers in certain scenarios." The authors concluded that "Oncotype DX RS is correlated strongly with the conventional clinicopathological parameters" (Lashen et al., 2023).

EndoPredict

EndoPredict® (Myriad Genetic, Inc.) is a breast cancer prognostic test that assesses the expression of eight target genes as compared to three housekeeping (normalization) genes and one additional control gene to detect contamination by residual DNA. The results of this testing are combined to produce a clinical score to determine whether the breast cancer is at high- or low-risk of possible recurrence within ten years (Myriad, 2024a; Warf et al., 2017).

According to the Myriad EndoPredict® Technical Specifications (Effective Date: December 21, 2021), if the test is used on resected tissue, the 12-gene molecular score is then "combined with tumor size and lymph node status to generate an EPclin Risk Score associated recurrence risks and estimated absolute benefit of chemotherapy" whereas "the molecular score and its associated 10-year risk of distance recurrence…are provided" if the sample is a biopsy specimen (Myriad, 2021). The target genes in the EndoPredict® test include the following (Filipits et al., 2019; Warf et al., 2017):

- *BIRC5*
- *DHCR7*
- *UBE2C*
- *AZGP1*
- *IL6ST*
- *MGP*
- *RBBP8*
- *STC2*

Filipits et al. (2011) validated EP's 11 gene version (only missing the one gene to control for DNA contamination, HBB). The investigators combined these gene expression results with nodal status and tumor size into a risk score ("EPClin"). Two cohorts of 378 and 1324 samples were used in this study, and both cohorts demonstrated that the risk score produced was an independent predictor of distant recurrence. The investigators calculated that EPClin had superior prognostic power compared to solely clinicopathologic factors. EPClin was also found to result in a 4% rate of recurrence for both low-risk cohorts in each sample, a 28% recurrence rate in the "high risk" portion of the 378-item sample, and a 22% recurrence rate in the "high risk" portion of the 1324-item sample. The authors concluded that "the multigene EP risk score provided additional prognostic information to the risk of distant recurrence of breast cancer patients, independent from clinicopathologic parameters. The EPclin score outperformed all conventional clinicopathologic risk factors (Filipits et al., 2011; Myriad, 2024b).

Two studies by Sestak et al. (2018)have also shown the clinical validity and utility of EndoPredict® testing in women with node-positive breast cancer (Sestak et al., 2018; Sestak et al., 2019). One study consisted of both node-positive and node-negative women (n=3746) to compare the performance of EPclin for predicting distant recurrence in women who had either received endocrine therapy (ET) alone or ET with chemotherapy (ET+C). "In this comparative non-randomised analysis, the rate of increase in DR with EPclin score was significantly reduced in women who received ET + C versus ET alone. Our indirect comparisons suggest that a high EPclin score can predict chemotherapy benefit in women with ER-positive, HER2-negative disease" (Sestak et al., 2019). In the other study, the researchers compared the performance of six different prognostic signatures for ER-positive breast cancer in two different patient groups—node-negative and node-positive. The six tests include EndoPredict®, Breast Cancer Index (BCI), Clinical Treatment Score (CTS), IHC4, ROR, and RS. Within the node-positive cohort (n=277), the authors report the highest univariate hazard ratio (HR) for EPclin (1.69) compared to 1.39 for RS (Sestak et al., 2018).

Another study (n=1702) compared the use of EndoPredict® in node-positive and node-negative women treated with endocrine therapy only. This study assessed both the 10- and 15-year distant recurrence-free rate (DRFR). Similar to the node-negative individuals, the 10-year DRFR for patients with one to three positive nodes improved significantly higher than for the full cohort (95.6% versus 80.9%, respectively). "The molecular and EPclin scores were significant predictors of DRFR after adjusting for clinical variables, regardless of nodal status" (Filipits et al., 2019).

Evidence of EndoPredict's ability to produce a change in therapeutic management abound. For example, Villarreal-Garza et al. (2020) enrolled 99 premenopausal women with a median age of 43 and with hormone receptor-positive, HER2-negative, T1-T2, and N0-N1 breast cancer, and the cases were presented to a multidisciplinary tumor board. The researchers reported that a "change in chemotherapy or endocrine therapy regimen was suggested in 19% and 20% of cases" when EPclin results were made available. Moreover, it was reported that a significant difference could be "found in the median EPclin score between patients with a low- vs. high-intensity chemotherapy and endocrine therapy regimen recommendation ($p = 0.049$ and $p = 0.0001$, respectively)", which may have contributed to high final treatment adherence (93%) in premenopausal patients (Villarreal-Garza et al., 2020).

Dubsky et al. (2020) used hormone receptor (HR)-positive, HER2-negative samples from patients in the ABCSG-34 randomized phase II trial to generate a 12-gene molecular score with the goal of assessing EndoPredict's ability to predict response to neoadjuvant chemotherapy (NaCT) or neoadjuvant endocrine therapy (NET). The patients in the study were assigned to receive either NaCT or NET based on menopausal status, HR expression, grade and Ki67, and their responses to their assigned therapies were measured by using residual cancer burden (RCB). It was reported that the 12-gene molecular score significantly predicted treatment response for NaCT and NET, boasting areas under the curve (AUC) of 0.736 and 0.726 respectively. Based on these results, it was concluded that "Tumours with low MS were unlikely to benefit from NaCT, whereas a high MS predicted resistance to NET", further motivating the use of EndoPredict to provide personalized treatment to patients (Dubsky et al., 2020).

Breast Cancer Index

The Breast Cancer Index (BCI) is a combination of two profiles, the HOXB13-to-IL17BR expression ratio (H:I ratio) and the Molecular Grade Index (MGI). This combination is intended to report "Whether or not an additional five years of anti-estrogen therapy is likely to help reduce [the] risk of the cancer returning" and "[the] individual risk of recurrence after 5 years based on [the] original tumor biology." The five genes the MGI

examines are *BUB1B*, *CENPA*, *NEK2*, *RACGAP1*, and *RRM2* (Biotheranostics, 2023). Using genome-wide microarray analysis, three differentially expressed genes that were associated with an increased risk of progression among ER-positive patients treated with tamoxifen were: the antiapoptotic homeobox B13 (HOXB13, overexpressed in tamoxifen recurrent cases) and both interleukin 17B receptor (IL17BR) and EST AI240933 (both overexpressed in tamoxifen nonrecurrent cases) (Foukakis & Bergh, 2022).

The MGI was validated by separate 410-sample and 323-sample cohorts, and its interaction with the H:I ratio was evaluated. The authors found that "high MGI was associated with significantly worse outcome only in combination with high HOXB13:IL17BR, and likewise, high HOXB13:IL17BR was significantly associated with poor outcome only in combination with high MGI." The investigators concluded that "the combination of MGI and HOXB13:IL17BR outperforms either alone and identifies a subgroup (~30%) of early stage estrogen receptor–positive breast cancer patients with very poor outcome despite endocrine therapy (Ma et al., 2008).

The BCI has been recently demonstrated to be discerning in other respects. The IDEAL trial attempted to answer the pressing question of the optimal duration of extended endocrine therapy (EET), after completing five years of initial aromatase inhibitor (AI)–based adjuvant therapy (Liefers et al., 2020). The IDEAL trial assessed the ability of BCI (H/I) to predict endocrine benefit of 2.5 vs. five years of extended letrozole using available tumor specimen samples from 908 HR+ patients (73% lymph node-positive, disease free at 2.5 years), with 88% and 68% of which were receiving prior treatment with an aromatase inhibitor (AI) or chemotherapy, respectively. It was found that BCI by H/I status (High vs. Low) was "significantly predictive of response from extended letrozole in the overall (N = 908) and pN+ [lymph node-positive] (N = 664) cohorts", leading the researchers to exalt the BCI as an important genomic tool for future use (Liefers et al., 2020).

Predictor Analysis of Microarray 50

The Predictor Analysis of Microarray 50 (PAM50, by Prosigna) is a 50-gene test that characterizes an individual tumor by intrinsic subtype. It was designed to determine the intrinsic subtype of a cancer using only 50 prespecified genes. The four subtypes are "luminal A, luminal B, HER2-enriched, and basal-like" (Parker et al., 2009). Results from the PAM50 are used to generate the risk of recurrence (ROR) score, which can stratify patients with ER-positive disease into high, medium, and low subsets. The test can be performed on formalin-fixed, paraffin-embedded tissue with a high degree of analytical validity (Foukakis & Bergh, 2022; Nielsen et al., 2010).

This test was validated by Parker et al. (2009)The investigators developed this 50-gene set from 189 prototype samples and evaluated 761 patients for prognosis. The four discrete subtypes were found to be prognostically significant and predicted neoadjuvant chemotherapy efficacy with a negative predictive value for pathologic complete response (to a taxane and anthracycline regimen) of 97%. The authors concluded "diagnosis by intrinsic subtype adds significant prognostic and predictive information to standard parameters for patients with breast cancer" (Parker et al., 2009).

Recently, the intrinsic molecular subtype generated by the Prosigna assay has been demonstrated to be useful in guiding the inclusion and exclusion of an anthracycline to CMF-based chemotherapy. The DBCG89D trial randomized 980 high-risk early breast cancer patients to adjuvant CMF (cyclophosphamide, methotrexate and fluorouracil) or CEF (cyclophosphamide, epirubicin and fluorouracil), ultimately using the data from 686 tumor samples. Continuous ROR score was associated with distant recurrence for CMF and CEF (hazard ratios of 1.20 and 1.04, respectively), and distant recurrence was reported to be significantly longer in those patients with HER2-enriched tumors treated with CEF than with CMF. The results suggested that the benefit of CEF as compared to CMF correlate with an increasing ROR score, and so "patients with Her2-enriched breast cancer derived substantial benefit from anthracycline chemotherapy whereas anthracyclines are not an essential component of chemotherapy for patients with luminal subtypes", and, similarly, "A benefit from substituting methotrexate with epirubicin is observed among patients with Her2-enriched or Basal-like subtypes whereas there is a lack of such benefit among patients with a Luminal A or Luminal B subtypes" (Jensen et al., 2020). However, the authors admit that the results of the study may potentially be skewed by the retrospective evaluation's use of only a small subset of patients' data and the lack of optimal instrumentation and testing, since one-third of the patients were ER-positive but endocrine therapies were not employed (Jensen et al., 2020).

MammaPrint

MammaPrint is a 70-gene test that assesses the risk of recurrence within the first 5-10 years of diagnosis. The test reports risk in one of two categories, "low" and "high." MammaPrint's validation places "low" risk at a 1.3% chance (95% confidence interval: 0-3.1%) that cancer will recur in five years whereas those classified as "high" risk have a 11.7% chance for the cancer to recur in five years (95% confidence interval: 6.6%-16.8%) (Agendia, 2014, 2024b). Agendia offers other breast cancer assessments, such as BluePrint. BluePrint is an 80-gene molecular subtyping assay, which "analyzes the activity" of its genes. It is proposed to identify a tumor's molecular "subtype": luminal A/B, basal, or HER2. Each subtype has varying molecular characteristics (ER-positive or

negative, HER2-positive or negative, et al), which is proposed to help clinicians provide targeted treatment for a patient (Agendia, 2024a).

MammaPrint was validated by Cardoso et al. (2016)The authors determined the risk recurrence of 6693 patients. Of these 6693 patients, 1550 of them were found to have high clinical risk and low genomic risk, and the rate of survival without distant metastasis in this cohort was 94.7% among those not receiving chemotherapy. The difference in survival rate between these patients and those receiving chemotherapy was 1.5%. The authors estimated that "given these findings, approximately 46% of women with breast cancer who are at high clinical risk might not require chemotherapy" (Cardoso et al., 2016).

However, it may be possible that new prognostic assays are beginning to make their foray. Prat et al. (2020) derived a prognostic assay that integrates multiple data types for predicting survival outcome in patients with newly diagnosed HER2-positive breast cancer to aid treatment decisions for those with early-stage HER2-positive breast cancer, where escalation or de-escalation of systemic therapy is a point of contention. Using clinical-pathological data on stromal tumor-infiltrating lymphocytes, PAM50 subtypes, and expression of 55 genes obtained from patients who participated in the Short-HER phase 3 trial, Prat et al. (2020) then evaluated their model based on a combined dataset of 267 patients with early-stage HER2-positive breast cancer treated with different neoadjuvant and adjuvant anti-HER2-based combinations and from four other studies (PAMELA, CHER-LOB, Hospital Clinic, and Padova) with disease-free survival outcome data. The final model, named HER2DX, was comprised of data from 435 (35%) of 1254 patients for tumor size (T1 vs rest), nodal status (N0 vs rest), number of tumor-infiltrating lymphocytes (continuous variable), subtype (HER2-enriched and basal-like vs rest), and 13 genes. The authors exalt the analytical power of HER2DX by reporting that it was "significantly associated with disease-free survival as a continuous variable" and reported that the 5-year disease-free survival in the HER2DX low-risk group was 93.5% (89.0-98.3%) whereas the high-risk group reported 81.1% (71.5-92.1). The researchers assert that HER2DX "identifies patients with early-stage, HER2-positive breast cancer who might be candidates for escalated or de-escalated systemic treatment" and that "Future clinical validation of HER2DX seems warranted to establish its use in different scenarios, especially in the neoadjuvant setting" (Prat et al., 2020).

DCISionRT®

The DCISionRT test out of Prelude Corporation aims to provide patients with three results: 1) their overall score (continuous risk score and low vs elevated category, 2) their rate of recurrence over 10 years, and 3) their benefit from radiation. The intended demographic is females aged 26 and older who have been diagnosed with ductal

carcinoma in situ (DCIS) of the breast, and who have been treated with breast conserving surgery or a core biopsy (PreludeDx, 2024).

Recognizing that patients diagnosed with DCIS are at risk of recurrence (30%) within 10 years following breast conserving surgery and that election of RT is not always beneficial, the SweDCIS randomized clinical trials also investigated the validity of the DCISionRT biosignature, which combines an evaluation of protein expression (PR, HER2, Ki67, FOXA1, p16/INK4A, SIAH2, COX2) with clinicopathologic factors (age, tumor size, margin status, palpability) and mapped it along a continuous Decision Score (DS) on a scale of 1 to 10 with corresponding 10-year risks after breast conserving surgery with and without radiation therapy (Wärnberg et al., 2021). The authors found that "using the pre-specified DS threshold of 3.0 to define DS Low and Elevated Risk groups, approximately 50% of women were classified into the Elevated Risk group", which is significant as many women who would have been erroneously sorted into one risk group based on classical clinicopathology were found to be better reclassified into opposite risk groups based on their DS. Finally, in the DS Elevated Risk group, the addition of radiation therapy was found to reduce absolute 10-year rates for TotBE by 15.9%, and for InvBE by 9.4%, while the DS Low Risk Group boasted 10-year DCIS rates of 0.7% and 5.2%, and InvBE rates of 6.5% and 7.7%, with and without RT, respectively, indicating to the authors that "Radiotherapy benefit was statistically significant in patients with elevated DS but not in patients with low DS by DCISionRT", though it is also admitted that this evaluation may not reflect modern practice and modern risk profiles because the women participating in the trials were randomly assigned between 1987 and 2000 (Wärnberg et al., 2021).

Bremer et al. (2018) reported on the development and the cross-validation of the DCISionRT biosignature using archived tissue samples from Uppsala University Hospital and Västmanland County Hospital, Sweden (UUH), from 1986 and 2004 and samples from between 1999 and 2008 at the University of Massachusetts, Worcester (UMass), in collaboration with PreludeDx. The study population comprised 721 female patients diagnosed with a primary DCIS and treated with BCS identified from 2 study sites, with mean and median follow-ups of 10 years and 9 years, respectively. Here, the authors found the Decision Score reclassified greater than 50% of the low- and intermediate-grade patients as elevated risk, and 33% of patients that were grade III or had tumor size >2.5 cm were reclassified as low risk. Moreover, it was found that in the low clinicopathological risk group—defined to have tumor size less than 25 mm, low or intermediate grade, and nonpalpable and clear margins—DS reclassified 42% of these patients into the Elevated Risk Group (DS > 3.0). When a linear panel using weighted clinicopathologic and molecular factors to assess recurrence risk was constructed for comparison with the proprietary biosignature, it could not identify a clinically meaningful low-risk group. The researchers reported that "These reclassified patients

had substantial 10-year risks, 23% (95% CI: 7%–36%) IBC and 31% (95% CI: 14%–45%) IBE, when not treated with RT" and that while patients in the DS Low Group had similar outcomes regardless of whether they received RT, patients treated with RT and classified into the Elevated Risk Group received a 70% or greater risk reduction than those without RT (Bremer et al., 2018). As such, Bremer et al. (2018) claims that these "findings reflect the effectiveness of the biological signature to identify patients with favorable tumor biology and separate them from the Elevated Risk Group of patients that have the highest likelihood of recurrence."

These results corroborate other investigations into the utility of DCISionRT in guiding treatment management. The prospective, multi-institutional PREDICT study found that DCISionRT testing can lead to great changes in radiation therapy recommendations, such that of the 539 women participating in the study, 69% were initially slated RT pre-test (n = 374) by treating clinicians based on traditional clinicopathology and 46% were recommended to not receive RT post-test (*n* = 165). But of those initially recommended to not receive RT (n = 165), 35% were then recommended to receive RT post-test (n = 58). As such, the authors reported a significant overall change in the RT recommendation for 42% of women (n = 539, $p < 0.001$) and a net reduction in the RT recommendation by 20% (Shah et al., 2021). Furthermore, after DCISionRT testing, elevated versus low DCISionRT score (DS>3 vs. DS ≤ 3) was found to be the strongest factor for an RT recommendation (odds ratio of 43.1) compared against all other factors (age, grade, tumor size, margin status, patient preference). However, the authors acknowledge that there exist limitations to the study, namely that long-term clinical outcomes and satisfaction ratings from patients and physicians are unavailable for analysis and that data for RT recommendations are limited to post-surgery/pre-testing and post-surgery/post-testing time points (Shah et al., 2021). Thus, while the results seem promising, the real predictive power of the DCISionRT test is still uncertain. Additional information will be critical to evaluating the test's value and valence—whether positive or negative—in ultimately determining the benefit of adjuvant RT following BCS.

Dabbs et al. (2023) studied the analytical validity, performance assessment, and clinical performance validation and clinical utility for the DCISionRT test. The authors performed an analytical validation assessment on multiple DCIS molecular assays; the analytical validation test was conducted based on Clinical and Laboratory Standards Institute guidelines. "The analytic validation showed that the molecular assays that are part of DCISionRT test have high sensitivity, specificity, and accuracy/reproducibility (≥95%)." The authors concluded that the DCISionRT has high analytical validity (Dabbs et al., 2023).

BBDRisk Dx®

The BBDRisk Dx® test is "the first genomic risk test designed specifically for women diagnosed with breast masses that have one or more of the following: Atypical Ductal Hyperplasias (ADH), Atypical Lobular Hyperplasia (ALH), Usual Ductal Hyperplasias (UDH), Papilloma and Sclerosing Adenosis" (BBDRiskDx, 2023). The test assays four cancer biomarker oncoproteins—Matrix Metalloproteinase-1 (MMP-1), Carcinoembryonic Antigen-related Cell Adhesion Molecule 6 (CEACAM6), Hyaluronoglucosaminidase 1 (HYAL1), Highly Expressed in Cancer Protein 1 (HEC1)—requiring a sample of formalin fixed paraffin embedded (FFPE) tissue for the immunohistochemistry. The four-marker profile is then rendered to produce a Cancer Risk Score between the range of 0 and 10, classifying the patient into the 'Low Risk' Group (Risk Score =< 0.5), the 'Intermediate Risk' group (>0.5 but =<5.4), and the 'High Risk' group (>5.4). These scores help provide the patient with information about their chances of developing future breast cancer over 5 to 10 years—as compared with women with similar profiles—and guide physician discretion in proposing prophylactic endocrine treatments and or surgery (Silbiotech, 2021).

Poola et al. (2019) explored the effectiveness of the four-marker signature of MMP-1, CEACAM6, HYAL1, and HEC1 in identifying the benefit that high-risk patients presenting atypical and nonatypical hyperplasias would derive from preventive therapies. A total of 440 tissues were included in the study and categorized into two groups: a cancer group of 162 specimens that developed DCIS after a minimum of one year and a maximum of 13 years, and a cancer-free group of 278 specimens from subjects who did not have prior breast cancer and did not develop for a minimum of five years and a maximum of 19 years. A 'cancer risk score' (composite value between 0 and 10) was obtained by combining the multiple marker levels through using piecewise linear regression (Poola et al., 2019). From constructing box plots and density plots of the scores generated from the 440 samples, the authors found that in the risk scores of the cancer-free group peaked around 0.5, while the scores peaked around 9.0 for the cancer group, suggesting that the "risk signature segregates the cancer group from the cancer-free group of tissues." Moreover, analysis of the risk scores between atypical and nonatypical tissues for any differences using *t* test, yielded significantly higher values in the cancer group of atypical tissues ($P = 0.02$) than the nonatypical tissues but the authors did not find any significant differences in the cancer-free groups. Plots produced from Kaplan-Meier survival analysis demonstrated that "In the first 5 years, 53% of subjects in the high-risk group, 12% in the intermediate-risk group, and 1% in the low-risk group developed cancer", and 73% of atypical subjects in high-risk group developed cancer in the first five years, while in the nonatypical group the cancer rates were only 34%. Meanwhile, mapping cancer risk scores to percent cancer rates in five years, 10 years, 15 years, and beyond 15 years produced a direct and positive correlation (Poola et al., 2019).

Clinical Utility and Validity

Blok et al. (2018) performed a meta-analysis of the MammaPrint, OncotypeDX, PAM50/Prosigna and Endopredict assays. The authors investigated the evidence available on both the clinical utility and economic value of these assays. Most of the observed studies concluded that genomic profiling contributed to less chemotherapy use. However, the authors caution that "absolute numbers should be interpreted carefully, since some tests are less frequently studied than others" especially as "the clinical consensus on adjuvant chemotherapy is that we are most likely over-treating our patients, since we are not capable of identifying patients that will or will not benefit from chemotherapy using the current clinicopathological parameters." The authors note that Petkov et al. (2016) retrospectively matched OncoType DX use with SEER registry data for over 40000 patients and found that "patients with node negative, HR+, HER2- breast cancer which underwent testing (n = 40,134, 22.7% chemotherapy) had no lower chemotherapy use compared to patients that were not tested (n = 144,056, 22.2% chemotherapy)." The authors noted that 90% of the 44 economic analyses concluded that genomic testing was cost-effective; however, various biases are mentioned (publication bias, publications using overlapping samples, and more) (Blok et al., 2018).

Gustavsen et al. (2014) performed an economic analysis of the Breast Cancer Index and found that "Costs associated with adjuvant chemotherapy, toxicity, followup, endocrine therapy, and recurrence were modeled over 10 years. The models examined cost utility compared with standard practice when used at diagnosis and in patients disease-free at 5 years post diagnosis." The authors calculated BCI to save approximately $3803 per patient for the newly diagnosed population and $1803 per patient in the five years post diagnosis population. These savings were projected to come from "adjuvant chemotherapy use, extended endocrine therapy use, and endocrine therapy compliance" (Gustavsen et al., 2014).

Camp et al. (2019) investigated a new reinterpretative technique of the PAM50 assay. The authors reorganized the gene expression data provided by the assay into five "quantitative, orthogonal, multi-gene breast tumor traits" and stated that these "dimensions" better represented breast cancer pedigrees. They re-assessed the data of the GEICAM/9906 clinical trial with these dimensions. After reorganization, the authors concluded that dimensions "PC1 and PC5" were associated with disease-free survival, and low "PC3 and PC4" indicated response to paclitaxel. However, high PC3 or PC4 did not show survival advantage (Camp et al., 2019).

Wuerstlein et al. (2019) evaluated the clinical utility of MammaPrint and its intended adjunct test, BluePrint. Physicians were asked to provide initial therapy

recommendations (based on clinicopathological factors), then were provided results of MammaPrint/BluePrint risk stratifiers. Then the same physicians provided new treatment recommendations, and the subsequent treatment was recorded. The authors identified a switch in treatment in 29.1% of cases. Physician adherence to MammaPrint risk assessment was over 90% in both groups of risk (low and high). Even when physicians and risk stratifiers disagreed, the physicians tended to recommend treatments based on the risk stratifier. Overall, the authors concluded that "MammaPrint and BluePrint test results strongly impacted physicians' therapy decisions in luminal EBC with up to three involved lymph nodes" (Wuerstlein et al., 2019).

Toole et al. (2014) evaluated the similarities and differences in genetic profiles between primary breast cancers in patients with multiple primaries. The authors also evaluated whether obtaining genetic profiling scores (OncoType DX) on each primary affected chemotherapy decisions. In total, 22 patients had multiple tumor samples sent for analysis, and the authors found that six patients had their chemotherapy recommendations changed based on differing OncoType scores (between their samples). The authors also noted that scores tended to differ more between tumors appearing simultaneously on different breasts compared to multiple tumors on the same breast (Toole et al., 2014).

Martin et al. (2016) compared the use of EndoPredict® (EPclin) and PAM50 risk of recurrence (ROR) scores in node-positive breast cancer to predict distant metastasis-free survival (MFS). ROR scores can be based using subtype (ROR-S); subtype and proliferation (ROR-P); subtype and tumor size (ROR-T); and subtype, proliferation, and tumor size combined (ROR-PT). They found that "Predictors including clinical information showed superior prognostic performance compared to molecular scores alone (10-year MFS, low-risk group: ROR-T 88 %; ROR-PT 92 %; EPclin 100 %). The EPclin-based risk stratification achieved a significantly improved prediction of MFS compared to ROR-T, but not ROR-PT" (Martin et al., 2016).

Ding et al. (2019) used the 21-gene recurrence score for patients with pure mucinous breast cancer (PMBC). This multi-year study of 8048 female PMBC patients categorized the patients based on RS risk groups of low, intermediate, and high RS risk groups. They found the distribution to be 64.9%, 31.9%, and 3.2%, respectively. For PMBC patients, the authors note that PMBC patients do show significantly different 5-year survival rates based on ER status. They do conclude that RS can be used with PMBC patients…"age, progesterone receptor status, and grade could independently predict RS" (Ding et al., 2019). These data support the findings of a considerably smaller comparative study (n=35 PMBC patients and n=70 IDC patients) that show on average PMBC patients score lower than IDC patients (average RS 21.26 and 24.40,

respectively); however, they authors do conclude that "patients with high RS in the PMBC group might be recommended to receive adjuvant chemotherapy" since 8.57% of PMBC patients have RS scores within the highest risk stratification (W. Wang et al., 2018). Both studies contradict an earlier, smaller study (n=10 TC patients and n=33 MC patients) that reported no patients within the high-risk RS category (Turashvili et al., 2017).

Kizy et al. (2018) conducted a large retrospective study of various subtypes of T1-T2N0 estrogen receptor-positive breast cancer (n=83665) was published in 2018. Both mucinous and tubular histologies were included in the study. The authors note considerable differences in RS scores between the various histologies; for example, they found that 1.0% and 28.5% of tubular adenocarcinoma patients had RS scores in the high and intermediate ranges, respectively, whereas mucinous adenocarcinoma patients fared worse with 3.4% and 28.8%, respectively. It should be noted, however, that only 2.1% of the patients included in the study had been diagnosed with mucinous adenocarcinoma and 0.6% with tubular adenocarcinoma (J. Wang et al., 2018). Similarly, a study by Kizy et al also reports that 4% of tubular carcinoma patients were classified as high risk based on the Trial Assigning Individualized Options for Treatment RS criteria (Kizy et al., 2018).

Harnan et al. (2019) released an in-depth systemic review of the effectiveness and cost-effectiveness of Oncotype DX®, MammaPrint®, Prosigna®, EndoPredict®, and immunohistochemistry 4 (IHC4). For this review, the authors included a total of 153 studies, including the MINDACT RCT for MammaPrint. The authors note that a limitation of this systemic review, and of the current field in general, is that only one RCT has been completed to date. Their results include the following:

- "There is limited and varying evidence that oncotype DX and MammaPrint can predict benefit from chemotherapy."
- "The health economic analysis suggests that the incremental cost-effectiveness ratios for the tests versus current practice are broadly favourable for the following scenarios: (1) oncotype DX, for the LN0 subgroup with a Nottingham Prognostic Index (NPI) of > 3.4 and the one to three positive lymph nodes (LN1–3) subgroup (if a predictive benefit is assumed); (2) IHC4 plus clinical factors (IHC4+C), for all patient subgroups; (3) Prosigna, for the LN0 subgroup with a NPI of > 3.4 and the LN1–3 subgroup; (4) *EndoPredict Clinical, for the LN1–3 subgroup only* [emphasis added]; and (5) MammaPrint, for no subgroups" (Harnan et al., 2019).

Qi et al. (2021) examined the efficacy of the 21-gene assay (Oncotype DX) in core needle biopsies (CNB) as compared to breast cancer resection specimens (the gold standard for obtaining IHC or other molecular information, as they exhibit the most

abundant tumor characteristics). The 21-gene assay can be applied to a CNB and CNBs do have the advantage of having a more standardized fixation protocol as compared to resection specimens, which have many different protocols available and often have a greater time between loss of blood supply and the initiation of fixation due to the complexity of surgery. While CNBs are more reliable in determining hormone receptor status due to decreased loss of antigens from the fixation process that resection specimens undergo, CNBs cannot account for crush artefacts which can lead to inaccurate results because of tumor heterogeneity. CNBs are also less accurate than resection specimens in determining the histological characteristics of a tumor, such as tumor invasiveness and tumor grade. It's also important to note that intratumoral heterogeneity can lead to an underestimation of tumor genomic characteristics because of varying gene expression signations found within different regions of the same tumor.

Qi et al. (2021) utilized a paired CNB and resection specimen approach to assess the concordance of the Oncotype DX results between the CNB and resection samples. They found that determination of 21-gene assay results from CNBs are reliable, providing results which closely parallel those obtained from resection specimens. However, there is no clear data indicating that tumor grade can be determined using a CNB sample (Qi et al., 2021).

Sennerstam et al. (2017) conducted retrospective study looking at the long-term outcomes of patients with breast cancer diagnosed with fine-needle aspiration biopsy (FNAB) vs CNB in the 1970s and the 1990s (diagnosis using FNAB from 1971-1976 (n=354); diagnosed using CNB from 1991-1995 (n=1,729)) shows a potentially alarming trend. The authors treatment, mammography screening, tumor size, DNA ploidy, and patient age matched samples from the two decades to get two strictly matched samples representing FNAB (n=181) and CNB (n=203), which were selected for a 15-year follow-up study. When comparing the rates of distant metastasis in the strictly matched patient groups, they found that 5-15 years after diagnosis of the primary tumor, patients diagnosed via CNB had significantly higher rates of metastases as compared to those diagnosed via FNAB (Sennerstam et al., 2017).

VI. Guidelines and Recommendations

American Society of Clinical Oncology (ASCO)

In 2016, ASCO provided recommendations on appropriate use of breast tumor biomarker assay results to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer. ASCO recommends that "in addition to estrogen and progesterone receptors and human epidermal growth factor receptor 2,

the panel found sufficient evidence of clinical utility for the biomarker assays Oncotype DX, EndoPredict, PAM50, Breast Cancer Index, and urokinase plasminogen activator and plasminogen activator inhibitor type 1 in specific subgroups of breast cancer" (Harris et al., 2016).

ASCO also made the following recommendations:

- "If a patient has ER/PgR-positive, HER2-negative (node-negative) breast cancer, the clinician may use the 21-gene recurrence score to guide decisions on adjuvant systemic chemotherapy. Type: evidence based. Evidence quality: high. Strength of recommendation: strong.
- If a patient has ER/PgR-positive, HER2-negative (node-positive) breast cancer, the clinician should not use the 21-gene RS to guide decisions on adjuvant systemic chemotherapy. Type: evidence based. Evidence quality: intermediate. Strength of recommendation: moderate.
- If a patient has HER2-positive breast cancer or TN breast cancer, the clinician should not use the 21-gene RS to guide decisions on adjuvant systemic therapy. Type: informal consensus. Evidence quality: insufficient. Strength of recommendation: strong
- If a patient has ER/PgR-positive, HER2-negative (node-positive) breast cancer, the clinician should not use the Breast Cancer Index, the PAM50-ROR, the 12-gene risk score, or the 21-gene RS, (EndoPredict), to guide decisions on adjuvant systemic therapy.
- If a patient has ER/PgR-positive, HER2-negative (node-negative) breast cancer, the clinician may use urokinase plasminogen activator (uPA), plasminogen activator inhibitor type 1 (PAI-1), the Breast Cancer Index, the PAM50 risk of recurrence (ROR) score, the 12-gene risk score (EndoPredict), and the 21-gene recurrence score (Oncotype DX) to guide decisions on adjuvant systemic therapy. If the patient has had 5 years of endocrine therapy without evidence of recurrence, the clinician should not use multiparameter gene expression or protein assays (Oncotype DX, EndoPredict, PAM50, Breast Cancer Index, or IHC4) to guide decisions on extended endocrine therapy.
- If a patient has HER2-positive breast cancer or TN breast cancer, the clinician should not use uPA, IHC4, the 12-gene risk score (EndoPredict), PAI-1, the 21-gene RS (Oncotype DX), the five-protein assay (Mammostrat), the Breast Cancer Index or TILs to guide decisions on adjuvant systemic therapy. The clinician should not use circulating tumor cells (CTCs) to guide decisions on adjuvant systemic therapy.

- If a patient has HER2-positive breast cancer, the clinician should not use the PAM50-ROR to guide decisions on adjuvant systemic therapy. Type: informal consensus. Evidence quality: insufficient. Strength of recommendation: strong
- If a patient has TN breast cancer, the clinician should not use the PAM50-ROR to guide decisions on adjuvant systemic therapy. Type: informal consensus. Evidence quality: insufficient. Strength of recommendation: strong" (Harris et al., 2016).

In 2017 the ASCO (Krop et al., 2017), based on a review of the MINDACT study publication, revised their guidelines on *Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer* to state the following:

"***Recommendation 1.1.1 (update of Recommendation 1.7).***

If a patient has **ER/PgR–positive, HER2-negative, node-negative,** breast cancer, the MammaPrint assay may be used in those with **high clinical risk** per MINDACT categorization to inform decisions on withholding adjuvant systemic chemotherapy due to its ability to identify a good prognosis population with potentially limited chemotherapy benefit (Type: evidence based; Evidence quality: high; Strength of recommendation: strong).

Recommendation 1.1.2 (update of Recommendation 1.7).

If a patient has **ER/PgR–positive, HER2-negative, node-negative,** breast cancer, the MammaPrint assay **should not** be used in those with **low clinical risk** per MINDACT categorization to inform decisions on withholding adjuvant systemic chemotherapy as women in the low clinical risk category had excellent outcomes and did not appear to benefit from chemotherapy even with a genomic high-risk cancer (Type: evidence based; Evidence quality: high; Strength of recommendation: strong)."

Recommendation 1.2.2: (update of 2016 recommendation 1.7): If a patient has **ER/PgR–positive, HER2-negative, node-positive**, breast cancer, the MammaPrint assay should not be used in patients with one to three positive nodes and at low clinical risk per MINDACT categorization to inform decisions on withholding adjuvant systemic chemotherapy. There are insufficient data on the clinical utility of MammaPrint in this specific patient population (Type: informal consensus; Evidence quality: low; Strength of recommendation: moderate).

Recommendation 1.3: (update of 2016 recommendation 1.8): If a patient has **HER2-positive** breast cancer, the clinician should not use the MammaPrint assay to guide decisions on adjuvant systemic therapy. Additional studies are required to address the role of MammaPrint in patients with this tumor subtype who are also

receiving HER2-targeted therapy (Type: informal consensus; Evidence quality: low; Strength of recommendation: moderate).

Recommendation 1.4: (update of 2016 recommendation 1.9): If a patient has **ER/PgR negative and HER2-negative (triple negative)** breast cancer, the clinician should not use the MammaPrint assay to guide decisions on adjuvant systemic chemotherapy (Type: informal consensus; Evidence quality: insufficient; Strength of recommendation: strong)" (Krop et al., 2017).

ASCO published an update regarding the "Role of Patient and Disease Factors in Adjuvant Systemic Therapy Decision Making for Early-Stage, Operable Breast Cancer", which was released in response to the results of two phase III trials, MINDACT and TAILORx. The updated guidelines state that the clinical trials found evidence of clinical utility for both node-positive and node-negative patients, but only for patients determined to be at high clinical risk. Therefore, the Panel did not recommend use of MammaPrint for patients determined to be of low clinical risk (Henry et al., 2019).

In a focused update published in 2019, the ASCO addressed the use of Oncotype DX in the following recommendation:

"For patients with hormone receptor–positive, axillary node–negative breast cancer whose tumors have Oncotype DX recurrence scores of less than 26, there is little to no benefit from chemotherapy, especially for patients older than age 50 years. Clinicians may recommend endocrine therapy alone for women older than age 50 years. For patients 50 years of age or younger with recurrence scores of 16 to 25, clinicians may offer chemoendocrine therapy. Patients with recurrence scores greater than 30 should be considered candidates for chemoendocrine therapy. Based on informal consensus, the panel recommends that oncologists may offer chemoendocrine therapy to these patients with recurrence scores of 26 to 30" (Andre et al., 2019).

In 2022, the ASCO released a guideline update on biomarkers for adjuvant endocrine and chemotherapy in early-stage breast cancer. The ASCO recommends that

- "**Recommendation 1.1.** If a patient has node-negative breast cancer, the clinician may use the Oncotype DX test to guide decisions for adjuvant endocrine and chemotherapy (Type: evidence-based; Evidence quality: high; Strength of recommendation: strong).
- **Recommendation 1.2.** In the group of patients in Recommendation 1.1 with Oncotype DX recurrence score greater or equal to 26, the clinician should offer chemoendocrine therapy (Type: evidence-based; Evidence quality: high; Strength of recommendation: strong).

- **Recommendation 1.3.** In the group of patients in Recommendation 1.1 who are 50 years of age or younger with Oncotype DX recurrence score 16 to 25, the clinician may offer chemoendocrine therapy (Type: evidence-based; Evidence quality: intermediate; Strength of recommendation: moderate).
- **Recommendation 1.4.** If a patient is postmenopausal and has node-positive breast cancer with 1-3 positive nodes, the clinician may use the Oncotype DX test to guide decisions for adjuvant endocrine and chemotherapy (Type: evidence-based; Evidence quality: high; Strength of recommendation: strong).
- **Recommendation 1.5.** In the group of patients in Recommendation 1.4, the clinician should offer chemoendocrine therapy for those whose Oncotype DX recurrence score is ≥ 26 (Type: evidence-based; Evidence quality: high; Strength of recommendation: strong).
- **Recommendation 1.6.** If a patient is premenopausal and has node-positive breast cancer with 1-3 positive nodes, Oncotype DX test should not be offered to guide decisions for adjuvant systemic chemotherapy (Type: evidence-based; Evidence quality: high; Strength of recommendation: moderate).
- **Recommendation 1.7.** If a patient has node-positive breast cancer with ≥ 4 positive nodes, the evidence on the clinical utility of routine Oncotype DX to guide decisions for adjuvant endocrine and chemotherapy is insufficient to recommend its use (Type: informal consensus; Evidence quality: insufficient; Strength of recommendation: moderate)" (Andre et al., 2022).

American Cancer Society (ACS)

In 2017 the American Cancer Society (ACS) released a revision to the American Joint Cancer Committee's Eighth Edition Cancer Staging Manual. The revision suggests that

"For patients with hormone receptor-positive, HER2-negative, and lymph node-negative tumors, a21-gene (Oncotype Dx) recurrence score less than 11, regardless of T size, places the tumor into the same prognostic category as T1a-T1b N0 M0, and the tumor is staged using the AJCC prognostic stage group table as stage I.

For patients with hormone receptor-positive, HER2-negative, and lymph node-negative tumors, a Mammaprint low-risk score, regardless of T size, places the tumor into the same prognostic category as T1a-T1b N0 M0.

For patients with hormone receptor-positive, HER2-negative, and lymph node-negative tumors, a12-gene (EndoPredict) low-risk score, regardless of T size, places the tumor into the same prognostic category as T1a-T1b N0 M0.

For patients with hormone receptor-positive, HER2-negative, and lymph node-negative tumors, aPAM50 risk–of-recurrence score in the low range, regardless of T size, places the tumor into the same prognostic category as T1a-T1b N0 M0.

For patients with hormone receptor-positive, HER2-negative, and lymph node-negative tumors, a Breast Cancer Index in the low-risk range, regardless of T size, places the tumor into the same prognostic category as T1a-T1b N0 M0" (Giuliano et al., 2017).

National Comprehensive Cancer Network (NCCN)

The NCCN Clinical Practice Guidelines for Breast Cancer consider five gene expression assays for adjuvant systemic therapy. The five are comprised of the 21-gene Oncotype Dx, the 70-gene MammaPrint, the 50-gene PAM50 (Prosigna), the 12-gene EndoPredict, and the Breast Cancer Index (BCI). All five gene expression assays achieved the NCCN Category of Evidence and Consensus grade of 2A (consensus of appropriateness of intervention based on lower-level evidence) or higher, with Oncotype Dx achieving grade 1 (consensus based on higher-level evidence) for predictive and prognostic use, and MammaPrint achieving grade 1 for prognostic use in patients who are diagnosed as node-negative or with 1 to 3 positive nodes. It should be noted that NCCN identifies only the Oncotype Dx as suitable for prediction of chemotherapy benefit; NCCN states that "Other prognostic gene assays can provide prognostic information but the ability to predict chemotherapy benefit is unknown." BCI is identified as suitable for predicting benefit of extended adjuvant endocrine therapy (NCCN, 2024).

The NCCN Panel "notes that the multigene assays provide prognostic and therapy-predictive information that complements TNM and biomarker information." Below is the table from their recommendations and guidelines based on risk scores and nodal statuses of the multigene assays (NCCN, 2024):

GENE EXPRESSION ASSAYS FOR CONSIDERATION OF ADJUVANT SYSTEMIC THERAPY[a,b]

Assay	Recurrence Risk	Treatment Implications
21-gene (Oncotype Dx) (for postmenopausal patients with pN0 and pN1 [1–3 positive nodes])[c]	<26	Patients with T1b/c–2, pN0, HR-positive, HER2-negative tumors, with risk scores (RS) between 0–10 have a risk of distant recurrence of <4% and those with RS 11–25 derived no benefit from the addition of chemotherapy to endocrine therapy in the prospective TAILORx study.[1] Postmenopausal patients with pT1–3, pN1, HR-positive, HER2-negative, with RS <26 derived no benefit from the addition of chemotherapy to endocrine therapy in the prospective RxPONDER study.[2]
	≥26	In postmenopausal patients with pT1–3, HR-positive, HER2-negative, and pN0 and pN1 (1–3 positive nodes) tumors and an RS ≥26, the addition of chemotherapy to endocrine therapy is recommended.[1,2]
21-gene (Oncotype Dx) (for premenopausal patients: pN0)	≤15	Premenopausal patients with T1b/c –2, pN0, HR-positive, HER2-negative tumors with RS <16 derived no benefit from the addition of chemotherapy to endocrine therapy in the prospective TAILORx study.[1]
	16–25	In premenopausal patients with RS between 16–25, a small benefit from the addition of chemotherapy could not be ruled out, but it is unclear if the benefit was due to the ovarian suppression effect promoted by chemotherapy in premenopausal patients.[1,2] For this group, consider chemotherapy followed by endocrine therapy or alternatively, ovarian function suppression combined with either tamoxifen or an AI.
	≥26	In premenopausal patients with HR-positive, HER2-negative, and pN0 tumors and an RS ≥26, the addition of chemotherapy to endocrine therapy is recommended.[1]
21-gene (Oncotype Dx) (for premenopausal patients with 1–3 positive nodes)[c]	<26	In premenopausal patients with pT1–3 and pN1 (1–3 positive nodes) tumors and an RS <26, the addition of chemotherapy to endocrine therapy was associated with a lower rate of distant recurrence compared with endocrine monotherapy[2] but it is unclear if the benefit was due to the ovarian suppression effects promoted by chemotherapy. For this group of patients, consider chemotherapy followed by endocrine therapy or alternatively, ovarian function suppression combined with either tamoxifen or an AI.[2]
	≥26	In premenopausal patients with HR-positive, HER2-negative, pT1–3 and pN1 (1–3 positive nodes) tumors and an RS ≥26, the addition of chemotherapy to endocrine therapy is recommended.[2]

[a] Gene expression assays provide prognostic and therapy-predictive information that complements T,N,M and biomarker information. Use of these assays is not required for staging. The 21-gene assay (Oncotype Dx) is preferred by the NCCN Breast Cancer Panel for prognosis and prediction of chemotherapy benefit. Other prognostic gene expression assays can provide prognostic information but the ability to predict chemotherapy benefit is unknown.
[b] See Special Considerations for Breast Cancer in Males (Sex Assigned at Birth) (BINV-J).
[c] In the overall study population of the RxPONDER trial, 10.3% had high-grade disease and 9.2% had 3 involved nodes.

GENE EXPRESSION ASSAYS FOR CONSIDERATION OF ADJUVANT SYSTEMIC THERAPY[a,b]

Assay	Recurrence Risk	Treatment Implications
70-gene (MammaPrint) (for pN0 and 1–3 positive nodes)	High	Patients with high clinical risk and low genomic risk were randomly assigned to receive chemotherapy (n = 749) or not (n = 748); this was the intention-to-treat population. The 8-year estimates for distant metastasis-free survival in the intention-to-treat population were 92.0% (95% CI, 89.6–93.8) for chemotherapy versus 89.4% (86.8–91.5) for no chemotherapy (HR, 0.66; 95% CI, 0.48–0.92). An exploratory analysis confined to the subset of patients with HR-positive, HER2-negative disease (1358 [90.7%] of 1497 randomly assigned patients, of whom 676 received chemotherapy and 682 did not) shows different effects of chemotherapy administration on 8-year distant metastasis-free survival according to age: 93.6% (95% CI, 89.3–96.3) with chemotherapy versus 88.6% (83.5–92.3) without chemotherapy in 464 patients aged ≤50 years (absolute difference 5.0 percentage points [SE, 2.8; 95% CI, −0.5–10.4]) and 90.2% (86.8–92.7) versus 90.0% (86.6–92.6) in 894 females >50 years (absolute difference 0.2 percentage points [2.1, −4.0–4.4]). The 8-year distant metastasis-free survival in the exploratory analysis by nodal status in these patients was 91.7% (95% CI, 88.1–94.3) with chemotherapy and 89.2% (85.2–92.2) without chemotherapy in 699 node-negative patients (absolute difference 2.5 percentage points [SE, 2.3; 95% CI, −2.1 –7.2]) and 91.2% (87.2–94.0) versus 89.9% (85.8–92.8) for 658 patients with one to three positive nodes (absolute difference 1.3 percentage points [2.4, −3.5–6.1]).[3]
	Low[c]	
50-gene (Prosigna) (for pN0 and 1–3 positive nodes)	Node negative: Low (0–40), Intermediate (41–60), High (61–100)	For patients with T1 and T2 HR-positive, HER2-negative, pN0 tumors, a risk of recurrence score in the low range, regardless of T size, places the tumor into the same prognostic category as T1a–T1b,N0,M0.[4]
	Node positive: Low (0–40)	In patients with HR-positive, HER2-negative, pN+ tumors (1–3 positive lymph nodes) with low risk of recurrence score, treated with endocrine therapy alone, the distant recurrence risk was less than 3.5% at 10 years and no distant recurrence was seen at 10 years in the TransATAC study in a similar group.[5]
	Node positive: High (41–100)	
12-gene (EndoPredict) (pN0 and 1–3 positive nodes)	Low (≤3.3)	For patients with T1 and T2 HR-positive, HER2-negative, and pN0 tumors, a 12-gene low-risk score, regardless of T size, places the tumor into the same prognostic category as T1a–T1b,N0,M0.[6] In ABCSG 6/8, patients in the low-risk group had risk of distant recurrence of 4% at 10 years and in the TransATAC study, patients with 1–3 positive nodes in the low-risk group had a 5.6% risk of distant recurrence at 10 years.[6,7] The assay is prognostic in endocrine and chemo-endocrine treated patients.[7]
	High (>3.3)	

[a] Gene expression assays provide prognostic and therapy-predictive information that complements T,N,M and biomarker information. Use of these assays is not required for staging. The 21-gene assay (Oncotype Dx) is preferred by the NCCN Breast Cancer Panel for prognosis and prediction of chemotherapy benefit. Other prognostic gene expression assays can provide prognostic information but the ability to predict chemotherapy benefit is unknown.
[b] See Special Considerations for Breast Cancer in Males (Sex Assigned at Birth) (BINV-J).
[2] Postmenopausal patients with UltraLow risk in the Stockholm Tamoxifen trial had a 20-year breast cancer specific survival of 97% with 2-5 years of Tamoxifen (Esserman LJ, et al. JAMA Oncology 2017;3:1503-1510). Patients with an ultralow-risk in the MINDACT trial have shown 8-year breast cancer specific survival above 99%. (Lopes Cardozo JMN, et al. J Clin Oncol 2022;40:1335-1345).

GENE EXPRESSION ASSAYS FOR CONSIDERATION OF EXTENDED ADJUVANT SYSTEMIC THERAPY[a,b]

Assay	Recurrence Risk/ Predictive Result	Treatment Implications
Breast Cancer Index (BCI)	BCI (H/I) Low	• For patients with T1 and T2 HR-positive, HER2-negative, and pN0 tumors, a BCI (H/I) in the low-risk range (0–5), regardless of T size, places the tumor into the same prognostic category as T1a–T1b, N0, M0. • Patients with BCI (H/I) low demonstrated a lower risk of distant recurrence (compared to BCI [H/I] high) and no significant improvement in disease-free survival (DFS) or OS compared to the control arm in terms of extending endocrine therapy duration.[8]
	BCI (H/I) High	• For patients with T1 HR-positive, HER2-negative, and pN0 tumors, a BCI (H/I) high (5.1–10) demonstrated significant rates of late distant recurrence. • In secondary analyses of the MA.17, Trans-aTTom, and IDEAL trials, patients with HR-positive, T1–T3, pN0 or pN+ who had a BCI (H/I) high demonstrated significant improvements in DFS when adjuvant endocrine therapy was extended, compared to the control arm.[8-11] • In contrast, BCI (H/I) low patients derived no benefit from extended adjuvant therapy.[8]

[a] Gene expression assays provide prognostic and therapy-predictive information that complements T,N,M and biomarker information. Use of these assays is not required for staging. The 21-gene assay (Oncotype Dx) is preferred by the NCCN Breast Cancer Panel for prognosis and prediction of chemotherapy benefit. Other prognostic gene expression assays can provide prognostic information but the ability to predict chemotherapy benefit is unknown.
[b] See Special Considerations for Breast Cancer in Males (Sex Assigned at Birth) (BINV-J).

The NCCN also had the following to summarize about the assays mentioned above and their respective interpretations and/or rationales:

For Oncotype DX in Node-Negative, HR-positive, HER2-negative disease: "Among patients with T1b/c and T2, lymph node-negative, HR-positive, HER2-negative tumors with RS between 0-10, the risk of distant recurrence is low and these patients derive no incremental benefit from the addition of adjuvant chemotherapy to endocrine therapy. At the other end of the spectrum, patients with lymph node-negative, HR-positive, HER2-negative cancers with high RS (≥31) have a higher risk of distant recurrence and secondary analyses of prospective studies demonstrate a clear benefit from adjuvant chemotherapy."

Further, "for those with intermediate RS (11-25), the recently reported TAILORx trial of postmenopausal patients (n=6711) with lymph node-negative, HR-positive, HER-2 negative breast cancer, showed similar disease-free survival rates at 9-years in those who received adjuvant chemotherapy followed by endocrine therapy compared with endocrine therapy alone."

Based on secondary analyses of studies involving the use of Oncotype DX, the NCCN states that for individuals with node-positive, HR-positive, HER2-negative disease, "results suggest that in patients with limited nodal disease (1-3 positive lymph nodes) and a low RS, the absolute benefit from chemotherapy is likely to be very small." On the other hand, "There is a clear benefit from adjuvant chemotherapy in patients with node-positive, HR-positive, HER2-negative tumors, if the RS is high (≥31)." However, "The absolute benefit of chemotherapy in patients with limited lymph node involvement and a RS ≤25 remains to be determined."

For MammaPrint: The NCCN claims that the "data suggest that the additional benefit of adjuvant chemotherapy in patients with high-clinical risk/low genomic risk is likely

to be small" and that the data suggest that "the results of the 70-gene signature do not provide evidence for making recommendations regarding chemotherapy for patients at low clinical risk."

For PAM50: for "patients with T1 and T2, HR-positive, HER2-negative, lymph node-negative tumors, a ROR in the low range, regardless of tumor size, places the individual into the same prognostic category as T1a-T1b, N0, M0 tumors.

In patients with 1-3 lymph-node positive, HR-positive, HER2-negative disease with low-risk of recurrence score, the distant recurrence risk was less than 3.5% at 10 years with endocrine therapy alone."

For EndoPredict: "patients with HR-positive, HER-2 negative, and lymph-node node-negative disease with a low-risk score by the 12-gene assay had risk of distant recurrence of 4% at 10 years" and "patients with T1 and T2 HR-positive, HER2-negative, and lymph node-negative tumors, a 12-gene low risk score, regardless of T size, places the tumor into the same prognostic category as T1a-T1b, N0, M0." Citing another study, the NCCN states the following for EndoPredict: "patients with 1-3 positive nodes in the low-risk group had a 5.6% risk of distant recurrence at 10 years, suggesting that chemotherapy would be of limited benefit in these patients."

For the BCI: "For patients with T1 and T2 HR-positive, HER2-negative, and lymph node-negative tumors, a BCI in the low-risk range, regardless of T size, places the tumor into the same prognostic category as T1a-T1b, N0, M0. There are limited data as to the role of BCI in HR-positive, HER2-negative, and lymph node-positive breast cancer" (NCCN, 2024).

The NCCN **strongly recommends (Category 1)** considering the use of the 21-gene reverse transcriptase polymerase chain reaction (RT-PCR) assay for determining the use of adjuvant chemotherapy in premenopausal and postmenopausal patients with the following tumor characteristics:

- Hormone receptor-positive;
- HER2 [human epidermal growth factor receptor 2]-negative;
- Ductal/NST, lobular, mixed or micropapillary histology;
- pT1, pT2 or pT3 stage; and pN0;
- Tumor >0.5 cm.

The assay may also be considered for pre- and postmenopausal, node-positive (up to three positive nodes) individuals if such individuals are candidates for chemotherapy.

Within a section on special considerations for breast cancer in men, the NCCN states (Category 2A), "Data are limited regarding the use of molecular assays to assess prognosis and to predict benefit from chemotherapy in males with breast cancer. Available data suggest 21-gene assay recurrence score provides prognostic information in males with breast cancer" (NCCN, 2024).

NCCN also notes that ER status should be determined for DCIS patients, but do not mention any gene expression tests for evaluation (NCCN, 2024).

National Institute for Health and Care Excellence (NICE)

NICE released three new guidelines regarding gene expression tests, which are as follows:

1. "EndoPredict (EPclin score), Oncotype DX Breast Recurrence Score and Prosigna are recommended as options for guiding adjuvant chemotherapy decisions for people with oestrogen receptor (ER)-positive, human epidermal growth factor receptor 2 (HER2)-negative and lymph node (LN)-negative (including micrometastatic disease; see section 5.4) early breast cancer, only if:
    - they have an intermediate risk of distant recurrence using a validated tool such as PREDICT or the Nottingham Prognostic Index
    - information provided by the test would help them choose, with their clinician, whether or not to have adjuvant chemotherapy taking into account their preference
    - the companies provide the tests to the NHS with the discounts agreed in the access proposals and
    - clinicians and companies make timely, complete and linkable record-level test data available to the National Cancer Registration and Analysis Service as described in the data collection arrangements agreed with NICE (see section 5.29)."
2. "MammaPrint is not recommended for guiding adjuvant chemotherapy decisions for people with ER-positive, HER2-negative and LN-negative early breast cancer because it is not cost effective" (NICE, 2018).

European Society for Medical Oncology (ESMO)

ESMO notes that "In cases of uncertainty regarding indications for adjuvant chemotherapy (after consideration of other tests), gene expression assays, such as MammaPrint, Oncotype DX, Prosigna and EndoPredict, may be used, where available. These assays can determine the individual's recurrence risk as well as potentially

predict the benefit of chemotherapy." This point was unchanged in the 2019 update" (Cardoso et al., 2019; Senkus et al., 2015).

In a 2019 update, ESMO elaborates on gene expression profiles for breast cancer. They state that "Gene expression profiles, such as MammaPrint, Oncotype DX Recurrence Score, Prosigna and Endopredict, may be used to gain additional prognostic and/or predictive information to complement pathology assessment and to predict the benefit of adjuvant chemotherapy." They also remark that Prosigna, Endopredict, and Oncotype are intended for ER-positive early breast cancer only and that Mammaprint and OncoType are still being evaluated for clinical utility (Cardoso et al., 2019).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

MammaPrint® received 510(k) clearance from the FDA on June 22, 2007. MammaPrint® is performed in Agendia laboratories and is FDA cleared for use in breast cancer patients who are under 61 years of age, with stage 1 or 2 breast cancer, tumor size ≤5 cm, and lymph node negative (FDA, 2007). MammaPrint® is a qualitative *in vitro* diagnostic test service which uses a 70 gene expression profile from fresh breast cancer tissue samples to assess a patient's risk for distant metastasis (up to 10 years in patients less than 61 years of age). It is indicated for use by physicians as a prognostic marker only, along with other clinicopathological factors. On December 11, 2009, the FDA approved modifications to the cleared device, adding five year prognostic information for breast cancer patients ≥61 years of age (FDA, 2009).

On January 23, 2015, the MammaPrint® FFPE received 510(k) clearance from the FDA. It is a qualitative *in vitro* diagnostic test, using the 70 gene expression profile obtained from FFPE breast cancer tissue samples to assess a patient's risk for distant metastasis within five years. Like the MammaPrint®, the MammaPrint® FFPE is performed for breast cancer patients who are under 61 years of age, with stage 1 or 2 breast cancer, tumor size ≤5 cm, and lymph node negative. It is indicated for use by physicians as a prognostic marker only, along with other clinicopathological factors (FDA, 2015).

The Prosigna™ Breast Cancer Prognostic Gene Signature Assay received 510(k) clearance from the FDA based on substantial equivalence to MammaPrint® on September 6, 2013. It is an *in vitro* diagnostic assay performed on the NanoString nCounter® Dx Analysis System and uses formalin-fixed, paraffin embedded breast tumor tissue that has previously been diagnosed as invasive breast carcinoma. This assay measures the RNA expression profile of 58 genes and weights the expression together with clinical variables to generate a risk category and numerical score, assessing a patient's risk of distant recurrence of disease (FDA, 2013).

On August 5, 2022, FDA approved Enhertu (fam-trastuzumab-deruxtecan-nxki) for the treatment of patients with unresectable or metastatic breast cancer that is also classified as HER2-low; establishing the first FDA-approved therapy for patients diagnosed with HER2-low breast cancer.

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81479	Unlisted molecular pathology procedure
81518	Oncology (breast), mRNA, gene expression profiling by real-time RT-PCR of 11 genes (7 content and 4 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithms reported as percentage risk for metastatic recurrence and likelihood of benefit from extended endocrine therapy Proprietary test: Breast Cancer Index Lab/manufacturer: Biotheranostics, Inc
81519	Oncology (breast), mRNA, gene expression profiling by real-time RT-PCR of 21 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as recurrence score Proprietary test: Oncotype DX® Lab/manufacturer: Genomic Health
81520	Oncology (breast), mrna gene expression profiling by hybrid capture of 58 genes (50 content and 8 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a recurrence risk score Proprietary test: Prosigna® Breast Cancer Assay Lab/manufacturer: NanoString Technologies, Inc.

CPT	Code Description
81521	Oncology (breast), mrna, microarray gene expression profiling of 70 content genes and 465 housekeeping genes, utilizing fresh frozen or formalin-fixed paraffin-embedded tissue, algorithm reported as index related to risk of distant metastasis Proprietary test: MammaPrint® Lab/Manufacturer: Agendia, Inc.
81522	Oncology (breast), mRNA, gene expression profiling by RT-PCR of 12 genes (8 content and 4 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as recurrence risk score Proprietary test: EndoPredict® Lab/Manufacturer: Myriad Genetic Laboratories, Inc.
81523	Oncology (breast), mRNA, next-generation sequencing gene expression profiling of 70 content genes and 31 housekeeping genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as index related to risk to distant metastasis Proprietary test: MammaPrint® Lab/Manufacturer: Agendia, Inc
81599	Unlisted multianalyte assay with algorithmic analysis
88360	Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, per specimen, each single antibody stain procedure; manual
88361	Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, per specimen, each single antibody stain procedure; using computer-assisted technology
88367	Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), using computer-assisted technology, per specimen; initial single probe stain procedure
88368	Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), manual, per specimen; initial single probe stain procedure
88381	Microdissection (ie, sample preparation of microscopically identified target); manual
0045U	Oncology (breast ductal carcinoma in situ), mRNA, gene expression profiling by real-time RT-PCR of 12 genes (7 content and 5 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as recurrence score Proprietary test: Oncotype DX® Breast DCIS Score™ Lab/manufacturer: Genomic Health, Inc.

CPT	Code Description
0067U	Oncology (breast), immunohistochemistry, protein expression profiling of 4 biomarkers (matrix metalloproteinase-1 [MMP-1], carcinoembryonic antigen-related cell adhesion molecule 6 [CEACAM6], hyaluronoglucosaminidase [HYAL1], highly expressed in cancer protein [HEC1]), formalin-fixed paraffin-embedded precancerous breast tissue, algorithm reported as carcinoma risk score Proprietary test: BBDRisk Dx™ Lab/manufacturer: Silbiotech, Inc.
0153U	Oncology (breast), mRNA, gene expression profiling by next-generation sequencing of 101 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a triple negative breast cancer clinical subtype(s) with information on immune cell involvement Proprietary test: Insight TNBCtype™ Lab/Manufacturer: Insight Molecular Labs
0295U	Oncology (breast ductal carcinoma in situ), protein expression profiling by immunohistochemistry of 7 proteins (COX2, FOXA1, HER2, Ki-67, p16, PR, SIAH2), with 4 clinicopathologic factors (size, age, margin status, palpability), utilizing formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as a recurrence risk score Proprietary test: DCISionRT® Lab/Manufacturer: PreludeDx™/Prelude Corporation
S3854	Gene expression profiling panel for use in the management of breast cancer treatment

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Agendia. (2014). *MammaPrint® FFPE Result*. https://www.agendia.com/pdf/high-risk.pdf

Agendia. (2024a). BluePrint: a test to predict likely tumor behavior. https://agendia.com/blueprint/

Agendia. (2024b). *MammaPrint: a test to predict risk of breast cancer recurrence*. https://agendia.com/mammaprint/

Andre, F., Ismaila, N., Allison, K. H., Barlow, W. E., Collyar, D. E., Damodaran, S., Henry, N. L., Jhaveri, K., Kalinsky, K., Kuderer, N. M., Litvak, A., Mayer, E. L., Pusztai, L., Raab, R., Wolff, A. C., & Stearns, V. (2022). Biomarkers for Adjuvant Endocrine and

Chemotherapy in Early-Stage Breast Cancer: ASCO Guideline Update. *J Clin Oncol*, *40*(16), 1816-1837. https://doi.org/10.1200/jco.22.00069

Andre, F., Ismaila, N., Henry, N. L., Somerfield, M. R., Bast, R. C., Barlow, W., Collyar, D. E., Hammond, M. E., Kuderer, N. M., Liu, M. C., Van Poznak, C., Wolff, A. C., & Stearns, V. (2019). Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer: ASCO Clinical Practice Guideline Update—Integration of Results From TAILORx. *Journal of Clinical Oncology*, *37*(22), 1956-1964. https://doi.org/10.1200/JCO.19.00945

BBDRiskDx. (2023). *What is the BBDRISK DX® Test?* https://bbdrisk.com/patients/bbdrisk-dx-test/bbdrisk-dx/

Biotheranostics. (2023). *About Breast Cancer Index (BCI)*. https://www.breastcancerindex.com/about-breast-cancer-index#

Blok, E. J., Bastiaannet, E., van den Hout, W. B., Liefers, G. J., Smit, V., Kroep, J. R., & van de Velde, C. J. H. (2018). Systematic review of the clinical and economic value of gene expression profiles for invasive early breast cancer available in Europe. *Cancer Treat Rev*, *62*, 74-90. https://doi.org/10.1016/j.ctrv.2017.10.012

Bremer, T., Whitworth, P. W., Patel, R., Savala, J., Barry, T., Lyle, S., Leesman, G., Linke, S. P., Jirström, K., Zhou, W., Amini, R. M., & Wärnberg, F. (2018). A Biological Signature for Breast Ductal Carcinoma In Situ to Predict Radiotherapy Benefit and Assess Recurrence Risk. *Clin Cancer Res*, *24*(23), 5895-5901. https://doi.org/10.1158/1078-0432.Ccr-18-0842

Camp, N. J., Madsen, M. J., Herranz, J., Rodriguez-Lescure, A., Ruiz, A., Martin, M., & Bernard, P. S. (2019). Re-interpretation of PAM50 gene expression as quantitative tumor dimensions shows utility for clinical trials: application to prognosis and response to paclitaxel in breast cancer. *Breast Cancer Res Treat*. https://doi.org/10.1007/s10549-018-05097-5

Cardoso, F., Kyriakides, S., Ohno, S., Penault-Llorca, F., Poortmans, P., Rubio, I. T., Zackrisson, S., & Senkus, E. (2019). Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-updagger. *Ann Oncol*, *30*(8), 1194-1220. https://doi.org/10.1093/annonc/mdz173

Cardoso, F., van't Veer, L. J., Bogaerts, J., Slaets, L., Viale, G., Delaloge, S., Pierga, J.-Y., Brain, E., Causeret, S., DeLorenzi, M., Glas, A. M., Golfinopoulos, V., Goulioti, T., Knox, S., Matos, E., Meulemans, B., Neijenhuis, P. A., Nitz, U., Passalacqua, R., . . . Piccart, M. (2016). 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. *New England Journal of Medicine*, *375*(8), 717-729. https://doi.org/10.1056/NEJMoa1602253

Chen, R., Xia, L., Tu, K., Duan, M., Kukurba, K., Li-Pook-Than, J., Xie, D., & Snyder, M. (2018). Longitudinal personal DNA methylome dynamics in a human with a chronic condition. *Nat Med*, *24*(12), 1930-1939. https://doi.org/10.1038/s41591-018-0237-x

Dabbs, D., Mittal, K., Heineman, S., Whitworth, P., Shah, C., Savala, J., Shivers, S. C., & Bremer, T. (2023). Analytical validation of the 7-gene biosignature for prediction of recurrence risk and radiation therapy benefit for breast ductal carcinoma in situ. *Front Oncol*, *13*, 1069059. https://doi.org/10.3389/fonc.2023.1069059

Darby, S., McGale, P., Correa, C., Taylor, C., Arriagada, R., Clarke, M., Cutter, D., Davies, C., Ewertz, M., Godwin, J., Gray, R., Pierce, L., Whelan, T., Wang, Y., & Peto, R. (2011). Effect of radiotherapy after breast-conserving surgery on 10-year recurrence and 15-year breast cancer death: meta-analysis of individual patient data for 10,801 women in 17 randomised trials. *Lancet*, *378*(9804), 1707-1716. https://doi.org/10.1016/s0140-6736(11)61629-2

Davies, C., Godwin, J., Gray, R., Clarke, M., Cutter, D., Darby, S., McGale, P., Pan, H. C., Taylor, C., Wang, Y. C., Dowsett, M., Ingle, J., & Peto, R. (2011). Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet*, *378*(9793), 771-784. https://doi.org/10.1016/s0140-6736(11)60993-8

Ding, S., Wu, J., Lin, C., Chen, W., Li, Y., Shen, K., & Zhu, L. (2019). Predictors for Survival and Distribution of 21-Gene Recurrence Score in Patients With Pure Mucinous Breast Cancer: A SEER Population-Based Retrospective Analysis. *Clin Breast Cancer*, *19*(1), e66-e73. https://doi.org/10.1016/j.clbc.2018.10.001

Dubsky, P. C., Singer, C. F., Egle, D., Wette, V., Petru, E., Balic, M., Pichler, A., Greil, R., Petzer, A. L., Bago-Horvath, Z., Fesl, C., Meek, S. M., Kronenwett, R., Rudas, M., Gnant, M., & Filipits, M. (2020). The EndoPredict score predicts response to neoadjuvant chemotherapy and neoendocrine therapy in hormone receptor-positive, human epidermal growth factor receptor 2-negative breast cancer patients from the ABCSG-34 trial. *Eur J Cancer*, *134*, 99-106. https://www.ejcancer.com/article/S0959-8049(20)30223-9/fulltext

FDA. (2007). *MammaPrint®: 510(k) Substantial Equivalence Determination Decision Summary*. https://www.accessdata.fda.gov/cdrh_docs/reviews/K070675.pdf

FDA. (2009). *MammaPrint®: 510(k) Substantial Equivalence Determination Decision Summary*. https://www.accessdata.fda.gov/cdrh_docs/reviews/K081092.pdf

FDA. (2013). *Prosigna(TM) Breast Cancer Prognostic Gene Signature Assay: 510(k) Substantial Equivalence Determination Decision Summary Assay and Instrument Combination Template*. https://www.accessdata.fda.gov/cdrh_docs/reviews/K130010.pdf

FDA. (2015). *MammaPrint® FFPE: 510(k) Substantial Equivalence Determination Decision Summary Assay and Instrument Combination Template*. https://www.accessdata.fda.gov/cdrh_docs/reviews/K141142.pdf

Filipits, M., Dubsky, P., Rudas, M., Greil, R., Balic, M., Bago-Horvath, Z., Singer, C. F., Hlauschek, D., Brown, K., Bernhisel, R., Kronenwett, R., Lancaster, J. M., Fitzal, F., & Gnant, M. (2019). Prediction of Distant Recurrence using EndoPredict among Women

with ER+, HER2- Node-Positive and Node-Negative Breast Cancer Treated with Endocrine Therapy Only. *Clinical Cancer Research*, clincanres.0376.2019. https://doi.org/10.1158/1078-0432.CCR-19-0376

Filipits, M., Rudas, M., Jakesz, R., Dubsky, P., Fitzal, F., Singer, C. F., Dietze, O., Greil, R., Jelen, A., Sevelda, P., Freibauer, C., Muller, V., Janicke, F., Schmidt, M., Kolbl, H., Rody, A., Kaufmann, M., Schroth, W., Brauch, H., . . . Gnant, M. (2011). A new molecular predictor of distant recurrence in ER-positive, HER2-negative breast cancer adds independent information to conventional clinical risk factors. *Clin Cancer Res*, *17*(18), 6012-6020. https://doi.org/10.1158/1078-0432.Ccr-11-0926

Forouzanfar, M. H., Foreman, K. J., Delossantos, A. M., Lozano, R., Lopez, A. D., Murray, C. J., & Naghavi, M. (2011). Breast and cervical cancer in 187 countries between 1980 and 2010: a systematic analysis. *Lancet*, *378*(9801), 1461-1484. https://doi.org/10.1016/s0140-6736(11)61351-2

Foukakis, T., & Bergh, J. (2022, November 9). *Prognostic and predictive factors in early, nonmetastatic breast cancer*. https://www.uptodate.com/contents/prognostic-and-predictive-factors-in-early-non-metastatic-breast-cancer

Giuliano, A. E., Connolly, J. L., Edge, S. B., Mittendorf, E. A., Rugo, H. S., Solin, L. J., Weaver, D. L., Winchester, D. J., & Hortobagyi, G. N. (2017). Breast Cancer-Major changes in the American Joint Committee on Cancer eighth edition cancer staging manual. *CA: A Cancer Journal for Clinicians*, *67*(4), 290-303. https://doi.org/10.3322/caac.21393

Gustavsen, G., Schroeder, B., Kennedy, P., Pothier, K. C., Erlander, M. G., Schnabel, C. A., & Ali, H. (2014). Health economic analysis of Breast Cancer Index in patients with ER+, LN- breast cancer. *Am J Manag Care*, *20*(8), e302-310. https://www.ajmc.com/view/health-economic-analysis-of-breast-cancer-index-in-patients-with-er-ln-breast-cancer

Harnan, S., Tappenden, P., Cooper, K., Stevens, J., Bessey, A., Rafia, R., Ward, S., Wong, R., Stein, R. C., & Brown, J. (2019). Tumour profiling tests to guide adjuvant chemotherapy decisions in early breast cancer: a systematic review and economic analysis. *Health Technol Assess*, *23*(30), 1-328. https://doi.org/10.3310/hta23300

Harris, L. N., Ismaila, N., McShane, L. M., Andre, F., Collyar, D. E., Gonzalez-Angulo, A. M., Hammond, E. H., Kuderer, N. M., Liu, M. C., Mennel, R. G., Van Poznak, C., Bast, R. C., & Hayes, D. F. (2016). Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol*, *34*(10), 1134-1150. https://doi.org/10.1200/jco.2015.65.2289

Henry, N. L., Somerfield, M. R., Abramson, V. G., Ismaila, N., Allison, K. H., Anders, C. K., Chingos, D. T., Eisen, A., Ferrari, B. L., Openshaw, T. H., Spears, P. A., Vikas, P., & Stearns, V. (2019). Role of Patient and Disease Factors in Adjuvant Systemic Therapy Decision Making for Early-Stage, Operable Breast Cancer: Update of the ASCO

Endorsement of the Cancer Care Ontario Guideline. *Journal of Clinical Oncology*, *37*(22), 1965-1977. https://doi.org/10.1200/JCO.19.00948

Jensen, M.-B., Lænkholm, A.-V., Balslev, E., Buckingham, W., Ferree, S., Glavicic, V., Dupont Jensen, J., Søegaard Knoop, A., Mouridsen, H. T., Nielsen, D., Nielsen, T. O., & Ejlertsen, B. (2020). The Prosigna 50-gene profile and responsiveness to adjuvant anthracycline-based chemotherapy in high-risk breast cancer patients. *npj Breast Cancer*, *6*(1), 7. https://doi.org/10.1038/s41523-020-0148-0

Kalinsky, K., Barlow, W. E., Meric-Bernstam, F., Gralow, J. R., Albain, K. S., Hayes, D., Lin, N., Perez, E. A., Goldstein, L. J., Chia, S., Dhesy-Thind, S., Rastogi, P., Alba, E., Delaloge, S., Martín, M., Gil, M. G., Arce-Salinas, C., Brain, E., Park, I. H., . . . Pusztai, L. (2021). Abstract GS3-00: First results from a phase III randomized clinical trial of standard adjuvant endocrine therapy (ET) +/- chemotherapy (CT) in patients (pts) with 1-3 positive nodes, hormone receptor-positive (HR+) and HER2-negative (HER2-) breast cancer (BC) with recurrence score (RS) < 25: SWOG S1007 (RxPonder). *Cancer Research*, *81*(4 Supplement), GS3-00-GS03-00. https://doi.org/10.1158/1538-7445.Sabcs20-gs3-00

Kizy, S., Huang, J. L., Marmor, S., Blaes, A., Yuan, J., Beckwith, H., Tuttle, T. M., & Hui, J. Y. C. (2018). Distribution of 21-Gene Recurrence Scores Among Breast Cancer Histologic Subtypes. *Arch Pathol Lab Med*, *142*(6), 735-741. https://doi.org/10.5858/arpa.2017-0169-OA

Krop, I., Ismaila, N., Andre, F., Bast, R. C., Barlow, W., Collyar, D. E., Hammond, M. E., Kuderer, N. M., Liu, M. C., Mennel, R. G., Van Poznak, C., Wolff, A. C., & Stearns, V. (2017). Use of Biomarkers to Guide Decisions on Adjuvant Systemic Therapy for Women With Early-Stage Invasive Breast Cancer: American Society of Clinical Oncology Clinical Practice Guideline Focused Update. *J Clin Oncol*, Jco2017740472. https://doi.org/10.1200/jco.2017.74.0472

Lashen, A., Toss, M. S., Fadhil, W., Oni, G., Madhusudan, S., & Rakha, E. (2023). Evaluation oncotype DX(®) 21-gene recurrence score and clinicopathological parameters: a single institutional experience. *Histopathology*, *82*(5), 755-766. https://doi.org/10.1111/his.14863

Liefers, G.-J., Noordhoek, I., Treuner, K., Putter, H., Zhang, Y., Kranenbarg, E. M. K., Wong, J., Velde, C. J. H. V. D., & Schnabel, C. A. (2020). Breast cancer index (BCI) predicts benefit of two-and-a-half versus five years of extended endocrine therapy in HR+ breast cancer patients treated in the ideal trial. *Journal of Clinical Oncology*, *38*(15_suppl), 512-512. https://doi.org/10.1200/JCO.2020.38.15_suppl.512

Ma, X.-J., Salunga, R., Dahiya, S., Wang, W., Carney, E., Durbecq, V., Harris, A., Goss, P., Sotiriou, C., Erlander, M., & Sgroi, D. (2008). A Five-Gene Molecular Grade Index and HOXB13:IL17BR Are Complementary Prognostic Factors in Early Stage Breast Cancer. *Clinical Cancer Research*, *14*(9), 2601. https://doi.org/10.1158/1078-0432.CCR-07-5026

Mamounas, E. P., Tang, G., Fisher, B., Paik, S., Shak, S., Costantino, J. P., Watson, D., Geyer, C. E., Jr., Wickerham, D. L., & Wolmark, N. (2010). Association between the 21-gene recurrence score assay and risk of locoregional recurrence in node-negative, estrogen receptor-positive breast cancer: results from NSABP B-14 and NSABP B-20. *J Clin Oncol*, *28*(10), 1677-1683. https://doi.org/10.1200/jco.2009.23.7610

Martin, M., Brase, J. C., Ruiz, A., Prat, A., Kronenwett, R., Calvo, L., Petry, C., Bernard, P. S., Ruiz-Borrego, M., Weber, K. E., Rodriguez, C. A., Alvarez, I. M., Segui, M. A., Perou, C. M., Casas, M., Carrasco, E., Caballero, R., & Rodriguez-Lescure, A. (2016). Prognostic ability of EndoPredict compared to research-based versions of the PAM50 risk of recurrence (ROR) scores in node-positive, estrogen receptor-positive, and HER2-negative breast cancer. A GEICAM/9906 sub-study. *Breast Cancer Res Treat*, *156*(1), 81-89. https://doi.org/10.1007/s10549-016-3725-z

Myriad. (2021, 12/10/2019). *Myriad EndoPredict® Technical Specifications*. Retrieved 01/27/2020 from https://myriad-library.s3.amazonaws.com/technical-specifications/EndoPredict-Technical-Specifications.pdf

Myriad. (2024a). *EndoPredict Executive Summary*. Myriad Genetics, Inc. Retrieved 01/21/2020 from https://myriad.com/managed-care/endopredict/

Myriad. (2024b). *WHAT IS ENDOPREDICT?* https://endopredict.com/overview/

NCCN. (2024, January 25, 2024). *NCCN Clinical Practice Guidelines in Oncology: Breast Cancer v1.2024*. NCCN. https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf

NICE. (2018). *Tumour profiling tests to guide adjuvant chemotherapy decisions in early breast cancer*. https://www.nice.org.uk/guidance/dg34/chapter/1-Recommendations

Nielsen, T. O., Parker, J. S., Leung, S., Voduc, D., Ebbert, M., Vickery, T., Davies, S. R., Snider, J., Stijleman, I. J., Reed, J., Cheang, M. C., Mardis, E. R., Perou, C. M., Bernard, P. S., & Ellis, M. J. (2010). A comparison of PAM50 intrinsic subtyping with immunohistochemistry and clinical prognostic factors in tamoxifen-treated estrogen receptor-positive breast cancer. *Clin Cancer Res*, *16*(21), 5222-5232. https://doi.org/10.1158/1078-0432.ccr-10-1282

Paik, S., Shak, S., Tang, G., Kim, C., Baker, J., Cronin, M., Baehner, F. L., Walker, M. G., Watson, D., Park, T., Hiller, W., Fisher, E. R., Wickerham, D. L., Bryant, J., & Wolmark, N. (2004). A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. *N Engl J Med*, *351*(27), 2817-2826. https://doi.org/10.1056/NEJMoa041588

Paik, S., Tang, G., Shak, S., Kim, C., Baker, J., Kim, W., Cronin, M., Baehner, F. L., Watson, D., Bryant, J., Costantino, J. P., Geyer, C. E., Jr., Wickerham, D. L., & Wolmark, N. (2006). Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. *J Clin Oncol*, *24*(23), 3726-3734. https://doi.org/10.1200/jco.2005.04.7985

Parker, J. S., Mullins, M., Cheang, M. C., Leung, S., Voduc, D., Vickery, T., Davies, S., Fauron, C., He, X., Hu, Z., Quackenbush, J. F., Stijleman, I. J., Palazzo, J., Marron, J. S., Nobel, A. B., Mardis, E., Nielsen, T. O., Ellis, M. J., Perou, C. M., & Bernard, P. S. (2009). Supervised risk predictor of breast cancer based on intrinsic subtypes. *J Clin Oncol*, *27*(8), 1160-1167. https://doi.org/10.1200/jco.2008.18.1370

Petkov, V. I., Miller, D. P., Howlader, N., Gliner, N., Howe, W., Schussler, N., Cronin, K., Baehner, F. L., Cress, R., Deapen, D., Glaser, S. L., Hernandez, B. Y., Lynch, C. F., Mueller, L., Schwartz, A. G., Schwartz, S. M., Stroup, A., Sweeney, C., Tucker, T. C., . . . Shak, S. (2016). Breast-cancer-specific mortality in patients treated based on the 21-gene assay: a SEER population-based study. *npj Breast Cancer*, *2*, 16017. https://doi.org/10.1038/npjbcancer.2016.17

Peto, R., Davies, C., Godwin, J., Gray, R., Pan, H. C., Clarke, M., Cutter, D., Darby, S., McGale, P., Taylor, C., Wang, Y. C., Bergh, J., Di Leo, A., Albain, K., Swain, S., Piccart, M., & Pritchard, K. (2012). Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. *Lancet*, *379*(9814), 432-444. https://doi.org/10.1016/s0140-6736(11)61625-5

Poola, I., Yue, Q., Gillespie, J. W., Sullivan, P. S., Aguilar-Jakthong, J., Rao, J., Shaaban, A. M., Sauter, E. R., & Ricci, A. J. (2019). Breast Hyperplasias, Risk Signature, and Breast Cancer. *Cancer Prev Res (Phila)*, *12*(7), 471-480. https://doi.org/10.1158/1940-6207.Capr-19-0051

Prat, A., Guarneri, V., Paré, L., Griguolo, G., Pascual, T., Dieci, M. V., Chic, N., González-Farré, B., Frassoldati, A., Sanfeliu, E., Cejalvo, J. M., Muñoz, M., Bisagni, G., Brasó-Maristany, F., Urso, L., Vidal, M., Brandes, A. A., Adamo, B., Musolino, A., . . . Conte, P. (2020). A multivariable prognostic score to guide systemic therapy in early-stage HER2-positive breast cancer: a retrospective study with an external evaluation. *Lancet Oncol*, *21*(11), 1455-1464. https://doi.org/10.1016/s1470-2045(20)30450-2

PreludeDx. (2024). *DCISionRT(R): Welcome to a New Paradigm for DCIS Management*. Prelude Corporation. https://preludedx.com/physicians/

Qi, P., Yang, Y., Bai, Q. M., Xue, T., Ren, M., Yao, Q. L., Yang, W. T., & Zhou, X. Y. (2021). Concordance of the 21-gene assay between core needle biopsy and resection specimens in early breast cancer patients. *Breast Cancer Res Treat*, *186*(2), 327-342. https://doi.org/10.1007/s10549-020-06075-6

Rueda, O. M., Sammut, S.-J., Seoane, J. A., Chin, S.-F., Caswell-Jin, J. L., Callari, M., Batra, R., Pereira, B., Bruna, A., Ali, H. R., Provenzano, E., Liu, B., Parisien, M., Gillett, C., McKinney, S., Green, A. R., Murphy, L., Purushotham, A., Ellis, I. O., . . . Curtis, C. (2019). Dynamics of breast-cancer relapse reveal late-recurring ER-positive genomic subgroups. *Nature*, *567*(7748), 399-404. https://doi.org/10.1038/s41586-019-1007-8

Savci-Heijink, C. D., Halfwerk, H., Koster, J., Horlings, H. M., & van de Vijver, M. J. (2019). A specific gene expression signature for visceral organ metastasis in breast cancer. *BMC Cancer*, *19*(1), 333. https://doi.org/10.1186/s12885-019-5554-z

Senkus, E., Kyriakides, S., Ohno, S., Penault-Llorca, F., Poortmans, P., Rutgers, E., Zackrisson, S., & Cardoso, F. (2015). Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, *26 Suppl 5*, v8-30. https://doi.org/10.1093/annonc/mdv298

Sennerstam, R. B., Franzen, B. S. H., Wiksell, H. O. T., & Auer, G. U. (2017). Core-needle biopsy of breast cancer is associated with a higher rate of distant metastases 5 to 15 years after diagnosis than FNA biopsy. *Cancer Cytopathol*, *125*(10), 748-756. https://doi.org/10.1002/cncy.21909

Sestak, I., Buus, R., Cuzick, J., Dubsky, P., Kronenwett, R., Denkert, C., Ferree, S., Sgroi, D., Schnabel, C., Baehner, F. L., Mallon, E., & Dowsett, M. (2018). Comparison of the Performance of 6 Prognostic Signatures for Estrogen Receptor-Positive Breast Cancer: A Secondary Analysis of a Randomized Clinical Trial. *JAMA Oncol*, *4*(4), 545-553. https://doi.org/10.1001/jamaoncol.2017.5524

Sestak, I., Martin, M., Dubsky, P., Kronenwett, R., Rojo, F., Cuzick, J., Filipits, M., Ruiz, A., Gradishar, W., Soliman, H., Schwartzberg, L., Buus, R., Hlauschek, D., Rodriguez-Lescure, A., & Gnant, M. (2019). Prediction of chemotherapy benefit by EndoPredict in patients with breast cancer who received adjuvant endocrine therapy plus chemotherapy or endocrine therapy alone. *Breast Cancer Res Treat*, *176*(2), 377-386. https://doi.org/10.1007/s10549-019-05226-8

Shah, C., Bremer, T., Cox, C., Whitworth, P., Patel, R., Patel, A., Brown, E., Gold, L., Rock, D., Riley, L., Kesslering, C., Brown, S., Gabordi, R., Pellicane, J., Rabinovich, R., Khan, S., Templeton, S., Majithia, L., Willey, S. C., . . . Vicini, F. A. (2021). The Clinical Utility of DCISionRT(®) on Radiation Therapy Decision Making in Patients with Ductal Carcinoma In Situ Following Breast-Conserving Surgery. *Ann Surg Oncol*, *28*(11), 5974-5984. https://doi.org/10.1245/s10434-021-09903-1

Siegel, R. L., Miller, K. D., & Jemal, A. (2019). Cancer statistics, 2019. *CA: A Cancer Journal for Clinicians*, *69*(1), 7-34. https://doi.org/10.3322/caac.21551

Silbiotech. (2021). DESCRIPTION OF THE BBDRisk Dx® TEST. In I. Silbiotech (Ed.): Silbiotech, Inc.

Simon, R. M., Paik, S., & Hayes, D. F. (2009). Use of archived specimens in evaluation of prognostic and predictive biomarkers. *J Natl Cancer Inst*, *101*(21), 1446-1452. https://doi.org/10.1093/jnci/djp335

Sparano, J. A., Gray, R. J., Makower, D. F., Pritchard, K. I., Albain, K. S., Hayes, D. F., Geyer, C. E., Jr., Dees, E. C., Goetz, M. P., Olson, J. A., Jr., Lively, T., Badve, S. S., Saphner, T. J., Wagner, L. I., Whelan, T. J., Ellis, M. J., Paik, S., Wood, W. C., Ravdin, P. M., . . . Sledge, G. W., Jr. (2018). Adjuvant Chemotherapy Guided by a 21-Gene Expression Assay in Breast Cancer. *N Engl J Med*. https://doi.org/10.1056/NEJMoa1804710

Sparano, J. A., Gray, R. J., Makower, D. F., Pritchard, K. I., Albain, K. S., Hayes, D. F., Geyer, C. E., Jr., Dees, E. C., Perez, E. A., Olson, J. A., Jr., Zujewski, J., Lively, T., Badve, S. S., Saphner, T. J., Wagner, L. I., Whelan, T. J., Ellis, M. J., Paik, S., Wood, W. C., . . . Sledge, G. W. (2015). Prospective Validation of a 21-Gene Expression Assay in Breast Cancer. *N Engl J Med*, *373*(21), 2005-2014. https://doi.org/10.1056/NEJMoa1510764

Steiling, K., & Christenson, S. (2023, October 3, 2023). *Tools for genetics and genomics: Gene expression profiling*. https://www.uptodate.com/contents/tools-for-genetics-and-genomics-gene-expression-profiling

Taghian, A., & Merajver, S. D. (2024, January 25). *Overview of the treatment of newly diagnosed, non-metastatic breast cancer*. https://www.uptodate.com/contents/overview-of-the-treatment-of-newly-diagnosed-invasive-non-metastatic-breast-cancer

Toole, M. J., Kidwell, K. M., & Van Poznak, C. (2014). Oncotype dx results in multiple primary breast cancers. *Breast Cancer (Auckl)*, *8*, 1-6. https://doi.org/10.4137/bcbcr.S13727

Turashvili, G., Brogi, E., Morrow, M., Hudis, C., Dickler, M., Norton, L., & Wen, H. Y. (2017). The 21-gene recurrence score in special histologic subtypes of breast cancer with favorable prognosis. *Breast Cancer Res Treat*, *165*(1), 65-76. https://doi.org/10.1007/s10549-017-4326-1

Villarreal-Garza, C., Lopez-Martinez, E., Deneken, Z., Maffuz-Aziz, A., Muñoz-Lozano, J., Barragan-Carrillo, R., Ramos-Elias, P., Diaz-Perez, H., Peña Curiel, O., Curiel-Valdez, J., & Bautista, V. (2020). Change in therapeutic management after the EndoPredict assay in a prospective decision impact study of Mexican premenopausal breast cancer patients. *PLOS ONE*, *15*, e0228884. https://doi.org/10.1371/journal.pone.0228884

Wang, J., He, Z. Y., Dong, Y., Sun, J. Y., Zhang, W. W., & Wu, S. G. (2018). The Distribution and Outcomes of the 21-Gene Recurrence Score in T1-T2N0 Estrogen Receptor-Positive Breast Cancer With Different Histologic Subtypes. *Front Genet*, *9*, 638. https://doi.org/10.3389/fgene.2018.00638

Wang, W., Chen, X., Lin, L., Fei, X., Garfield, D. H., Hong, J., Gao, W., Zhu, S., Wu, J., Huang, O., He, J., Li, Y., Zhu, L., Chen, W., & Shen, K. (2018). Distribution and Clinical Utility of the 21-gene Recurrence Score in Pure Mucinous Breast Cancer Patients: a case-control study. *J Cancer*, *9*(18), 3216-3224. https://doi.org/10.7150/jca.27291

Warf, M. B., Rajamani, S., Krappmann, K., Doedt, J., Cassiano, J., Brown, K., Reid, J. E., Kronenwett, R., & Roa, B. B. (2017). Analytical validation of a 12-gene molecular test for the prediction of distant recurrence in breast cancer. *Future Science OA*, *3*(3), FSO221. https://doi.org/10.4155/fsoa-2017-0051

Wärnberg, F., Karlsson, P., Holmberg, E., Sandelin, K., Whitworth, P. W., Savala, J., Barry, T., Leesman, G., Linke, S. P., Shivers, S. C., Vicini, F., Shah, C., Weinmann, S., Mann, G. B., & Bremer, T. (2021). Prognostic Risk Assessment and Prediction of Radiotherapy Benefit for Women with Ductal Carcinoma In Situ (DCIS) of the Breast, in a

Randomized Clinical Trial (SweDCIS). *Cancers*, *13*(23), 6103. https://www.mdpi.com/2072-6694/13/23/6103

Wuerstlein, R., Kates, R., Gluz, O., Grischke, E. M., Schem, C., Thill, M., Hasmueller, S., Köhler, A., Otremba, B., Griesinger, F., Schindlbeck, C., Trojan, A., Otto, F., Knauer, M., Pusch, R., & Harbeck, N. (2019). Strong impact of MammaPrint and BluePrint on treatment decisions in luminal early breast cancer: results of the WSG-PRIMe study. *Breast Cancer Res Treat*, *175*(2), 389-399. https://doi.org/10.1007/s10549-018-05075-x

1) For the prognosis of gliomas, the following tests **MEET COVERAGE CRITERIA:**
   a) Array-based genomic copy number testing or fluorescence in situ hybridization (FISH) for the co-deletion of 1p and 19q.
   b) *ATRX* mutation testing via gene sequencing **or** loss of ATRX protein expression via immunohistochemistry.
   c) *BRAF* fusion and mutation testing, including *BRAF* V600E common variant.
   d) *IDH1* and *IDH2* testing.
   e) *MGMT* promoter methylation testing.
   f) *TERT* promotor mutation testing.
2) For the prognosis of diffuse midline gliomas, the following tests **MEET COVERAGE CRITERIA:**
   a) *H3-3A* and *HIST1H3B* gene sequencing.
   b) *H3-3A* mutation testing by immunohistochemistry using an H3-3A K27M histone antibody.
3) For the prognosis of ependymomas, *ZFTA* fusion testing using either RNA sequencing analysis (RNA-Seq) or FISH **MEETS COVERAGE CRITERIA**.
4) *ATRX* mutation co-testing using **both** immunohistochemistry and gene sequencing **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
AG	Anaplastic glioma
ATRX	*Alpha-Thalassemia/Mental Retardation Syndrome X-Linked* gene
BRAF	*B-Raf proto-oncogene*
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare and Medicaid
CNA	Copy number alterations
CNS	Central nervous system

CSF	Cerebrospinal fluid
ddPCR	Digital droplet polymerase chain reaction
DIPG	Diffuse intrinsic pontine gliomas
EANO	European Association of Neuro-Oncology
ELISA	Enzyme-linked immunoassay
ESMO	European Society for Medical Oncology
FISH	Fluorescence in situ hybridization
H3-3A	*H3.3 histone A* gene
H3F3A	Previous gene name of *H3.3 histone A*
H3FA	*H3 clustered histone 1* gene
IDH1	*Isocitrate Dehydrogenase 1* gene
IDH2	*Isocitrate Dehydrogenase 2* gene
LDTs	Laboratory-developed tests
MGMT	*O-6-Methylguanine-Dna Methyltransferase* gene
NCCN	National Comprehensive Cancer Network
NICE	National Institute for Health and Care Excellence
NFkB	Nuclear factor kappa B
NGS	Next-generation sequencing
NICE	National Institute for Health and Care Excellence
PCR	Polymerase chain reaction
PFA	Posterior fossa ependymoma group A
PFB	Posterior fossa ependymoma group B
RELA	*RELA proto-oncogene, NF-kB subunit*
RNA-Seq	Ribonucleic acid sequencing analysis
SNVs	Single nucleotide variants
sPD-L1	Soluble programmed cell death ligand 1
TERT	*Telomerase reverse transcriptase*
TMB	Tumor mutational burden
TP53	*Tumor protein 53*
WHO	World Health Organization
YAP1	*Yes1 associated transcriptional regulator* gene
ZFTA	*Zinc finger translocation associated* gene

V. Scientific Background

According to the American Cancer Society, an estimated 25,400 adults in the United States were diagnosed with malignant tumors of the brain and spinal cord in 2023.

Additionally, The American Cancer Society reported that there was an estimated 18,760 deaths from brain and spinal cord tumors in 2023. (American Cancer Society, 2024).

Studies over the past two decades have clarified the genetic basis of tumorigenesis in the common, and some rarer, brain tumor entities (Louis et al., 2016), and identified clinically relevant molecular genetic characterizations that complement standard histologic analysis providing additional diagnostic and prognostic information to improve diagnostic accuracy, influence treatment selection, and improve survival. Molecular and/or genetic characterization do not replace standard histologic assessment, but rather serve as a complimentary approach (NCCN, 2023).

Isocitrate dehydrogenase (IDH1/2) mutations

Metabolic enzymes, IDH one and two, oxidize isocitrate to alpha-ketoglutarate, and are important in the mitigation of cellular oxidative damage (Horbinski, 2013b). Mutations in genes encoding these enzymes leads to the aberrant production of D-2 hydroxyglutarate (Dang et al., 2009), an oncometabolite that causes epigenetic modifications in affected cells (Horbinski, 2013b).

The *IDH* mutations are a defining feature of WHO grade II and III astrocytomas and oligodendrogliomas (Louis et al., 2016). Their presence distinguishes lower grade gliomas from primary glioblastomas, which are *IDH* wild type. *IDH* mutations are commonly associated with O-6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation and are also associated with a relatively favorable prognosis (Brat et al., 2015; Eckel-Passow et al., 2015).

O-6-methylguanine-DNA methyltransferase (MGMT) methylation

The DNA repair enzyme, MGMT, reverses the DNA damage caused by alkylating agents, resulting in tumor resistance to temozolomide and nitrosourea-based chemotherapy. Methylation of the *MGMT* promoter silences *MGMT,* making the tumor more sensitive to treatment with alkylating agents (Esteller et al., 2000; Gusyatiner & Hegi, 2018).

The *MGMT* promoter methylation is strongly associated with *IDH* mutation and genome-wide epigenetic change (Eckel-Passow et al., 2015); it is also associated with longer survival in patients with glioblastoma who receive alkylating agents (Hegi et al., 2005; Zhao et al., 2016). *MGMT* promoter methylation is particularly useful in treatment decisions for elderly patients with high grade gliomas (Malmstrom et al., 2012; Wick et al., 2012; Wick et al., 2014).

Codeletion of chromosomes 1p and 19q

The codeletion of 1p and 19q represents an unbalanced translocation (1;19) (q10;p10) leading to the whole arm deletion of chromosome 1p and chromosome 19q (Jenkins et al., 2006). Codeletion of 1p and 19q is a defining feature of oligodendroglial tumors, is strongly associated with oligodendroglial histology, and helps to confirm the oligodendroglial character of tumors with equivocal or mixed histologic features (Brat et al., 2015; Burger et al., 2001; Eckel-Passow et al., 2015). Combined loss involving chromosomes 1p and 19q is significantly associated with both favorable therapeutic response and longer recurrence-free survival after chemotherapy (Cairncross et al., 1998).

Alpha-thalassemia/mental retardation syndrome X-linked (ATRX) mutations

Mutations in the chromatin regulator gene, *ATRX*, enable alternative lengthening of telomeres (Abedalthagafi et al., 2013). ATRX is a switch/sucrose helicase that assists with H3.3 chromatin deposition in telomeric regions. Disruption of this gene leads to the alternative lengthening of telomeres stated above and is thought to represent an early event in gliomagenesis (Batchelor & Louis, 2023).

The *ATRX* mutations in glioma are strongly associated with *IDH* and *TP53* mutations and are nearly always mutually exclusive with 1p19q codeletion (Reuss et al., 2015). ATRX deficiency, coupled with *IDH* mutation, is typical of astrocytoma (Brat et al., 2015).

Tumor protein 53 (TP53) mutation

Tumor protein 53 is essential for regulating cell division and preventing tumor formation (Parikh et al., 2014). Missense mutations in the *TP53* gene are present in the clear majority of IDH-mutant astrocytomas (Brat et al., 2015). Immunopositivity for mutant p53 is not entirely sensitive or specific for a *TP53* mutation, however, and loss of *ATRX* expression may be a more reliable marker of astrocytic differentiation (Louis et al., 2023).

Telomerase reverse transcriptase (TERT) mutations

Telomerase reverse transcriptase encodes the catalytic active site of telomerase, the enzyme responsible for maintaining telomere length in dividing cells. *TERT* mutations in the noncoding promoter region cause increased expression of the TERT protein and are one of the major mechanisms of telomerase activation in gliomas (Arita et al., 2013). *TERT* mutations are strongly associated with 1p19q codeletion and are found in most glioblastomas. A *TERT* mutation in combination with an *IDH* mutation and 1p19q codeletion is characteristic of oligodendroglioma. The absence of a *TERT* mutation, coupled with an *IDH* mutation, designates astrocytoma (Eckel-Passow et al., 2015). In

terms of survival, mutation in the *TERT* promoter is generally unfavorable in the absence of *IDH* mutation and favorable in the presence of *IDH* mutation and 1p/19q codeletion. *TERT* promoter mutation is associated with an older age of the patient at presentation, regardless of whether *IDH* mutation is present (Eckel-Passow et al., 2015).

Histone (H3FA) mutations

A lysine to methionine substitution in the *H3F3A* gene (*H3K27M*) is the most common histone mutation in brain tumors and inhibits the trimethylation of H3.3 histone (Sturm et al., 2012), arresting cells in a primitive state refractory to differentiation induction (Weinberg et al., 2017). *G34R/G34V* mutations in the *H3F3A* gene are more common in cortical gliomas in children (Schwartzentruber et al., 2012). *H3FA* mutations can be useful in the diagnosis of infiltrative glioma (Sturm et al., 2012). The *H3K27M* mutation is an adverse prognostic marker in children and adults (Meyronet et al., 2017). The *G34* mutation does not appear to have any prognostic significance once the diagnosis of a glioblastoma has been established (Sturm et al., 2012).

A similar mutation to *H3K27M* may also occur in the *HIST1H3B/C* gene, which encodes the histone H3.1 variant. However, the mutation at *HIST1H3B/C* is about one third as common as *H3F3A* and often confers a better prognosis than its *H3F3A* counterpart (Louis et al., 2023).

B-Raf proto-oncogene (BRAF) mutations

The serine-threonine protein kinase, BRAF, is involved in cell survival, proliferation, and differentiation (Davies et al., 2002). Activating mutations in *BRAF*, most often V600E, have been discovered in most pediatric and some adult gliomas (Chappe et al., 2013; Horbinski, 2013a), including approximately 80% of pleomorphic xanthoastrocytomas, 20% of gangliogliomas, 10% of pilocytic astrocytomas, and occasionally diffuse gliomas (Chi et al., 2013). Tandem duplication of chromosome 7q34 resulting in an activating fusion of the *BRAF* and *KIAA1549* genes occur in 60-80% of pilocytic astrocytoma (Jones et al., 2008).

The presence of a *BRAF* fusion is reliable evidence that the tumor is a pilocytic astrocytoma and predicts a better clinical outcome (Hawkins et al., 2011). A *BRAF* mutation is more complicated, as it can occur in a variety of tumors and requires integration with histology. Tumors with *BRAF* mutations may respond to BRAF inhibitors; however, in pediatric gliomas, *BRAF* V600E indicates poor prognosis when treated with current adjuvant therapy, especially in combination with a *CDKN2A* mutation (Lassaletta et al., 2017).

v-rel avian reticuloendotheliosis viral oncogene homolog A (RELA, p65, NFKB3) fusion

Fusion between the *C11orf95* and *RELA* genes defines approximately 70 percent of all childhood supratentorial ependymomas (Louis et al., 2023). These fusions are associated with increased NF-kappa-B (NFkB) signaling and poor outcome (Malgulwar et al., 2018). Normally, NFkB is an inactive transcription factor in the cytoplasm. When its inhibitor degrades, it activates transcription of certain genes, *RELA* among them (NCBI, 2011). New research supports the hypothesis that the status of *RELA* fusion and p53 overexpression are significantly associated with the prognosis of supratentorial extraventricular ependymomas (Wang et al., 2019).

New Tests

Assessment of gliomas is incredibly difficult, and new methods of molecular analyses for gliomas are consistently being developed. For example, Miller et al. (2019) devised a liquid-biopsy based method to evaluate cerebrospinal fluid from 42 (of 85) patients. The genomic profile developed from the cerebrospinal fluid (CSF) samples closely matched established profiles, such as the characteristic 1p/19q codeletion and *IDH1/2* mutations. The authors stated that the ability to monitor the glioma genome in real time could be useful in management of this condition (Miller et al., 2019). Other researchers report that "A cerebrospinal fluid ct-DNA liquid biopsy approach may virtually support all the stages of glioma management, from facilitating molecular diagnosis when surgery is not feasible, to monitoring tumor response, identifying early recurrence, tracking longitudinal genomic evolution, providing a new molecular characterization at recurrence and allowing patient selection for targeted therapies" (Simonelli et al., 2020).

Clinical Utility and Validity

Nikiforova et al. (2016) validated GlioSeq, a commercial next generation sequencing (NGS) panel of 30 genes, in 54 patients with CNS tumors against fluorescence in-situ hybridization (FISH), Sanger sequencing, and reverse transcription polymerase chain reaction (PCR). GlioSeq correctly identified 71/71 (100%) genetic alterations known to be present by conventional techniques. The assay sensitivity was three to five percent for mutant alleles of single nucleotide variants (SNVs), and one to five percent for gene fusions. Likewise, Zacher et al. (2017) developed an NGS panel of 20 genes that allowed for molecular classification of 121 gliomas. The researchers conclude that gene panel NGS is a promising diagnostic technique that may facilitate integrated histological and molecular glioma classification.

Ramkissoon et al. (2017) used OncoPanel and OncoCopy to identify targetable alterations in tumors for the establishment of best practices in routine clinical pediatric oncology. They analyzed 117 samples by OncoPanel and 146 by OncoCopy; further, 60 tumors were subjected to both methodologies. OncoPanel revealed clinically relevant

alterations in 56% of patients (44 cancer mutations and 20 rearrangements), including *BRAF* alterations that directed the use of targeted inhibitors. Rearrangements in *MYB-QKI, MYBL1, BRAF*, and *FGFR1* were also detected. Furthermore, while copy number profiles differed across histologies, the combined use of OncoPanel and OncoCopy identified subgroup-specific alterations in 89% (17/19) of medulloblastomas.

Ryall et al. (2016) evaluated the prognostic impact of H3K27M and MAPK pathway aberrations in 64 gliomas (44 low grade, 22 high grade). Tumors are designated as low-grade if the cells are well differentiated, are less aggressive overall, and suggest a better prognosis for the patient. Five low grade gliomas contained the *H3F3A/HIST1H3B K27M* (*H3K27M*) mutation, and 11 high grade gliomas contained the *H3K27M* mutation. Survival analysis evaluated the median survival at 9.12 years for wildtype H3 patients compared to 1.02 years for patients with the *H3K27M* mutation. MAPK pathway mutations (through *BRAF* or *FGFR1* mutation) were associated with long-term survival in absence of *H3K27M* mutations. Further, H3K27M status and high-grade histology were found to be the most significant independent predictors of poor overall survival with hazard ratios of 6.945 and 7.721 respectively. MAPK pathway activation was a predictor of "favourable patient outcome," but dependent on other factors (Ryall et al., 2016).

Houdova Megova et al. (2017) evaluated the prognostic value of the *IDH1/2* mutation in glioblastomas. A total of 37 *IDH* mutations were examined and studied. The authors found that *IDH1* mutations were positively associated with *MGMT* methylation (odds ratio [OR]: 3.08), 1p/19q co-loss (OR: 8.85), and negatively associated with *EGFR* amplification. IDH-mutant patients had an overall survival of 25 months compared to only nine months for IDH-wildtype gliomas (Houdova Megova et al., 2017).

Johnson et al. (2017) performed comprehensive genomic profiling of 282 pediatric gliomas: 157 high-grade and 125 low-grade. The investigators used a 315 gene panel and calculated the tumor mutational burden (TMB). In low grade gliomas, *BRAF* was the most frequent mutation found (48%), followed by *FGFR* missense (17.6%), *NF1* loss of function (8.8%), and *TP53* (5.6%). Rearrangements were found in 35% of low-grade gliomas. In high-grade gliomas, *TP53* was the most frequent mutation found (49%), followed by *H3F3A* (37.6%), *ATRX* (24.2%), *NF1* (22.2%), and *PDGFRA* (21.7%). *H3F3A* mutations were found to be the K28M variant. Approximately six percent of the high-grade gliomas were found have a TMB of >20 mutations/Mb ("hypermutated") (Johnson et al., 2017).

Back et al. (2020) studied the pattern of failure in anaplastic glioma (AG) patients with an *IDH1/2* mutation. A total of 156 patients participated in the study, with data collected from 2008 to 2014; the median follow-up time was 5.1 years. Of all 156

patients, 75% were found to have an *IDH1 or IDH2* mutation. The authors concluded that "patients with *IDH*-mutated AG have improved outcomes"; however, this population also had a greater number of distant relapses approximately two years after intensity-modulated radiation therapy compared to individuals with *IDH* wild type mutations (Back et al., 2020).

Ji et al. (2021) studied the clinical utility of comprehensive genomic profiling to detect CNS tumors in children and young adults using the OncoKids next-generation sequencing panel, chromosomal microarray analysis, and germline testing. NGS was performed on 222 samples and CMA was performed on 146 of the 222 samples. The OncoKids NGS panel identified diagnostic biomarkers in 138/222 samples (62%), prognostic information in 49/222 cases (22%), and targetable genomic alterations in 41/222 samples (18%). Additionally, CMA revealed prognostic copy number alterations (CNA) in 101/146 cases (69%). Further, germline cancer predisposition testing was performed in 57 of 212 patients which identified 20 patients which a confirmed germline pathogenic/likely pathogenic variant of genes *TP53, NF1, SMARCB1, NF2, MSH6, PMS2*, and a patient with Klinefelter syndrome. Overall, the authors conclude that there is "significant clinical utility of integrating genomic profiling into routine clinical testing for pediatric and young adult patients with CNS tumors" (Ji et al., 2021).

Muralidharan et al. (2021) studied the diagnostic utility of a novel digital droplet PCR (ddPCR) assay for detection of two TERT promoter mutations (*C228T* and *C250T*) and monitoring of gliomas. In comparison with the gold-standard tumor tissue-based detection of *TERT* mutations, the ddPCR assay had an overall sensitivity of 62.5% and a specificity of 90%. Longitudinal monitoring of five patients demonstrated that the peripheral *TERT* mutant allele frequency reflects the clinical course of the disease. *TERT* mutant alleles decreased after surgical intervention and pharmacotherapy but increased with tumor progression. The authors conclude that the ddPCR assay has feasibility in "detecting circulating cfDNA TERT promoter mutations in patients with glioma with clinically relevant sensitivity and specificity" (Muralidharan et al., 2021).

Cabezas-Camarero et al. (2021) studied the levels of soluble PD-L1 (sPD-L1) in patients with gliomas according to histologic grade and IDH mutation status, evaluating its predicted role and dynamic changes in sPD-L1. Plasma samples were obtained prior to and after radiotherapy/chemotherapy and were evaluated using ELISA. The authors compared 12 healthy controls with 57 patients with grade II to IV gliomas. They found that sPD-L1 levels were numerically higher in glioma patients as compared to the healthy control group. The authors found that elevated sPD-L1 levels pre- and post-treatment associated with a worse prognosis in IDH-MUT gliomas. Dynamics of SPDL1 and other immune-biomarkers should still be explored in gliomas (Cabezas-Camarero et al., 2021).

Rios et al. (2022) studied the health and economic impacts of using tumor molecular testing for guiding treatments for pediatric patients with low grade glioma. With their microsimulation for modeling health and cost outcomes for 100,000 simulated patients, they found that there was a statistically significant increase in life expectancy in those who received molecular testing for the BRAF mutation (40.08 vs 39.01 in those who did not receiving testing), and an increase of 0.38 quality-adjusted life-years (QALY) and $1384 reduction in costs due to likely avoidance of adverse events associated with radiation, like stroke and other neoplastic transformations. This study ultimately demonstrates that in this subset of patients, there could be long-term benefits in the treatment plans derived for childhood cancers, including the increased timely use of *BRAF*-specific biologic agents versus standard treatment with radiation (Rios et al., 2022).

VI. Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

The NCCN published Clinical Practice Guidelines in Oncology (2023) for Central Nervous System Cancers which recommend:

IDH1 and *IDH2* mutation

Recommendation: *IDH* mutation testing is required for the workup of glioma.

"The most common *IDH1* mutation (R132H) is reliably screened by mutation specific immunohistochemistry (IHC), which is recommended for all glioma patients. If the R132H immunostain result is negative, in the appropriate clinical context, sequencing of *IDH1* and *IDH2* is highly recommended to detect less common *IDH1* and *IDH2* mutations. Prior to age 55 years, sequencing of *IDH1* and *IDH2* is required if the R132H immunostain result is negative, or if the glioma is only grade 2 or 3 histologically. Standard sequencing methods include Sanger sequencing, pyrosequencing, and next-generation sequencing (NGS), and should be performed on formalin fixed, paraffin embedded tissue" (NCCN, 2023).

MGMT promoter methylation

Recommendation: *MGMT* promoter methylation is an essential part of molecular diagnostics for all high-grade gliomas (grade 3 and 4). The NCCN also notes that "*MGMT* promoter methylation is strongly associated with *IDH* mutations and genome-wide epigenetic changes (G-CIMP phenotype)" (NCCN, 2023).

"There are multiple ways to test for *MGMT* promoter methylation, including methylation-specific polymerase chain reaction (PCR), methylation-specific high-resolution melting, pyrosequencing, and droplet-digital PCR" (NCCN, 2023).

"MGMT promoter methylation testing is particularly useful in treatment decisions for older adult patients with high-grade gliomas (grades 3–4) " (NCCN, 2023).

Codeletion of 1p and 19q

Recommendation: 1p/19q testing is an essential part of molecular diagnostics for oligodendroglioma.

"The codeletion of 1p and 19q is detectable by array-based genomic copy number testing (preferable), or fluorescence in situ hybridization (FISH) … IDH-mutated gliomas that do NOT show loss of *ATRX* (for example, by IHC) should be strongly considered for 1p/19q testing, even if not clearly oligodendroglial by histology. Conversely, *IDH1* wild-type gliomas do not contain true whole-arm 1p/19q codeletion. Therefore, 1p/19q testing is unnecessary if a glioma is definitely *IDH*-wild-type, and a glioma should not be regarded as 1p19q-codeleted without an accompanying *IDH* mutation, regardless of the test results" (NCCN, 2023).

ATRX mutation

"Recommendation: *ATRX* mutation testing is required for the workup of glioma."

"*ATRX* mutations can be detected by IHC for wild-type *ATRX* (loss of wild type expression) and/or sequencing. ATRX mutations in glioma are strongly associated with *IDH* mutations and are nearly always mutually exclusive with 1p/19q codeletion. *ATRX* deficiency, coupled with IDH mutation and *TP53* mutation, is typical of astrocytoma. A lack of *ATRX* immunostaining in glioblastoma should trigger *IDH1/2* sequencing if *IDH1* R132H immunostaining is negative, due to frequent co-occurrence of *ATRX* and *IDH* mutations" (NCCN, 2023).

TERT mutation

"Recommendation: *TERT* promoter mutation testing is required for the workup of gliomas."

"*TERT* promoter mutations are nearly always present in 1p/19q codeleted oligodendroglioma and are found in most glioblastomas. *TERT* promoter mutation, in combination with *IDH* mutation and 1p/19q codeletion, is characteristic of oligodendroglioma. Absence of *TERT* promoter mutation, coupled with the presence of mutant *IDH*, strongly suggests astrocytoma"(NCCN, 2023).

H3F3A and *HIST1H3B* mutation

"Recommendation: *H3-3A* and *HIST1H3B* mutation testing is recommended in the appropriate clinical context."

"Diffuse midline gliomas should be screened for *H3-3A* mutations, specifically the H3K27M mutation. While sequencing is the gold standard, H3K27M-specific IHC, paired with H3K27 trimethylation immunostaining, is a reasonable alternative, especially when tissue is scarce. In these gliomas, H3K27M immunopositivity should be associated with loss of histone trimethylation immunostaining."

"Although a K27M histone antibody is available, it is not 100% specific and interpretation can be difficult for non-experts. Therefore, screening by *H3F3A* and *HIST1H3B* sequencing is a viable alternative and preferred approach, especially since it will also detect mutations in G34" (NCCN, 2023).

"Diagnostic value: Histone mutations most commonly occur in pediatric midline gliomas (e.g., diffuse intrinsic pontine gliomas [DIPG]), although midline gliomas in adults can also contain histone modifications. Their presence can be considered solid evidence of an infiltrative glioma, which is often helpful in small biopsies of midline lesions that may not be fully diagnostic with light microscopy or do not fully resemble infiltrative gliomas."

"Prognostic value: The *K27M* gliomas typically do not have MGMT promoter methylation, and the mutation is an adverse prognostic marker in children and adults. The *G34* mutation does not appear to have any prognostic significance once the diagnosis of a glioblastoma has been established" (NCCN, 2023).

BRAF mutation

"Recommendation: *BRAF* fusion and/or mutation testing is recommended in the appropriate clinical context."

"*BRAF* V600E is best detected by sequencing, and *BRAF* fusions can be detected with RNA-Seq or other PCR-based breakpoint methods that capture the main 16-9, 15-9, and 16-11 breakpoints between *BRAF* and its main fusion partner, *KIAA1549*. FISH is too unreliable to detect *BRAF* fusions" (NCCN, 2023).

"The presence of a *BRAF* fusion is reliable evidence that the tumor is a [pilocytic astrocytoma], provided the histology is compatible. *BRAF* V600E is more complicated, as it can occur in a variety of tumors over all four WHO grades and requires integration with histology."

"Tumors with *BRAF* fusions tend to be indolent, with occasional recurrence but only rare progression to lethality. *BRAF* V600E tumors show a much greater range of outcomes and need to be considered in context with other mutations and clinicopathologic findings (e.g., CDKN2A/B deletion). *BRAF* V600E tumors may respond to BRAF inhibitors, such as vemurafenib, but comprehensive clinical trials are still ongoing" (NCCN, 2023).

ZFTA fusion

"Testing for *ZFTA* and *YAP1* fusions is recommended in the appropriate clinical context. Ependymomas arising in the supratentorium often contain activating fusions of *ZFTA*. This leads to increased NF-kappa-B signaling and more aggressive behavior. This event is more common in children than in adults, and occurs only in the supratentorium, not the posterior fossa or spine" (NCCN,2022).

"*ZFTA* fusion can be detected with RNA sequencing or a break-apart FISH probe set… Detection of *ZFTA* fusion is not required for the diagnosis of ependymoma, as this entity is still diagnosed by light microscopy."

"*ZFTA* fusion-positive ependymomas are now a distinct entity in the WHO classification of CNS tumors, as this subset of ependymomas tends to be far more aggressive than other supratentorial ependymomas, including those with *YAP1* fusions. PFA vs. PFB via methylation profiling is reasonable for posterior fossa ependymoma" (NCCN, 2023).

Finally, the NCCN states there are no identified targeted agents with demonstrated efficacy in glioblastoma (NCCN, 2023).

National Institute for Health and Care Excellence (NICE)

NICE recommends the following molecular markers for investigation of gliomas: *IDH1/2* mutations, *ATRX* mutations, 1p/19q co-deletion, histone H3.3 K27M, *BRAF* mutation, and *MGMT* promoter methylation (for prognosis). NICE also notes that testing *IDH* wild type gliomas for *TERT* promoter mutations may be considered (NICE, 2021).

European Society for Medical Oncology (ESMO)

The ESMO has published clinical practice guidelines for the diagnosis, treatment, and follow-up of high-grade gliomas. They state that *MGMT* promoter methylation status, *IDH1/2* mutation status, and 1p/19q codeletions are "commonly determined" for assessment of gliomas (ESMO, 2014).

World Health Organization (WHO)

In 2016 and 2021, the WHO published guidelines on the classification of central nervous system tumors. These WHO guidelines, for the first time, incorporated molecular testing in the diagnosis of gliomas and medulloblastomas. The following key points were given by the WHO regarding molecular testing:

- "*IDH1* R132H, which accounts for approximately 90% of *IDH* mutations, can be detected immunohistochemically. If this testing is negative, sequencing of *IDH1* and *IDH2* is necessary to ensure that no other *IDH* mutations are present.
- Given the importance of *IDH* mutational status in the diagnosis or gliomas, at a minimum, it will be important that most institutions have the capacity to both stain tumor specimens for *IDH1* R132H by immunohistochemistry and, ideally, sequence those tumors that are negative for both *IDH1* and *IDH2* mutations" (Wen & Huse, 2016).
- "Because of the growing importance of molecular information in CNS tumor classification, diagnoses and diagnostic reports need to combine different data types into a single, 'integrated' diagnosis. To display the full range of diagnostic information available, the use of layered (or tiered) diagnostic reports is strongly encouraged. Such reports feature an integrated diagnosis at the top, followed by layers that display histological, molecular, and other key types of information" (Louis et al., 2021).
- Certain tumors (Diffuse astrocytoma, MYB- or MYBL1-altered; Angiocentric glioma; Polymorphous low-grade neuroepithelial tumor of the young; and Diffuse low-grade glioma, MAPK pathway-altered) "require molecular characterization and the integration of histopathological and molecular information in a tiered diagnostic format as molecular work-up helps to characterize the lesion as one type or the other" (Louis et al., 2021).
- For other tumors such as Myxopapillary ependymoma and Subependymoma, "although these can be identified with methylome studies, molecular classification does not provide added clinicopathological utility for these two tumors" (Louis et al., 2021).
- "Several molecular biomarkers are also associated with classification and grading of meningiomas, including SMARCE1 (clear cell subtype), BAP1 (rhabdoid and papillary subtypes), and KLF4/TRAF7 (secretory subtype) mutations, TERT promoter mutation and/or homozygous deletion of CDKN2A/B62, H3K27me3 loss of nuclear expression (potentially worse prognosis), and methylome profiling (prognostic subtyping)" (Louis et al., 2021).

European Association of Neuro-Oncology (EANO)

In 2021, the EANO published guidelines regarding diagnosis and management of adult patients with diffuse gliomas. The following recommendations were made on molecular testing:

- "Patients with relevant germline variants or suspected hereditary cancer syndromes should receive genetic counselling and might subsequently be referred for molecular genetic testing.
- Immunohistochemistry for mutant *IDH1* R132H protein and nuclear expression of *ATRX* should be performed routinely in the diagnostic assessment of diffuse gliomas.
- If immunohistochemistry for *IDH1* R132H is negative, sequencing of *IDH1* codon 132 and *IDH2* codon 172 should be conducted in all WHO grade 2 and 3 diffuse astrocytic and oligodendroglial gliomas as well as in all glioblastomas of patients aged < 55 years to enable integrated diagnoses according to the WHO classification and to guide treatment decisions.
- 1p/19q codeletion status should be determined in all *IDH*-mutant gliomas with retained nuclear expression of *ATRX*.
- *MGMT* promoter methylation status should be determined in glioblastoma, notably in elderly or frail patients, to aid in decision-making for the use of temozolomide.
- *CDKN2A/B* homozygous deletions should be explored in *IDH*-mutant astrocytomas.
- Combined chromosome seven gain and chromosome 10 loss (+7/–10 signature), *EGFR* amplification and *TERT* promoter mutation should be tested in *IDH*-wild-type diffuse gliomas lacking microvascular proliferation and necrosis as histological features of WHO grade 4 to allow for a diagnosis of *IDH*-wild-type glioblastoma" (Weller et al., 2021).

In addition, EANO published a table to summarize the molecular markers used for the diagnosis and management of gliomas.

Table 1: Molecular Markers for the Diagnosis and Management of Gliomas (Weller et al., 2021)

Molecular Marker	**Diagnostic Roles**
IDH1 R132 or IDH2 R172 mutation	"Distinguishes diffuse gliomas with IDH mutation from IDH-wild-type glioblastomas and other IDH-wild-type gliomas
1p/19q codeletion	Distinguishes oligodendroglioma, IDH-mutant and 1p/19q-codeleted from astrocytoma, IDH-mutant

Loss of nuclear ATRX	Loss of nuclear ATRX in an IDH-mutant glioma is diagnostic for astrocytic lineage tumours
Histone H3 K27M mutation	Defining molecular feature of diffuse midline glioma, H3 K27M-mutant
Histone H3.3 G34R/V mutation	Defining molecular feature of diffuse hemispheric glioma, H3.3 G34-mutant
MGMT promoter methylation	None, but is a predictive biomarker of benefit from alkylating chemotherapy in patients with IDH-wild-type glioblastoma
Homozygous deletion of CDKN2A/CDKN2B	A marker of poor outcome and WHO grade 4 disease in IDH-mutant astrocytomas
EGFR amplification	EGFR amplification occurs in ~40–50% of glioblastoma, IDH wild type Molecular marker of glioblastoma, IDH wild type, WHO grade 4
TERT promotor mutation	TERT promoter mutation occurs in ~70% of glioblastoma, IDH wild type and >95% of oligodendroglioma, IDH-mutant and 1p/19q-codeleted Molecular marker of glioblastoma, IDH wild type, WHO grade 4
+7/–10 cytogenetic signature	Molecular marker of glioblastoma, IDH wild type, WHO grade 4
$BRAF^{V600E}$ mutation	Rare in adult diffuse gliomas but amenable to pharmacological intervention" (Weller et al., 2021).

College of American Pathologists

In 2022, The College of American Pathologists in Collaboration with the American Association of Neuropathologists, Association for Molecular Pathology, and Society for Neuro-Oncology published guidelines that suggests these following recommendations for molecular biomarker testing for the diagnosis of diffuse gliomas:

- "IDH mutational testing must be performed on all diffuse gliomas (DG) (Strong recommendation)"
- "ATRX status should be assessed in all IDH-mutant DG unless they show 1p/19q codeletion (Strong recommendation)"
- "TP53 status should be assessed in all IDH-mutant DGs unless they show 1p/19q codeletion (Conditional recommendation)"
- "1p/19q codeletion must be assessed in IDH-mutant DGs unless they show ATRX loss or *TP53* mutations (Strong recommendation)"

- "*CDKN2A/B* homozygous deletion testing should be performed on IDH-mutant astrocytomas (Conditional recommendation)"
- "*MGMT* promoter methylation testing should be performed on all glioblastoma (GBM), IDH-wild type (WT) (Strong recommendation)"
- "For IDH-mutant DG, *MGMT* promoter methylation testing may not be necessary (Conditional recommendation)"
- "*TERT* promoter mutation testing may be used to provide further support for the diagnosis of oligodendroglioma and IDH-WT GBM (Conditional recommendation)"
- "For histologic grade 2-3 DGs that are IDH-WT, testing should be performed for whole chromosome 7 gain/whole chromosome 10 loss, *EGFR* amplification, and *TERT* promoter mutation to establish the molecular diagnosis of GBM, IDH-WT, grade IV (Strong recommendation)"
- "H3 K27M testing must be performed in DGs that involve the midline in the appropriate clinical and pathologic setting (Strong recommendation)"
- "H3 G34 testing may be performed in pediatric and young adult patients with IDH-WT DG (Conditional recommendation)"
- "*BRAF* mutation testing (V600) may be performed in DGs that are IDH-WT and H3-WT (Conditional recommendation)"
- "*MYB/MYBL1* AND *FGFR1* testing may be performed in children and young adults with DGs that are histologic grade 2-3 and are IDH-WT and H3-WT (Conditional recommendation)" (Brat et al., 2022).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration

has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81120	IDH1 (isocitrate dehydrogenase 1 [NADP+], soluble) (eg, glioma), common variants (eg, R132H, R132C)
81121	IDH2 (isocitrate dehydrogenase 2 [NADP+], mitochondrial) (eg, glioma), common variants (eg, R140W, R172M)
81210	BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer, melanoma), gene analysis, V600 variant(s);
81287	MGMT (O-6-methylguanine-DNA methyltransferase) (eg, glioblastoma multiforme) promoter methylation analysis
81345	TERT (telomerase reverse transcriptase) (eg, thyroid carcinoma, glioblastoma multiforme) gene analysis, targeted sequence analysis (eg, promoter region)
81479	Unlisted molecular pathology procedure ***H3F3A gene sequencing*** ***RELA Testing*** ***H3F3A testing using a K27M histone antibody***
88341	Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)
88342	Immunohistochemistry or immunocytochemistry, per specimen; initial single antibody stain procedure
88374	Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), using computer-assisted technology, per specimen; each multiplex probe stain procedure
88377	Morphometric analysis, in situ hybridization (quantitative or semi-quantitative), manual, per specimen; each multiplex probe stain procedure

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Abedalthagafi, M., Phillips, J. J., Kim, G. E., Mueller, S., Haas-Kogen, D. A., Marshall, R. E., Croul, S. E., Santi, M. R., Cheng, J., Zhou, S., Sullivan, L. M., Martinez-Lage, M., Judkins,

A. R., & Perry, A. (2013). The alternative lengthening of telomere phenotype is significantly associated with loss of ATRX expression in high-grade pediatric and adult astrocytomas: a multi-institutional study of 214 astrocytomas. *Mod Pathol*, *26*(11), 1425-1432. https://doi.org/10.1038/modpathol.2013.90

American Cancer Society. (2024). Key Statistics for Brain and Spinal Cord Tumors. https://www.cancer.org/cancer/types/brain-spinal-cord-tumors-adults/about/key-statistics.html

Arita, H., Narita, Y., Takami, H., Fukushima, S., Matsushita, Y., Yoshida, A., Miyakita, Y., Ohno, M., Shibui, S., & Ichimura, K. (2013). TERT promoter mutations rather than methylation are the main mechanism for TERT upregulation in adult gliomas. *Acta Neuropathol*, *126*(6), 939-941. https://doi.org/10.1007/s00401-013-1203-9

Back, M., Jayamanne, D., Brazier, D., Newey, A., Bailey, D., Schembri, G., Hsiao, E., Khasraw, M., Wong, M., Kastelan, M., Brown, C., & Wheeler, H. (2020). Pattern of failure in anaplastic glioma patients with an IDH1/2 mutation. *Strahlenther Onkol*, *196*(1), 31-39. https://doi.org/10.1007/s00066-019-01467-0 (Rezidivmuster bei Patienten mit anaplastischem Glioma mit einer IDH1/2-Mutation.)

Batchelor, T., & Louis, D. N. (2023, March 16). *Molecular pathogenesis of diffuse gliomas*. https://www.uptodate.com/contents/molecular-pathogenesis-of-diffuse-gliomas

Brat, D. J., Aldape, K., Bridge, J. A., Canoll, P., Colman, H., Hameed, M. R., Harris, B. T., Hattab, E. M., Huse, J. T., Jenkins, R. B., Lopez-Terrada, D. H., McDonald, W. C., Rodriguez, F. J., Souter, L. H., Colasacco, C., Thomas, N. E., Yount, M. H., van den Bent, M. J., & Perry, A. (2022). Molecular Biomarker Testing for the Diagnosis of Diffuse Gliomas. *Arch Pathol Lab Med*, *146*(5), 547-574. https://doi.org/10.5858/arpa.2021-0295-CP

Brat, D. J., Verhaak, R. G., Aldape, K. D., Yung, W. K., Salama, S. R., Cooper, L. A., Rheinbay, E., Miller, C. R., Vitucci, M., Morozova, O., Robertson, A. G., Noushmehr, H., Laird, P. W., Cherniack, A. D., Akbani, R., Huse, J. T., Ciriello, G., Poisson, L. M., Barnholtz-Sloan, J. S., . . . Radenbaugh, A. J. (2015). Comprehensive, Integrative Genomic Analysis of Diffuse Lower-Grade Gliomas. *N Engl J Med*, *372*(26), 2481-2498. https://doi.org/10.1056/NEJMoa1402121

Burger, P. C., Minn, A. Y., Smith, J. S., Borell, T. J., Jedlicka, A. E., Huntley, B. K., Goldthwaite, P. T., Jenkins, R. B., & Feuerstein, B. G. (2001). Losses of chromosomal arms 1p and 19q in the diagnosis of oligodendroglioma. A study of paraffin-embedded sections. *Mod Pathol*, *14*(9), 842-853. https://doi.org/10.1038/modpathol.3880400

Cabezas-Camarero, S., García-Barberán, V., Pérez-Alfayate, R., Lopez Cade, I., Tejerina-Peces, J., Casado-Fariñas, I., & Pérez-Segura, P. (2021). 34P Dynamic changes in plasma PD-L1 in patients with gliomas: Prognostic value and association with IDH status. *Annals of Oncology*. https://doi.org/10.1016/j.annonc.2021.10.050

Cairncross, J. G., Ueki, K., Zlatescu, M. C., Lisle, D. K., Finkelstein, D. M., Hammond, R. R., Silver, J. S., Stark, P. C., Macdonald, D. R., Ino, Y., Ramsay, D. A., & Louis, D. N. (1998). Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. *J Natl Cancer Inst*, *90*(19), 1473-1479. https://doi.org/10.1093/jnci/90.19.1473

Chappe, C., Padovani, L., Scavarda, D., Forest, F., Nanni-Metellus, I., Loundou, A., Mercurio, S., Fina, F., Lena, G., Colin, C., & Figarella-Branger, D. (2013). Dysembryoplastic neuroepithelial tumors share with pleomorphic xanthoastrocytomas and gangliogliomas BRAF(V600E) mutation and expression. *Brain Pathol*, *23*(5), 574-583. https://doi.org/10.1111/bpa.12048

Chi, A. S., Batchelor, T. T., Yang, D., Dias-Santagata, D., Borger, D. R., Ellisen, L. W., Iafrate, A. J., & Louis, D. N. (2013). BRAF V600E mutation identifies a subset of low-grade diffusely infiltrating gliomas in adults. *J Clin Oncol*, *31*(14), e233-236. https://doi.org/10.1200/jco.2012.46.0220

Dang, L., White, D. W., Gross, S., Bennett, B. D., Bittinger, M. A., Driggers, E. M., Fantin, V. R., Jang, H. G., Jin, S., Keenan, M. C., Marks, K. M., Prins, R. M., Ward, P. S., Yen, K. E., Liau, L. M., Rabinowitz, J. D., Cantley, L. C., Thompson, C. B., Vander Heiden, M. G., & Su, S. M. (2009). Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature*, *462*(7274), 739-744. https://doi.org/10.1038/nature08617

Davies, H., Bignell, G. R., Cox, C., Stephens, P., Edkins, S., Clegg, S., Teague, J., Woffendin, H., Garnett, M. J., Bottomley, W., Davis, N., Dicks, E., Ewing, R., Floyd, Y., Gray, K., Hall, S., Hawes, R., Hughes, J., Kosmidou, V., . . . Futreal, P. A. (2002). Mutations of the BRAF gene in human cancer. *Nature*, *417*(6892), 949-954. https://doi.org/10.1038/nature00766

Eckel-Passow, J. E., Lachance, D. H., Molinaro, A. M., Walsh, K. M., Decker, P. A., Sicotte, H., Pekmezci, M., Rice, T., Kosel, M. L., Smirnov, I. V., Sarkar, G., Caron, A. A., Kollmeyer, T. M., Praska, C. E., Chada, A. R., Halder, C., Hansen, H. M., McCoy, L. S., Bracci, P. M., . . . Jenkins, R. B. (2015). Glioma Groups Based on 1p/19q, IDH, and TERT Promoter Mutations in Tumors. *N Engl J Med*, *372*(26), 2499-2508. https://doi.org/10.1056/NEJMoa1407279

ESMO. (2014). High-grade glioma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. https://www.annalsofoncology.org/article/S0923-7534(19)34077-3/fulltext

Esteller, M., Garcia-Foncillas, J., Andion, E., Goodman, S. N., Hidalgo, O. F., Vanaclocha, V., Baylin, S. B., & Herman, J. G. (2000). Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. *N Engl J Med*, *343*(19), 1350-1354. https://doi.org/10.1056/nejm200011093431901

Gusyatiner, O., & Hegi, M. E. (2018). Glioma epigenetics: From subclassification to novel treatment options. *Semin Cancer Biol*, *51*, 50-58. https://doi.org/10.1016/j.semcancer.2017.11.010

Hawkins, C., Walker, E., Mohamed, N., Zhang, C., Jacob, K., Shirinian, M., Alon, N., Kahn, D., Fried, I., Scheinemann, K., Tsangaris, E., Dirks, P., Tressler, R., Bouffet, E., Jabado, N., & Tabori, U. (2011). BRAF-KIAA1549 fusion predicts better clinical outcome in pediatric low-grade astrocytoma. *Clin Cancer Res*, *17*(14), 4790-4798. https://doi.org/10.1158/1078-0432.ccr-11-0034

Hegi, M. E., Diserens, A. C., Gorlia, T., Hamou, M. F., de Tribolet, N., Weller, M., Kros, J. M., Hainfellner, J. A., Mason, W., Mariani, L., Bromberg, J. E., Hau, P., Mirimanoff, R. O., Cairncross, J. G., Janzer, R. C., & Stupp, R. (2005). MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med*, *352*(10), 997-1003. https://doi.org/10.1056/NEJMoa043331

Horbinski, C. (2013a). To BRAF or not to BRAF: is that even a question anymore? *J Neuropathol Exp Neurol*, *72*(1), 2-7. https://doi.org/10.1097/NEN.0b013e318279f3db

Horbinski, C. (2013b). What do we know about IDH1/2 mutations so far, and how do we use it? *Acta Neuropathol*, *125*(5), 621-636. https://doi.org/10.1007/s00401-013-1106-9

Houdova Megova, M., Drabek, J., Dwight, Z., Trojanec, R., Koudelakova, V., Vrbkova, J., Kalita, O., Mlcochova, S., Rabcanova, M., & Hajduch, M. (2017). Isocitrate Dehydrogenase Mutations are Better Prognostic Marker than O6-methylguanine-DNA Methyltransferase Promoter Methylation in Glioblastomas - a Retrospective, Single-centre Molecular Genetics Study of Gliomas. *Klin Onkol*, *30*(5), 361-371. https://doi.org/10.14735/amko2017361 (Mutace isocitratdehydrogenazy jsou lepsi prognosticky marker nez metylace promotoru O6-metylguanin-DNA-metyltransferazy u glioblastomu - retrospektivni molekularne geneticka studie gliomu z jednoho centra.)

Jenkins, R. B., Blair, H., Ballman, K. V., Giannini, C., Arusell, R. M., Law, M., Flynn, H., Passe, S., Felten, S., Brown, P. D., Shaw, E. G., & Buckner, J. C. (2006). A t(1;19)(q10;p10) mediates the combined deletions of 1p and 19q and predicts a better prognosis of patients with oligodendroglioma. *Cancer Res*, *66*(20), 9852-9861. https://doi.org/10.1158/0008-5472.can-06-1796

Ji, J., Kaneva, K., Hiemenz, M. C., Dhall, G., Davidson, T. B., Erdreich-Epstein, A., Hawes, D., Hurth, K., Margol, A. S., Mathew, A. J., Robison, N. J., Schmidt, R. J., Tran, H. N., Judkins, A. R., Cotter, J. A., & Biegel, J. A. (2021). Clinical utility of comprehensive genomic profiling in central nervous system tumors of children and young adults. *Neuro-Oncology Advances*, *3*(1). https://doi.org/10.1093/noajnl/vdab037

Johnson, A., Severson, E., Gay, L., Vergilio, J. A., Elvin, J., Suh, J., Daniel, S., Covert, M., Frampton, G. M., Hsu, S., Lesser, G. J., Stogner-Underwood, K., Mott, R. T., Rush, S. Z., Stanke, J. J., Dahiya, S., Sun, J., Reddy, P., Chalmers, Z. R., . . . Ramkissoon, S. H. (2017). Comprehensive Genomic Profiling of 282 Pediatric Low- and High-Grade Gliomas Reveals Genomic Drivers, Tumor Mutational Burden, and Hypermutation Signatures. *Oncologist*, *22*(12), 1478-1490. https://doi.org/10.1634/theoncologist.2017-0242

Jones, D. T., Kocialkowski, S., Liu, L., Pearson, D. M., Backlund, L. M., Ichimura, K., & Collins, V. P. (2008). Tandem duplication producing a novel oncogenic BRAF fusion gene defines the majority of pilocytic astrocytomas. *Cancer Res*, *68*(21), 8673-8677. https://doi.org/10.1158/0008-5472.can-08-2097

Lassaletta, A., Zapotocky, M., Mistry, M., Ramaswamy, V., Honnorat, M., Krishnatry, R., Guerreiro Stucklin, A., Zhukova, N., Arnoldo, A., Ryall, S., Ling, C., McKeown, T., Loukides, J., Cruz, O., de Torres, C., Ho, C. Y., Packer, R. J., Tatevossian, R., Qaddoumi, I., . . . Tabori, U. (2017). Therapeutic and Prognostic Implications of BRAF V600E in Pediatric Low-Grade Gliomas. *J Clin Oncol*, *35*(25), 2934-2941. https://doi.org/10.1200/jco.2016.71.8726

Louis, D., Perry, A., Reifenberger, G., von Deimling, A., Figarella-Branger, D., Cavenee, W. K., Ohgaki, H., Wiestler, O. D., Kleihues, P., & Ellison, D. W. (2016). The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol*, *131*(6), 803-820. https://doi.org/10.1007/s00401-016-1545-1

Louis, D., Schiff, D., & Batchelor, T. (2023, August 18). *Classification and pathologic diagnosis of gliomas, glioneuronal tumors, and neuronal tumors*. https://www.uptodate.com/contents/classification-and-pathologic-diagnosis-of-gliomas-glioneuronal-tumors-and-neuronal-tumors

Louis, D. N., Perry, A., Wesseling, P., Brat, D. J., Cree, I. A., Figarella-Branger, D., Hawkins, C., Ng, H. K., Pfister, S. M., Reifenberger, G., Soffietti, R., von Deimling, A., & Ellison, D. W. (2021). The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. *Neuro-Oncology*, *23*(8), 1231-1251. https://doi.org/10.1093/neuonc/noab106

Malgulwar, P. B., Nambirajan, A., Pathak, P., Faruq, M., Rajeshwari, M., Singh, M., Suri, V., Sarkar, C., & Sharma, M. C. (2018). C11orf95-RELA fusions and upregulated NF-KB signalling characterise a subset of aggressive supratentorial ependymomas that express L1CAM and nestin. *J Neurooncol*, *138*(1), 29-39. https://doi.org/10.1007/s11060-018-2767-y

Malmstrom, A., Gronberg, B. H., Marosi, C., Stupp, R., Frappaz, D., Schultz, H., Abacioglu, U., Tavelin, B., Lhermitte, B., Hegi, M. E., Rosell, J., & Henriksson, R. (2012). Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy in patients older than 60 years with glioblastoma: the Nordic randomised, phase 3 trial. *Lancet Oncol*, *13*(9), 916-926. https://doi.org/10.1016/s1470-2045(12)70265-6

Meyronet, D., Esteban-Mader, M., Bonnet, C., Joly, M. O., Uro-Coste, E., Amiel-Benouaich, A., Forest, F., Rousselot-Denis, C., Burel-Vandenbos, F., Bourg, V., Guyotat, J., Fenouil, T., Jouvet, A., Honnorat, J., & Ducray, F. (2017). Characteristics of H3 K27M-mutant gliomas in adults. *Neuro Oncol*, *19*(8), 1127-1134. https://doi.org/10.1093/neuonc/now274

Miller, A. M., Shah, R. H., Pentsova, E. I., Pourmaleki, M., Briggs, S., Distefano, N., Zheng, Y., Skakodub, A., Mehta, S. A., Campos, C., Hsieh, W.-Y., Selcuklu, S. D., Ling, L., Meng, F., Jing, X., Samoila, A., Bale, T. A., Tsui, D. W. Y., Grommes, C., . . . Mellinghoff, I. K. (2019). Tracking tumour evolution in glioma through liquid biopsies of cerebrospinal fluid.

Muralidharan, K., Yekula, A., Small, J. L., Rosh, Z. S., Kang, K. M., Wang, L., Lau, S., Zhang, H., Lee, H., Bettegowda, C., Chicoine, M. R., Kalkanis, S. N., Shankar, G. M., Nahed, B. V., Curry, W. T., Jones, P. S., Cahill, D. P., Balaj, L., & Carter, B. S. (2021). TERT Promoter Mutation Analysis for Blood-Based Diagnosis and Monitoring of Gliomas. *Clinical Cancer Research*, *27*(1), 169-178. https://doi.org/10.1158/1078-0432.Ccr-20-3083

NCBI. (2011). RELA - RELA proto-oncogene, NF-kB subunit. https://www.ncbi.nlm.nih.gov/gene/5970/ortholog/?scope=117570

NCCN. (2023, March 24). *NCCN Clinical Practice Guidelines in Oncology; Central Nervous System Cancers v1.2023*. National Comprehensive Cancer Network. https://www.nccn.org/professionals/physician_gls/pdf/cns.pdf

NICE. (2021). Brain tumours (primary) and brain metastases in adults. https://www.nice.org.uk/guidance/ng99/chapter/Recommendations

Nikiforova, M. N., Wald, A. I., Melan, M. A., Roy, S., Zhong, S., Hamilton, R. L., Lieberman, F. S., Drappatz, J., Amankulor, N. M., Pollack, I. F., Nikiforov, Y. E., & Horbinski, C. (2016). Targeted next-generation sequencing panel (GlioSeq) provides comprehensive genetic profiling of central nervous system tumors. *Neuro Oncol*, *18*(3), 379-387. https://doi.org/10.1093/neuonc/nov289

Parikh, N., Hilsenbeck, S., Creighton, C. J., Dayaram, T., Shuck, R., Shinbrot, E., Xi, L., Gibbs, R. A., Wheeler, D. A., & Donehower, L. A. (2014). Effects of TP53 Mutational Status on Gene Expression Patterns Across Ten Human Cancer Types. *J Pathol*, *232*(5), 522-533. https://doi.org/10.1002/path.4321

Ramkissoon, S. H., Bandopadhayay, P., Hwang, J., Ramkissoon, L. A., Greenwald, N. F., Schumacher, S. E., O'Rourke, R., Pinches, N., Ho, P., Malkin, H., Sinai, C., Filbin, M., Plant, A., Bi, W. L., Chang, M. S., Yang, E., Wright, K. D., Manley, P. E., Ducar, M., . . . Ligon, K. L. (2017). Clinical targeted exome-based sequencing in combination with genome-wide copy number profiling: precision medicine analysis of 203 pediatric brain tumors. *Neuro Oncol*, *19*(7), 986-996. https://doi.org/10.1093/neuonc/now294

Reuss, D. E., Sahm, F., Schrimpf, D., Wiestler, B., Capper, D., Koelsche, C., Schweizer, L., Korshunov, A., Jones, D. T., Hovestadt, V., Mittelbronn, M., Schittenhelm, J., Herold-Mende, C., Unterberg, A., Platten, M., Weller, M., Wick, W., Pfister, S. M., & von Deimling, A. (2015). ATRX and IDH1-R132H immunohistochemistry with subsequent copy number analysis and IDH sequencing as a basis for an "integrated" diagnostic approach for adult astrocytoma, oligodendroglioma and glioblastoma. *Acta Neuropathol*, *129*(1), 133-146. https://doi.org/10.1007/s00401-014-1370-3

Rios, J. D., Velummailum, R., Bennett, J., Nobre, L., Tsang, D. S., Bouffet, E., Hawkins, C., Tabori, U., Denburg, A., & Pechlivanoglou, P. (2022). Clinical and economic impact of molecular testing for BRAF fusion in pediatric low-grade Glioma. *BMC Pediatr*, *22*(1), 13. https://doi.org/10.1186/s12887-021-03069-1

Ryall, S., Krishnatry, R., Arnoldo, A., Buczkowicz, P., Mistry, M., Siddaway, R., Ling, C., Pajovic, S., Yu, M., Rubin, J. B., Hukin, J., Steinbok, P., Bartels, U., Bouffet, E., Tabori, U., & Hawkins, C. (2016). Targeted detection of genetic alterations reveal the prognostic impact of H3K27M and MAPK pathway aberrations in paediatric thalamic glioma. *Acta Neuropathol Commun*, *4*(1), 93. https://doi.org/10.1186/s40478-016-0353-0

Schwartzentruber, J., Korshunov, A., Liu, X. Y., Jones, D. T., Pfaff, E., Jacob, K., Sturm, D., Fontebasso, A. M., Quang, D. A., Tonjes, M., Hovestadt, V., Albrecht, S., Kool, M., Nantel, A., Konermann, C., Lindroth, A., Jager, N., Rausch, T., Ryzhova, M., . . . Jabado, N. (2012). Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature*, *482*(7384), 226-231. https://doi.org/10.1038/nature10833

Simonelli, M., Dipasquale, A., Orzan, F., Lorenzi, E., Persico, P., Navarria, P., Pessina, F., Nibali, M. C., Bello, L., Santoro, A., & Boccaccio, C. (2020). Cerebrospinal fluid tumor DNA for liquid biopsy in glioma patients' management: Close to the clinic? *Crit Rev Oncol Hematol*, *146*, 102879. https://doi.org/10.1016/j.critrevonc.2020.102879

Sturm, D., Witt, H., Hovestadt, V., Khuong-Quang, D. A., Jones, D. T., Konermann, C., Pfaff, E., Tonjes, M., Sill, M., Bender, S., Kool, M., Zapatka, M., Becker, N., Zucknick, M., Hielscher, T., Liu, X. Y., Fontebasso, A. M., Ryzhova, M., Albrecht, S., . . . Pfister, S. M. (2012). Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. *Cancer Cell*, *22*(4), 425-437. https://doi.org/10.1016/j.ccr.2012.08.024

Wang, L., Liu, L., Li, H., Wang, P., Hu, Z., Wei, Y., Zhang, M., Wen, W., Li, Z., Liu, L., Zhao, L., Lu, D., & Teng, L. (2019). RELA Fusion in Supratentorial Extraventricular Ependymomas: A Morphologic, Immunohistochemical, and Molecular Study of 43 Cases. *Am J Surg Pathol*, *43*(12), 1674-1681. https://doi.org/10.1097/pas.0000000000001342

Weinberg, D. N., Allis, C. D., & Lu, C. (2017). Oncogenic Mechanisms of Histone H3 Mutations. *Cold Spring Harb Perspect Med*, *7*(1). https://doi.org/10.1101/cshperspect.a026443

Weller, M., van den Bent, M., Preusser, M., Le Rhun, E., Tonn, J. C., Minniti, G., Bendszus, M., Balana, C., Chinot, O., Dirven, L., French, P., Hegi, M. E., Jakola, A. S., Platten, M., Roth, P., Rudà, R., Short, S., Smits, M., Taphoorn, M. J. B., . . . Wick, W. (2021). EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. *Nature Reviews Clinical Oncology*, *18*(3), 170-186. https://doi.org/10.1038/s41571-020-00447-z

Wen, P. Y., & Huse, J. T. (2016). 2016 World Health Organization Classification of Central Nervous System Tumors. *Continuum (Minneap Minn)*, *23*(6, Neuro-oncology), 1531-1547. https://doi.org/10.1212/con.0000000000000536

Wick, W., Platten, M., Meisner, C., Felsberg, J., Tabatabai, G., Simon, M., Nikkhah, G., Papsdorf, K., Steinbach, J. P., Sabel, M., Combs, S. E., Vesper, J., Braun, C., Meixensberger, J., Ketter, R., Mayer-Steinacker, R., Reifenberger, G., & Weller, M. (2012). Temozolomide chemotherapy alone versus radiotherapy alone for malignant astrocytoma in the elderly: the NOA-08 randomised, phase 3 trial. *Lancet Oncol*, *13*(7), 707-715. https://doi.org/10.1016/s1470-2045(12)70164-x

Wick, W., Weller, M., van den Bent, M., Sanson, M., Weiler, M., von Deimling, A., Plass, C., Hegi, M., Platten, M., & Reifenberger, G. (2014). MGMT testing--the challenges for biomarker-based glioma treatment. *Nat Rev Neurol*, *10*(7), 372-385. https://doi.org/10.1038/nrneurol.2014.100

Zacher, A., Kaulich, K., Stepanow, S., Wolter, M., Kohrer, K., Felsberg, J., Malzkorn, B., & Reifenberger, G. (2017). Molecular Diagnostics of Gliomas Using Next Generation Sequencing of a Glioma-Tailored Gene Panel. *Brain Pathol*, *27*(2), 146-159. https://doi.org/10.1111/bpa.12367

Zhao, H., Wang, S., Song, C., Zha, Y., & Li, L. (2016). The prognostic value of MGMT promoter status by pyrosequencing assay for glioblastoma patients' survival: a meta-analysis. *World J Surg Oncol*, *14*(1), 261. https://doi.org/10.1186/s12957-016-1012-4

Molecular Markers in Fine Needle Aspirates of the Thyroid

Policy Number: AHS – M2108 – Molecular Markers in Fine Needle Aspirates of the Thyroid	Prior Policy Name and Number, as applicable:
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Thyroid nodules are growths or enlargements on the thyroid gland. These nodules may be caused by several different disorders such as thyroiditis or cysts. However, these nodules may also be caused by thyroid cancer, which occurs in 4-6.5% of nodules. A biopsy is often performed to assess the histological components of this nodule, usually through a fine needle aspiration (FNA). A 23 to 27-gauge (commonly 25 gauge) needle is used, with or without local anesthesia. This technique can obtain an acceptable sample in 90-97% of solid nodules (Ross, 2023).

Molecular markers, such as genetic mutations or microRNA (miRNA) expression, may be used to help identify malignant nodules. Mutational analysis by sequencing or PCR can identify mutations, such as *BRAF*, *RAS*, *RET/PTC*, and *PAX8/PPARG*. Another tool is a gene expression classifier, which measures mRNA to determine the activity level of several genes and uses an algorithm to predict malignancy based on gene expression (Nikiforov et al., 2013).

II. Related Policies

Policy Number	Policy Title
AHS-G2045	Thyroid Disease Testing
AHS-M2066	Genetic Cancer Susceptibility Using Next Generation Sequencing
AHS-M2078	Genetic Testing for Germline Mutations of the RET Proto-Oncogene
AHS-M2087	Genetic Testing for PTEN Hamartoma Tumor Syndrome

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) To assist in patient management decisions for individuals 18 years of age or older being evaluated for thyroid carcinoma, mutation analysis (e.g., *BRAF* V600, *RET/PTC*, *RAS*, *PAX8/PPARG*) and/or the use of a gene expression classifier in fine-needle aspirates (FNAs) of the thyroid **MEETS COVERAGE CRITERIA** when the FNA is cytologically characterized as **any** of the following:
   a) Bethesda-III (atypia of undetermined significance [AUS] or follicular lesion of undetermined significance [FLUS]).
   b) Bethesda-IV (follicular neoplasm [FN] or suspicious for follicular neoplasm [SFN]).

2) For individuals 18 years of age or older being evaluated for thyroid carcinoma, mutation analysis (e.g., *BRAF* V600, *RET/PTC*, *RAS*, *PAX8/PPARG*) and/or the use of a gene expression classifier in FNAs of the thyroid) **DOES NOT MEET COVERAGE CRITERIA** when the FNA is cytologically characterized as **any** of the following:
   a) Bethesda-I (nondiagnostic or unsatisfactory).
   b) Bethesda-II (benign).
   c) Bethesda-V (suspicious for malignancy).
   d) Bethesda-VI (malignant).

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.

3) For individuals under 18 years of age, mutation analysis (e.g., *BRAF* V600, *RET/PTC*, *RAS*, *PAX8/PPARG*) or the use of a gene expression classifier in FNAs of the thyroid **DOES NOT MEET COVERAGE CRITERIA**.

4) MicroRNA profiling tests (e.g., ThyraMIR) in FNAs of the thyroid **DO NOT MEET COVERAGE CRITERIA**.

5) For all other situations not discussed above, mutation analysis (e.g., *BRAF* V600, *RET/PTC*, *RAS*, *PAX8/PPARG*) or the use of a gene expression classifier **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note: For 5 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
AACE	American Association of Clinical Endocrinologists
AAP	American Academy of Pediatrics
ACE	American College of Endocrinology
ALK	*Anaplastic lymphoma kinase* gene
ALS	*Argininosuccinate lyase* gene
AME	Associazione medici endocrinologi
ARID1A	*AT-rich interaction domain 1A* gene
ATA	American Thyroid Association
ATC	Anaplastic thyroid cancer
ATM	*ATM serine/threonine kinase* gene
AUS	Atypia of undetermined significance
BRAF	*B-Raf proto-oncogene* gene
CDKN2A	*Cyclin dependent kinase inhibitor 2A* gene
CLIA/CAP	Clinical Laboratory Improvement Amendments/College of American Pathologists
CMS	Centers for Medicare and Medicaid Services
CTNNB1	*Catenin beta 1* gene
DNA	Deoxyribonucleic acid
ERBB2	*Erb-b2 receptor tyrosine kinase 2* gene
ERBB4	*Erb-b2 receptor tyrosine kinase 4* gene
ESMO	European Society for Medical Oncology
EU-TIRADS	European Thyroid Imaging Reporting and Data System
ETA	European Thyroid Association
FISH	Fluorescence in situ hybridization
FLUS	Follicular lesion of undetermined significance
FN	Follicular neoplasm
FNA	Fine needle aspiration
FNAs	Fine needle aspirates
GC	Genomic classifier
GEC	Gene expression classifier
GSC	Genomic sequencing classifier
HRAS	*HRAS proto-oncogene*

KRAS	*KRAS proto-oncogene*
LDTs	Laboratory-developed tests
MEN1	*Menin 1*
MET	*Met proto-oncogene, receptor tyrosine kinase* gene
MPTX	Thygenext expanded mutation panel
mRNA	Messenger ribonucleic acid
miRNA	Micro-ribonucleic acid
MSI	Microsatellite instability
MTC	Medullary thyroid cancer
NCCN	National Comprehensive Cancer Network
NGS	Next-generation sequencing
NICE	National Institute of Care and Health Excellence
NIFTP	Noninvasive follicular thyroid neoplasm with papillary-like nuclear features
NKX2-1	NK2 homeobox 1
NPV	Negative predictive value
NTRK	*Neurotrophic tyrosine receptor kinase* gene
PAX8/PPARG	*Paired box gene 8/peroxisome proliferator-activated receptor gamma* gene
PCR	Polymerase chain reaction
PD-L1	Programmed death-ligand 1
PIK3CA	*Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha* gene
PTEN	*Phosphatase and tensin homolog* gene
PPV	Positive predictive value
RAS	Rat sarcoma virus
RET/PTC	*Rearranged during transfection)/papillary thyroid carcinoma type 1* gene
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
SFN	Suspicious for follicular neoplasm
SMAD4	*SMAD family member 4* gene
SMO	*Smoothened, frizzled class receptor* gene
SRC	*Proto-oncogene tyrosine-protein kinase Src* gene
TBP	TATA-box binding protein
TERT	*Telomerase reverse transcriptase* gene
Tg	Thyroglobulin
TMB	Tumor mutation burden
US	Ultrasound

USP33	Ubiquitin specific peptidase 33
XA	Xpression atlas

V. Scientific Background

Fine needle aspiration (FNA) is a traditional diagnostic approach to differentiate malignant thyroid nodules that need surgery from benign nodules that do not require surgery. It offers definitive diagnosis in most cases; however, 20–30% of nodules yield an indeterminate cytologic diagnosis in which cancer cannot be ruled out, and such nodules can exhibit malignancy risk ranging from 12% to 33% (Jackson et al., 2020). This may lead to suboptimal management of these patients and can result in unnecessary resections and surgical interventions (Nikiforov et al., 2013). FNA results are normally categorized according to the National Cancer Institute into six categories. They are, in order of severity: nondiagnostic or unsatisfactory (Bethesda-I), benign (II), atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS) (III), follicular neoplasm (FN) or suspicious for follicular neoplasm (SFN) (IV), suspicious for malignancy (V), and malignant, which includes lymphomas and carcinomas (VI) (Ali et al., 2023). A benign result is reported in 60-70% of FNAs. Nondiagnostic does not refer to an absence of cancer; rather it means that the sample provided was inadequate for a conclusive result and another sample must be provided. Categories III and IV are typically referred to as "indeterminate" and typically have a malignancy risk of as high as 40%. Patients with indeterminate nodules will frequently have a surgery to treat the issue; however, up to 95% of these nodules are ultimately evaluated as benign. Further testing must be done with indeterminate cases to categorize these lesions (Cibas & Ali, 2009; Douglas, 2023).

Molecular markers have been used to identify the true status of an indeterminate FNA. Assessing components of FNA aspirates, such as micro-RNA, mutational status of certain genes, or genomic sequencing could prove useful, especially as these components can be reliably detected during the FNA itself (Hodak & Rosenthal, 2013; Xing et al., 2013). Prior to the emergence of molecular markers as an indicator, a repeat FNA was often performed, ultimately leading to a surgery to remove the nodule. Molecular markers could thus reduce unnecessary surgeries as well as provide better risk stratification (Douglas, 2023).

Proprietary Testing

Commercially available panels of molecular markers utilizing FNA specimens from the thyroid include the following tests:

ThyGeNEXT and ThyraMIR

ThyGeNEXT (Interpace Diagnostics, Parsippany, NJ) is a specific oncogene, mutational panel that tests genetic alterations across 10 genes associated with papillary carcinoma and follicular carcinoma. ThyGeNEXT uses a next generation sequencing (NGS) platform to identify genetic alterations across those 10 genes, which are as follows: *ALK, BRAF, GNAS, HRAS, KRAS, NRAS, PIK3CA, PTEN, RET, TERT*. This test is primarily for category III or IV nodules. Recently, Interpace Diagnostics has developed a new molecular test, ThyraMIR. This test is based on microRNA analysis of the expression of 10 microRNAs. The manufacturer claims that this test can identify malignancy in nodules that otherwise evince no or "weak" mutation for ThyGeNEXT. According to Interpace Diagnostics, combined test performance has negative predictive value of 99%, positive predictive value of 96%, with 98% sensitivity and 98% specificity. The sensitivity and specificity for the ThyGeNEXT panel alone is 63% and 84%, respectively, for cases of indeterminant cytology (Interpace, 2023).

Lupo et al. (2020) featured a blinded multicenter study centered around the performance of the ThyGeNext expanded mutation panel (MPTX) and the expanded panel with the microRNA classifier ThyraMIR by comparing them to the histopathology diagnosis by three pathologists. The expanded mutation panel included *NTRK* and *ALS* fusions that have targeted therapies as well as proto-oncogenes *TERT* and *RET*, which are indicative of aggressive disease. The study found that the performance of the expanded mutation panel (ThyGeNext) alone unsatisfactory due to numerous false positives, 90% of which were attributed to individual *RAS* mutations primarily found in benign adenomas, with a few additional errors due to *TERT* mutations in benign disease. It was therefore reported that the expanded mutation panel of ThyGeNext alone demonstrated an NPV of 81% while the PPV was even less stellar, a mere 56% (Lupo et al., 2020). This contrasts with the reported high sensitivity for malignancy (95%) in negative mutation panel testing results and high (90%) specificity in nodules with Bethesda III and IV cytology, purportedly driving an increase in the NPV to 97% and the PPV to 75%, as aforementioned above. The researchers suggest that the increases in NPV and PPV from the inclusion of the microRNA classifier, therefore, possess great power and potential in ruling in and out the need for surgery for indeterminate thyroid nodule cytology, leading them to conclude that ancillary use of the three category MPTX approach can be leveraged to accurately inform the need for surgery in four out of five indeterminate nodules tested (Lupo et al., 2020).

The same optimism may be found in Jackson et al. (2020), where researchers aimed to better understand the incremental use of using expanded mutation panels, along with the integration of microRNA classifier testing to provide additional and more accurate diagnostic information. Using molecular results from two consecutive cohorts of patients totaling 12993 members, who had FNAs performed on thyroid nodules resulting in Bethesda Diagnostic categories III or IV cytology results, underwent either

focused mutation panel testing (n=8619) alone or expanded mutation panel testing (n=4374), the latter of which included the microRNA classifier test ThyraMIR. The study found that 89% of patients who underwent the simple focused panel testing lacked detectable oncogenic mutations and fusions as compared to the 74% in the cohort who underwent the expanded panel testing ($P < 0.001$). Moreover, weak drivers were more frequently identified in patients who underwent expanded (20%) compared with focused (9%) panel testing ($P < 0.001$), and strong drivers were likewise more frequent in patients who underwent expanded (6%) compared with focused (2%) panel testing ($P < 0.001$). Thus, the power of the expanded panel was not limited to detecting weak drivers, as 16% of those who underwent focused panel testing had strong drivers, while 24% of those who underwent expanded panel testing had strong drivers ($P < 0.001$). Inclusion of less common mutations in the expanded panel increased the detection of multiple coexisting mutations by 4%, which provides increased utility in identifying aggressive forms of thyroid cancer. However, the study concluded broadly that all oncogenic changes can contribute to neoplastic growth and progression, and therefore both strong and weak drivers should be considered clinically important. From the results of the study, the researchers contend that subsequent microRNA testing can help overcome uncertainty as to the presence of cancer, as approximately half of nodules with weak drivers had positive microRNA results consistent with a higher risk of malignancy, and 33% of those with positive microRNA results likewise had strong positive microRNA levels specific for malignancy that are prevalent in nodules with strong drivers (Jackson et al., 2020).

Ciarletto et al. (2021) used ThyraMIR to study if pairwise comparisons of differentially expressed miRNAs can identify medullary thyroid carcinoma in FNA. Differential pairwise analysis was performed on 10 miRNAs in 7557 specimens. Nine differential pairs were determined to have significant power to differentiate medullary thyroid carcinoma and non- medullary thyroid carcinoma samples. The test accuracy was 100%, "the assay correctly classified all MTC [medullary thyroid carcinoma] and non-MTC samples." The authors conclude that "pairwise miRNA expression analysis of ThyraMIR results were found to accurately predict medullary thyroid carcinoma in thyroid FNA samples, including those with indeterminate FNA findings" (Ciarletto et al., 2021).

ThyroSeq v3

ThyroSeq is a test intended for assessment of thyroid nodules with undetermined cytology initially designed to target 12 cancer genes with 284 mutational hotspots. The latest version of this test, ThyroSeq v3, is based on NGS of DNA and RNA. This test detects 4 types of alterations; mutations, gene fusions, expression alterations, and copy number alterations. This test analyzes 112 genes, providing information on more than

12,000 mutation hotspots and more than 120 gene fusion types. First, the sample is ensured to have enough material to proceed (such as amount of thyroid follicular cells). Then, the NGS is performed and reviewed by a pathologist. Finally, the report is sent to the patient in about 2 weeks (ThyroSeq, 2024).

ThyroSeq test

In a multicenter study, this test was produced a negative predictive value of 97%, positive predictive value of 66%, with 94% sensitivity and 82% specificity in 257 cases of Bethesda-III and -IV nodules. The authors concluded that up to 61% of patients with indeterminate cytology could avoid diagnostic surgery through multigene genomic classifier testing (Steward et al., 2018). Other NGS-based molecular tests for thyroid nodules are available from other labs.

Nikiforova et al. (2018) evaluated the analytical performance of ThyroSeq v3 (intended to analyze 112 genes for alterations). A genomic classifier (GC) is used to differentiate malignant nodules from benign. 238 tissue samples and 175 FNA samples were included in the cohort. ThyroSeq detected over 100 genetic alterations. The GC cutoff identified malignant nodules in the tissue samples from benign at 93.9% sensitivity, 89.4% specificity, and 92.1% accuracy. For the FNA sample, the GC sensitivity was 98.0%, the specificity was 81.8%, and the accuracy was 90.9% (Nikiforova et al., 2018).

Afirma Series

The Afirma Genomic Sequencing Classifier (GSC) is offered by Afirma. This test is intended to assess indeterminate nodules (Bethesda categories III and IV) and "conclusively rule out" surgery (Afirma, 2022a). This test is based on the Afirma Gene Expression Classifier (GEC), which measured the activity level of 167 genes. The GSC added several features to better classify nodules, such as classifiers for medullary thyroid cancer, parathyroid lesions, *BRAF V600E* mutations, and overall better discrimination of Hurthle cell neoplasms (Douglas, 2023). Wei et al note that the GSC is an "updated" version of the GEC, available since 2011. Wei et al also published a study discussing proposed advantages of the GSC over the GEC, namely its improved specificity (as a weakness of the GEC was its inability to identify true oncocytic lesions in the "suspicious" category). The authors compared the results of indeterminate FNA specimens (Bethesda categories III and IV) that were tested by Afirma GEC or GSC. 272 tests (194 GEC, 78 GSC) were evaluated. 221 samples were classified as AUS/FLUS and 51 were classified as FN/SFN. Out of the 194 samples tested with GEC, 88 were considered benign (45.4%) whereas 52 of the 78 GSC samples were considered benign (66.7%). In the AUS/FLUS category, 47.1% of cases were considered benign by GEC whereas 71.2% were considered benign by GSC (Wei et al., 2019).

A component of the Afirma GSC is the medullary thyroid cancer (MTC) component. This component includes 108 genes and is performed along with the primary GSC at no extra charge (Afirma, 2021a, 2021b). Randolph et al. performed a validation of this test using 211 samples (21 MTC cases, 190 controls), and all 211 samples were identified correctly. These sample results were confirmed by surgery (for positive cases) and pathology (for negative cases) (Randolph, 2017).

Another test is the Afirma Xpression Atlas. This test is a comprehensive panel encompassing 511 genes, 761 variants, and 130 fusion pairs. Genes such as *BRAF, RET*, and the *RAS* pathway of genes are included in this panel. This test is intended to inform surgical and therapeutic decisions for high-risk patients. Afirma lists three populations for this test; those who are "suspicious" per the Afirma GSC test, Bethesda category V, and Bethesda category VI. Angell et al validated this test. The XA was compared to the results of multiple other methodologies (such as whole-transcriptome RNA-seq, targeted DNA-seq, et al) and concordance was evaluated. 943 blinded FNA samples were used to compare the DNA and RNA detection and 695 blinded FNAs were used to compare the fusion detections. At the cutoff of 5% variant frequency, 74% of allele variants detected by traditional methods were also detected by the XA, with 88% detected by the XA at a 20% variant frequency cutoff. 82% of fusions detected by the targeted RNA fusion assay were detected by the XA. From an analytical validity standpoint, intra-plate reproducibility was found to be 89%-94%, and inter-plate reproducibility was found to be 86%-91% (Angell et al., 2019). More recently, Krane et al. (2020) compiled evidence for the analytical validity, clinical validity, and clinical utility of the Afirma series in an evidence-based assessment. Analytical validation of the Afirma XA test using 69 variant-positive FNA samples demonstrated that there was high accuracy between the detection of variants (90%) and detection of fusion (94%) across two different laboratories. Clinical validation on the Afirma XA test's ability to detect genomic variants was measured against those of currently accepted methods of DNA and RNA sequencing using the aforementioned 943 FNA-blinded samples. However, it should be noted that 95% or more of the variants and fusions identified by Afirma XA can be identified simply through the reference DNA and RNA method. Moreover, some variants that were identified by DNA were absent or poorly expressed in RNA, and important promoter variants such as *TERT* are not identified by said test.

Jug et al. (2018) evaluated the performances of ThyroSeq and Afirma GEC within the context of ultrasonographic features and with the incorporation of the "noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) nomenclature." The ultrasonographic pattern evaluations were derived from the 2015 American Thyroid Association guidelines. A total of 304 cases were evaluated, with 119 resections. All cases that met criteria for NIFTP were considered high-risk by both tests.

However, when these NIFTP cases were moved from the malignant to the non-malignant category, the positive predictive value of ThyroSeq dropped from 42.9% to 14.3% and Afirma's dropped from 30.1% to 25.3% (Jug et al., 2018).

Endo et al. (2019) compared Afirma's GEC to its Gene Sequencing Classifier (GSC), using cytologically indeterminate nodules. 343 GEC-tested nodules and 164 GSC-tested nodules were identified. The GSC was found to have a "statistically significant higher benign call rate (76.2% vs. 48.1%), PPV (60.0% vs. 33.3%), and specificity (94.3% vs. 61.4%)." The authors noted that the improvement was statistically significant for Bethesda III and IV nodules. The GSC benign call rate was significantly higher in Hürthle cell changes (88.8% vs 25.7%). 52.5% of indeterminate nodules went to surgery when using the GEC compared to only 17.6% when using the GSC (Endo et al., 2019). Vuong et al. (2021) completed a meta-analysis of seven studies comparing the clinical impact and diagnostic performance of Afirma's GEC and GSC. Similarly, this study showed that GSC had a higher benign cell rate, particularly in Hürthle cell predominated nodules, as well as a lower resection rate and higher risk of malignancy. The authors conclude that "the specificity (43.0% vs 25.1%; P = .003) and PPV (63.1% vs 41.6%; P = .004) of Afirma GSC were significantly improved while it still maintained a high sensitivity (94.3%) and a high NPV (90.0%)" compared to the GEC (Vuong et al., 2021).

RosettaGX Reveal

The RosettaGX Reveal test from Rosetta Genomics is a micro-RNA-based diagnostic test that evaluates indeterminate thyroid nodules. The test measures 24 sequences of miRNA through quantitative RT-PCR, as well as a medullary carcinoma marker (hsa-miR-375). Each sample is classified as benign or suspicious for malignancy.

The test has a claimed negative predictive value of 99%, a 98% sensitivity, and a 78% specificity from a sample of 150 in which three pathologists all agreed on the evaluation. Out of the 189 total samples, the negative predictive value was 91%, sensitivity was 85%, and specificity was 72%. The nodule sizes were >0.5 cm. The authors concluded that this test may be able to differentiate between malignant and benign from a previously evaluated indeterminate sample (Lithwick-Yanai et al., 2017). This test has been discontinued.

NeoGenomics

NeoGenomics offers a "NeoTYPE" thyroid profile, intended for evaluation of "fine needle aspirates of thyroid nodules that are indeterminate or suspicious on cytology." NeoGenomics states that FISH detects mutations and other gene rearrangements, and that *BRAF* mutation V600E is associated with poor prognosis of papillary thyroid

carcinoma. The test measures the following genetic features: "*AKT1, ALK, ARID1A, ATM, BRAF, CDKN2A, CTNNB1, ERBB2, ERBB4, HRAS, KRAS, MEN1, MET*, Microsatellite Instability (MSI), *NF1, NF2, NRAS, PIK3CA, PTEN, RET, SMAD4, SMO, SRC, TERT* Promoter, *TP53, TSC1, TSC2,* Tumor Mutation Burden (TMB)", *MET* and *RET* by FISH, PD-L1, and Pan-TRK (NeoGenomics, 2023).

Clinical Utility and Validity

A study focusing on detection of *BRAF*, *RAS*, *RET/PTC*, and *PAX8/PPARg* mutations found that detection of any mutation resulted in a risk of histologic malignancy of 88%, 87%, and 95% for samples of Bethesda categories III, IV, and V, respectively. 967 samples were taken, and 87 mutations were found. The risk of cancer in mutation-negative samples was 6%, 14%, and 28%, respectively. Unfortunately, there was also a 14% false negative rate, limiting the usefulness of this panel (Douglas, 2023; Nikiforov et al., 2011).

Additional point mutations (including *TERT*, *TP53*, and others), as well as gene fusions that occur in thyroid cancer were found in another study using a next-generation sequencing (NGS) assay. 143 samples with a classification of follicular neoplasm or suspicious for follicular neoplasm were assessed, and the NGS panel "ThyroSeq v2" was used, which tests for point mutations in 13 genes and 42 types of gene fusions. 104 samples were found to be benign with the other 39 sample results being malignant. Overall, the NGS panel performed at a negative predictive value of 96%, a positive predictive value of 83%, 90% sensitivity, 93% specificity, and 92% accuracy for FN/SFN nodules (Nikiforov et al., 2014).

Another method that has been used is assessment of mRNA expression through a gene expression classifier. This is the basis of the Afirma genomic sequencing classifier, which identifies markers for features, such as medullary thyroid cancer, as well as distinguishes between Hürthle cell neoplasms from non-neoplastic Hürthle cell neoplasms. The second version of this test was assessed with 191 indeterminate samples. The sensitivity and specificity were 91% and 68%, respectively, and the negative and positive predictive values were 96% and 47%, respectively (Douglas, 2023; Patel et al., 2018). Afirma has produced a similar gene expression classifier "Xpression Atlas" that can be used to assess B-III to B-VI category neoplasms. Xpression Atlas evaluates a total of 761 gene variants and 130 fusion pairs (Afirma, 2022b; Douglas, 2023).

The above methods may be combined to better evaluate neoplasms, for instance, an miRNA gene expression classifier using both mutation analysis and miRNA expression. Labourier et al. (2015) evaluated a diagnostic algorithm that combined mutation analysis and miRNA expression to assess preoperative FNAs. This algorithm contained

17 validated gene alterations using the *miRInform* thyroid test and a 10-miRNA gene expression classifier. 109 samples of either AUS/FLUS or FN/SFN were evaluated with this combined algorithm, and this algorithm correctly identified 64% of malignant samples and 98% of benign ones. The sensitivity and specificity were 89% and 85%, respectively. For AUS/FLUS and FN/SFN, the negative predictive value was 97% and 91%, and the positive predictive value for malignancy was 68% and 82%, respectively (Douglas, 2023; Labourier et al., 2015).

No single methodology has achieved clinical utility to reliably resolve all indeterminate cytology, and thus several professional organizations, including the American Association of Clinical Endocrinologists (AACE), the American Thyroid Association (ATA), and the National Comprehensive Cancer Network (NCCN), have published guidelines for the evaluation of thyroid nodules, all of which endorse a similar multistep strategy suggesting molecular markers can be of use when cytology is indeterminate, yet acknowledging its current limitations (Gharib et al., 2016; Haugen et al., 2016; NCCN, 2023).

Banizs and Silverman (2019) evaluated the utility of "combined mutation analysis and microRNA classification in reclassifying cancer risk of cytologically indeterminate thyroid nodules." A three-tiered microRNA threshold was determined based on nodules with known disease status, and an expected rate of malignancy calculated from mutation analysis and the microRNA approach was validated in an independent cohort of "atypia of undetermined significance or follicular lesion of undetermined significance (AUS/FLUS) and follicular neoplasm or suspicious for follicular neoplasm (FN/SFN) nodules with surgically derived outcomes." From there, 2685 patients were included in the intended analysis. 82% of these samples lacked mutations, with *BRAF, PIK3CA, PAX8/PPARg*, and *RET/PTC* mutations all comprising 2% or less. The maximum expected risk of malignancy in these nodules without mutation was 9% for AUS/FLUS nodules and 17% for FN/SFN nodules, but with positive microRNA status, these rates increased to 36% and 54% respectively. *RAS* mutations occurred in 15% of mutations, and the expected malignancy rates in nodules with *RAS* or *PAX8/PPARg* mutations was 49% for AUS/FLUS nodules and 65% for FN/SFN nodules. With positive microRNA status, these rates increased to 85% and 91%, respectively (Banizs & Silverman, 2019).

VI. Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

NCCN guidelines for thyroid carcinoma state that molecular diagnostic testing to detect individual mutations (such as *BRAF V600E* or *RET/PTC*) or pattern recognition approaches using molecular classifiers may be useful in the evaluating indeterminate FNA samples to assist in management decisions. NCCN states molecular diagnostics

may be used to reclassify indeterminate lesions such as AUS/FLUS. Molecular markers should be interpreted within the context of each patient. The NCCN states that molecular testing may be considered to drive treatment decisions, and some mutations may have prognostic importance.

When FNA results suggest atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS, Bethesda III) and there is not a high clinical and/or radiographic suspicion of malignancy, the NCCN recommends that one considers repeat FNA with second opinion pathology, diagnostic lobectomy, molecular diagnostics, or node surveillance as recommended by the ATA or TI-RADS as treatment. However, they remind readers that "Molecular markers should be interpreted with caution and in the context of clinical, radiographic, and cytologic features of each individual patient. If molecular diagnostics are technically inadequate or not done, then repeat FNA" (NCCN, 2023).

The NCCN further clarifies that "The choice of the precise molecular test depends on the cytology and the clinical question being asked. Indeterminate groups include: 1) follicular or oncocytic neoplasms; and 2) AUS/FLUS." The NCCN panel recommends molecular diagnostic testing for evaluating FNA results that are suspicious for follicular cell neoplasms or AUS/FLUS (category 2A), including oncocytic carcinoma, as studies historically do not perform well on Hürthle neoplasms. However, the NCCN has acknowledged promising studies for assessing oncocytic carcinoma neoplasms, with both the Afirma and ThyroSeq v3 tests providing better evaluations than their predecessors. The NCCN notes that molecular diagnostic testing may include individual mutation analysis or a multigene assay such as a gene expression classifier. Molecular markers may also help with treatment decisions or with eligibility in clinical trials.

However, they also note that "The diagnosis of follicular carcinoma or oncocytic carcinoma requires evidence of either vascular or capsular invasion, which cannot be determined by FNA. Molecular diagnostics may be useful to allow reclassification of follicular lesions (i.e., follicular neoplasm, AUS, FLUS) as either more or less likely to be benign or malignant based on the genetic profile. If molecular testing suggests papillary thyroid carcinoma, especially in the case of BRAF V600E, see PAP-1 Given the challenges of cytology to explicitly diagnose MTC in limited samples, molecular tests may be used to identify them. If molecular testing, in conjunction with clinical and ultrasound features, predicts a risk of malignancy comparable to the risk of malignancy seen with a benign FNA cytology (approximately 5% or less), consider nodule surveillance. Molecular markers should be interpreted with caution and in the context of clinical, radiographic, and cytologic features of each individual patient. If molecular diagnostics are technically inadequate or not done, then repeat FNA" (NCCN, 2023).

For individuals with or suspected with having papillary carcinoma, the NCCN recommends the following procedures as part of the diagnostic workup: thyroid and neck ultrasound (including central and lateral compartments), if not previously done, CT/MRI with contrast for locally advanced disease or vocal cord paresis, Consider assessment of vocal cord mobility (ultrasound, mirror indirect laryngoscopy, or fiberoptic laryngoscopy), FNA for suspicious lateral neck nodes (NCCN, 2023). FNA is important to consider in this case as "Tg washout is useful in diagnosis of lymph node metastases and recommended if cytology is negative" (NCCN, 2023).

The NCCN further notes that active surveillance may be an option for patients whose molecular diagnostics demonstrate a risk of malignancy under 5% and that the predictive value of molecular diagnostics may be influenced by pre-test probability of disease based on various FNA cytology groups. The NCCN states that molecular diagnostic testing may be useful for follicular cell carcinomas and diagnosing NIFTP [noninvasive follicular thyroid neoplasm with papillary-like nuclear features], although current tests for assessing NIFTP have not been validated. Molecular testing is not recommended for diagnosing anaplastic thyroid carcinoma. Finally, the NCCN highlights that the diagnostic utility of molecular diagnostics in pediatric patients is still unclear because most of the published literature is on adult patients with thyroid nodules (NCCN, 2023).

American Thyroid Association (ATA)

The 2015 ATA guidelines on the management of adult patients with thyroid nodules and differentiated thyroid cancer make the following recommendations on the use of molecular markers:

- "If molecular testing is being considered, patients should be counseled regarding the potential benefits and limitations of testing and about the possible uncertainties in the therapeutic and long-term clinical implications of results." (Strong recommendation; low-quality evidence)
- "If intended for clinical use, molecular testing should be performed in Clinical Laboratory Improvement Amendments/College of American Pathologists (CLIA/CAP)-certified molecular laboratories, or the international equivalent, because reported quality assurance practices may be superior compared to other settings." (Strong recommendation; low-quality evidence)
- "For nodules with AUS/FLUS cytology, after consideration of worrisome clinical and sonographic features, investigations such as repeat FNA or molecular testing may be used to supplement malignancy risk assessment in lieu of proceeding directly with a strategy of either surveillance or diagnostic surgery. Informed

patient preference and feasibility should be considered in clinical decision-making." (Weak recommendation; moderate-quality evidence)

- "Diagnostic surgical excision is the long-established standard of care for the management of FN/SFN cytology nodules. However, after consideration of clinical and sonographic features, molecular testing may be used to supplement malignancy risk assessment data in lieu of proceeding directly with surgery. Informed patient preference and feasibility should be considered in clinical decision-making." (Weak recommendation; moderate-quality evidence)
- In general, only nodules >1 cm should be evaluated as they have a greater chance to become a clinically significant cancer. However, there are some cases where nodules <1 cm may be evaluated due to other clinical symptoms. The ATA states that "attempts to diagnose and treat all such small thyroid cancers in an effort to prevent exceedingly rare outcomes is deemed to cause more harm than good."
- "If the nodule is benign on cytology, further immediate diagnostic studies or treatment are not required."
- "Each nodule that is >1 cm carries an independent risk of malignancy and therefore multiple nodules may require FNA" (Haugen et al., 2016).

The guidelines also state that "there is currently no single optimal molecular test that can definitively rule in or rule out malignancy in all cases of indeterminate cytology, and long-term outcome data proving clinical utility are needed" (Haugen et al., 2016).

The ATA Guidelines Task Force on Pediatric Thyroid Cancer have developed unique guidelines for children and adolescents with thyroid tumors. They have presented 34 recommendations including recommendations on molecular markers testing and nodules. The ATA panel recommended the pediatric age to be limited to a patient that is $\leq$ 18 years of age to more accurately define the impact of the physiologic changes of growth and development on tumor behavior. These guidelines note that a size criterion is more difficult in children as age affects volume greatly and size of the nodule is not predictive of malignancy. Overall, studies focusing on molecular diagnostics in children have not been validated and so cannot be recommended at this time (Francis et al., 2015).

The 2021 ATA Guidelines for Management of Patients with Anaplastic Thyroid Cancer notes that FNA cytology can be an important diagnostic tool for ATC diagnosis but recommends a parallel core biopsy to obtain sufficient material for molecular testing and accurate diagnosis (ATA, 2021; Bible et al., 2021). Other notable recommendations are reported below.

"RECOMMENDATION 2

Every effort should be made to establish a diagnosis via biopsy before proceeding with surgical resection, as surgical resection may be inappropriate.
Strength of Recommendation: Strong
Quality of Evidence: Low"

Moreover, the ATA asserts that "Generous sampling of the tumor, including its advancing edge, is indicated and necessary to fully characterize the neoplasm and increase the probability of finding any coexistent carcinoma and/or other pathologic findings. Additional or repeat immunohistochemical analysis should be performed as needed." Consequently, surgical pathology evaluation is indicated in the below situations.

"**RECOMMENDATION 3**
Routine surgical pathology evaluation of resection specimens should focus on confirming a definitive diagnosis of ATC, documenting the extent of disease, and defining the presence of any coexisting DTC and/or other pathologies. The proportion of tumor that represents ATC should also be documented.
Strength of Recommendation: Strong
Quality of Evidence: Low

RECOMMENDATION 4
Once ATC diagnosis is considered, assessment of *BRAF*^{V600E} mutation should be expeditiously performed by IHC and confirmed/expeditiously assessed by molecular testing.
Strength of Recommendation: Strong
Quality of Evidence: Moderate"

Regarding the timing of molecular profiling, the ATA offers the following recommendation:

"**RECOMMENDATION 5**
Molecular profiling should be performed at the time of ATC diagnosis to inform decisions related to the use of targeted therapies, especially as there are now FDA-approved mutation-specific therapies in this context.
Strength of Recommendation: Strong
Quality of Evidence: Moderate."

For initial evaluations, "There are a number of preoperative staging procedures of importance in ATC." The ATA propounds the below procedures.

"**RECOMMENDATION 6**
Initial radiological tumor staging should include cross-sectional imaging, in particular, CT neck, chest, abdomen, and pelvis with contrast (or MRI), and, if available, FDG PET/CT. Contrast-enhanced imaging of the brain (MRI preferred) should also be performed, if clinically indicated.
Strength of Recommendation: Strong
Quality of Evidence: Moderate" (Bible et al., 2021).

American Academy of Pediatrics (AAP)

The AAP endorsed the guidelines of the American Thyroid Association Guidelines Task Force on Pediatric Thyroid Cancer (as presented in Francis et al. (2015)) in a publication released in 2018 (AAP, 2018).

American Association of Clinical Endocrinologists (AACE), American College of Endocrinology (Interpace) and Associazione Medici Endocrinologi

These joint guidelines recommend the following:

- "Molecular testing should be considered to complement, not replace cytologic evaluation, and only if the results are expected to influence clinical management. As a general rule, molecular testing is not recommended in nodules with established benign or malignant cytologic characteristics."
- FNA is recommended for high ultrasound (US) risk lesions of ≥10 mm, intermediate risk lesions of >20 mm, and low risk lesions >20 mm and growing or with a risk history.
- "FNA is not recommended for nodules that are functional on scintigraphy."
- Repeat FNA is recommended in benign nodules with suspicious clinical or US findings, a nondiagnostic initial FNA on a solid nodule, and nodules that become symptomatic or increase in volume by 50%. Pregnancy does not affect cytologic diagnostic criteria. Routine repeat FNA is generally not necessary.
- Nodules with a major diameter of <5 mm should be monitored instead of biopsied.
- Consider the detection of *BRAF* and *RET/PTC* and possibly *PAX8/PPARG* and *RAS* mutations if available.
- There is no stance on gene expression classifiers for indeterminate nodules, due to insufficient evidence and limited follow-up.
- There is also insufficient evidence to take any stance on mutation testing to guide surgery decisions, except on mutations with a PPV approaching 100% for papillary thyroid carcinoma such as BRAFV600E (Gharib et al., 2016).

National Institute for Care and Health Excellence (NICE)

The guidelines concerning the use of fine needle aspiration in the assessment and management of thyroid cancer are reported below.

"**Management options based on ultrasound results**
1.2.9 Offer fine needle aspiration cytology (FNAC) to people who meet the threshold using an established system for grading ultrasound appearance.
1.2.10 Consider FNAC or active surveillance for people who do not meet the threshold for FNAC on ultrasound grading alone if there are other reasons for clinical concern." (NICE, 2022)

"**FNAC testing**
Performing and reporting FNAC

1.2.11 See the recommendation on using ultrasound [1.2.9, 1.2.10] guidance when performing FNAC in the NICE guideline on thyroid disease.
1.2.12 Use liquid-based cytology, direct smear or both when processing FNAC samples.
1.2.13 Use the Royal College of Pathologists modification of the British Thyroid Association (BTA) reporting system to report cytology results.
1.2.14 Consider rapid on-site evaluation of FNAC adequacy rates to improve the diagnostic yield of samples if the Thy1 (inadequate) rate for the centre or individual clinicians is higher than 15% (when Thy1c is excluded)" (NICE, 2022).

"**Management and further sampling after initial FNAC**

"1.2.15 Use the initial FNAC results to determine further management and sampling options, as shown in table 1."

Table 1 is captured below. Repeat sampling with FNAC is recommended for Thy1c (cystic lesion).

Table 1 Management options after initial fine-needle aspiration cytology

Initial fine-needle aspiration cytology result	Management and further sampling
Thy1 (inadequate)	Offer repeat sampling Consider core-needle biopsy (CNB) or fine-needle aspiration cytology (FNAC) with rapid on-site evaluation (ROSE) as the first choice Consider FNAC alone if ROSE is unavailable and CNB is unavailable or inappropriate Consider diagnostic hemithyroidectomy if the repeat sample is also Thy1
Thy1c (cystic lesion)	Offer repeat sampling with FNAC Consider diagnostic hemithyroidectomy if the repeat sample is also Thy1c and the ultrasound appearances are concerning
Thy2 and Thy2c (benign)	Consider repeat ultrasound Offer repeat sampling with FNAC if the second ultrasound also reaches the threshold for FNAC Consider CNB as an alternative to FNAC Discharge people if there is no evidence of malignancy after all investigations are complete, unless there are other reasons for clinical concern
Thy3a (atypia, neoplasia possible)	Offer repeat sampling Consider CNB (or FNAC if CNB unavailable or inappropriate) Consider diagnostic hemithyroidectomy or active surveillance if repeated samples are still Thy3a
Thy3f (suggesting follicular neoplasm)	Consider diagnostic hemithyroidectomy
Thy4 (suspicion of malignancy) and Thy5 (malignant)	Offer diagnostic hemithyroidectomy, or treatment with therapeutic hemithyroidectomy or total thyroidectomy

(NICE, 2022)

European Thyroid Association (ETA)

The ETA states that molecular testing for *BRAF, RET/PTC* and possibly *PAX8*, *PPARG,* and *RAS* mutations may be considered for cytologically indeterminate lesions. The search for molecular markers in B-II class lesions is not recommended, although one member of the panel did not agree with this non-recommendation. A GEC test cannot be recommended to exclude malignancy to replace diagnostic surgery or close surveillance, although one member did not agree with this non-recommendation. The targeted NGS approach is considered the most promising, with larger panels potentially becoming a rule-in and rule-out test if >95% negative predictive value can be reached. The ETA notes that molecular diagnostics may reduce completion thyroidectomies or other surgeries due to clearer assessments of an indeterminate lesion (Paschke et al., 2017).

In their 2023 Clinical Practice Guidelines for thyroid nodule management, the ETA stated that

- "Combine clinical factors, laboratory evaluation, and US risk stratification when defining the indication for FNA, in a shared decision with the patient *(Ungraded good practice statement. Agreement: 9/9 (100%); round: 1)*
- US guidance and use of either capillary action or suction is recommended when performing thyroid nodule FNA *(Strength of recommendation: 1; quality of evidence: ØØØO. Agreement: 9/9 (100%); round: 1)*
- FNA indication should be based on the following size cut-offs:
  - EU-TIRADS 5: >10 mm
  - EU-TIRADS 4: >15 mm
  - EU-TIRADS 3: >20 mm

  (*Strength of recommendation: 2; quality of evidence: ØOOO. Agreement: 9/9 (100%); round: 1*)
- Repeat FNA should be considered in case of a first non-diagnostic sample (excluding the solitary cyst), a Bethesda class III cytology, discrepancy between US risk score (i.e. high risk) and cytological findings (i.e. benign cytology), and significant nodule growthc *(Strength of recommendation: 1; quality of evidence: ØØØO. Agreement: 8/9 (88.9%); round: 1)*
- FNA is recommended in suspicious lymph nodes, with thyroglobulin or calcitonin washout dependent on phenotype *(Strength of recommendation: 1; quality of evidence: ØØØO. Agreement: 9/9 (100%); round: 1)*
- Core-needle biopsy should not be used as a first-line tool to assess thyroid nodules after US but could be considered a second-line procedure for specific conditions *(Strength of recommendation: 1; quality of evidence: ØØOO. Agreement: 8/9 (88.9%); round: 1)*" (Durante et al., 2023)

Regarding cytopathology-based management, the ETA recommend that practitioners "Correlate the cytological diagnosis with clinical, ultrasound and laboratory results *(Ungraded good practice statement. Agreement: 9/9 (100%); round: 1).*" Moreover, "Clinical surveillance of benign thyroid nodules which do not require therapeutic intervention, should be followed up according to the management schedule" below.

- Bethesda I
  - EU-TIRADS 3 (>20 mm): repeat FNA. If still non-diagnostic, consider CNB. If still non-diagnostic, re-evaluate the nodule within 1 year or offer surgery
  - EU-TIRADS 4 (>15 mm) and 5 (>10 mm): repeat FNA. If still non-diagnostic, consider CNB or, molecular testing (if available and sufficient material). If still non-diagnostic, offer active surveillance or surgery

(*Strength of recommendation: 2; quality of evidence: ØOOO. Agreement: 7/9 (77.8%); round: 2*)

- Bethesda II
  - EU-TIRADS 3 (>20 mm) and 4 (>15 mm): re-evaluate the nodule in 3–5 years[d]
  - EU-TIRADS 5 (>10 mm): repeat FNA[f]

(*Strength of recommendation: 2; quality of evidence: ØOOO. Agreement: 9/9 (100%); round: 2*)

- Bethesda III
  - Repeat FNA regardless of EU-TIRADS class
  - EU-TIRADS 3 (>10 mm), with repeat Bethesda III: re-evaluate the nodule within 1 year, consider molecular testing if available or offer surgery
  - EU-TIRADS 4 and 5 (>10 mm), with repeat Bethesda III: offer surgery or active surveillance; molecular testing if available

(*Strength of recommendation: 2; quality of evidence: ØOOO. Agreement: 9/9 (100%); round: 2*)

- Bethesda IV
  - All nodules, regardless of EU-TIRADS class: offer surgery[g]; molecular testing if available

(*Strength of recommendation: 2; quality of evidence: ØØOO. Agreement: 8/9 (88.9%); round: 1*)

- Bethesda V and VI
  - All nodules, regardless of EU-TIRADS class: recommend surgery[h]
  - Active surveillance and MIT may be considered in patients with 5-10 mm nodules, in the absence of suspicious lymph nodes or risk of extra-thyroidal extension[i]
  - Multidisciplinary workup is warranted in case of advanced cancer

(*Strength of recommendation: 1; quality of evidence: ØØØO. Agreement: 8/9 (88.9%); round: 1*)

(Extracted from Table 1 of Durante et al. (2023).)

However, the ETA also states that "Molecular testing may be considered in cytologically indeterminate nodules, if available *(Strength of recommendation: 1; quality of evidence: ØØØO. Agreement: 9/9 (100%); round: 1).*"

Regarding the use of non-ultrasound imaging modalities, it is recommended that FNA be avoided in specific instances: "Thyroid scintigraphy should be performed when serum TSH is subnormal in order to diagnose functioning nodules and/or multinodularity, avoid FNA and determine eligibility for RAI as an alternative to surgery *(Strength of recommendation: 1; quality of evidence: ØØØO. Agreement: 9/9 (100%); round: 1)*" (Durante et al., 2023).

European Society for Medical Oncology (ESMO)

The ESMO remarks that preoperative FNA for cytology is "not required" for nodules 1 cm or smaller. ESMO states that FNA diagnosis "can be facilitated" by assessment of malignancy markers and molecular alterations. Specifically designed gene panels are "reportedly" useful for identifying malignancy with indeterminate samples (Filetti et al., 2019).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81210	BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer, melanoma), gene analysis, V600 variant(s)
81401	Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)
81445	Solid organ neoplasm, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants and copy number variants or rearrangements, if performed; DNA analysis or combined DNA and RNA analysis
81455	Solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes, genomic sequence analysis panel, interrogation for sequence variants and copy number variants or rearrangements, or isoform

	expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis
81479	Unlisted molecular pathology procedure
81546	Oncology (thyroid), mRNA, gene expression analysis of 10,196 genes, utilizing fine needle aspirate, algorithm reported as a categorical result (eg, benign or suspicious) Proprietary test: Afirma® Genomic SequencingClassifier Lab/Manufacturer: Veracyte, Inc
81599	Unlisted multianalyte assay with algorithmic analysis
0018U	Oncology (thyroid), microRNA profiling by RT-PCR of 10 microRNA sequences, utilizing fine needle aspirate, algorithm reported as a positive or negative result for moderate to high risk of malignancy Proprietary test: ThyraMIR Lab/Manufacturer: Interpace Diagnostics
0026U	Oncology (thyroid), DNA and mRNA of 112 genes, next-generation sequencing, fine needle aspirate of thyroid nodule, algorithmic analysis reported as a categorical result ("Positive, high probability of malignancy" or "Negative, low probability of malignancy") Proprietary test: Thyroseq Genomic Classifier Lab/Manufacturer: CBLPath, Inc/University of Pittsburgh Medical Center
0204U	Oncology (thyroid), mRNA, gene expression analysis of 593 genes (including BRAF, RAS, RET, PAX8, and NTRK) for sequence variants and rearrangements, utilizing fine needle aspirate, reported as detected or not detected Proprietary test: Afirma Xpression Atlas Lab/Manufacturer: Veracyte, Inc
0245U	Oncology (thyroid), mutation analysis of 10 genes and 37 RNA fusions and expression of 4 mRNA markers using next-generation sequencing, fine needle aspirate, report includes associated risk of malignancy expressed as a percentage Proprietary test: ThyGeNEXT® Thyroid Oncogene Panel Lab/Manufacturer: Interpace Diagnostics
0287U	Oncology (thyroid), DNA and mRNA, next-generation sequencing analysis of 112 genes, fine needle aspirate or formalin-fixed paraffin-embedded (FFPE) tissue, algorithmic prediction of cancer recurrence, reported as a categorical risk result (low, intermediate, high) Proprietary test: ThyroSeq® CRC Lab/Manufacturer: CBLPath, Inc/University of Pittsburgh Medical Center

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

AAP. (2018). Management Guidelines for Children With Thyroid Nodules and Differentiated Thyroid Cancer. *Pediatrics*, *142*(6). https://doi.org/10.1542/peds.2018-3063

Afirma. (2021a). Case Study: Genomic Analysis Identifies Medullary Thyroid Carcinoma. https://www.afirma.com/physicians/practice-tools/newsletter/case-study-genomic-analysis-identifies-medullary-thyroid-carcinoma/

Afirma. (2021b). Clinical Validation of the Afirma Genomic Sequencing Classifier for Medullary Thyroid Cancer. https://www.afirma.com/physicians/ata-2017/qa-6/

Afirma. (2022a). https://www.afirma.com/physicians/why-afirma/#a1

Afirma. (2022b). *NEW Afirma GSC + Xpression Atlas*. https://www.afirma.com/physicians/practice-resources/#a6

Ali, S. Z., Baloch, Z. W., Cochand-Priollet, B., Schmitt, F. C., Vielh, P., & VanderLaan, P. A. (2023). The 2023 Bethesda System for reporting thyroid cytopathology. *J Am Soc Cytopathol*, *12*(5), 319-325. https://doi.org/10.1016/j.jasc.2023.05.005

Angell, T. E., Wirth, L. J., Cabanillas, M. E., Shindo, M. L., Cibas, E. S., Babiarz, J. E., Hao, Y., Kim, S. Y., Walsh, P. S., Huang, J., Kloos, R. T., Kennedy, G. C., & Waguespack, S. G. (2019). Analytical and Clinical Validation of Expressed Variants and Fusions From the Whole Transcriptome of Thyroid FNA Samples [10.3389/fendo.2019.00612]. *Frontiers in Endocrinology*, *10*, 612. https://www.frontiersin.org/article/10.3389/fendo.2019.00612

ATA. (2021). 2021 American Thyroid Association Guidelines for Management of Patients with Anaplastic Thyroid Cancer. *Thyroid*, *31*(3), 337-386. https://doi.org/10.1089/thy.2020.0944

Banizs, A. B., & Silverman, J. F. (2019). The utility of combined mutation analysis and microRNA classification in reclassifying cancer risk of cytologically indeterminate thyroid nodules. *Diagn Cytopathol*, *47*(4), 268-274. https://doi.org/10.1002/dc.24087

Bible, K. C., Kebebew, E., Brierley, J., Brito, J. P., Cabanillas, M. E., Clark, T. J., Jr., Di Cristofano, A., Foote, R., Giordano, T., Kasperbauer, J., Newbold, K., Nikiforov, Y. E., Randolph, G., Rosenthal, M. S., Sawka, A. M., Shah, M., Shaha, A., Smallridge, R., & Wong-Clark, C. K. (2021). 2021 American Thyroid Association Guidelines for Management of Patients with Anaplastic Thyroid Cancer. *Thyroid*, *31*(3), 337-386. https://doi.org/10.1089/thy.2020.0944

Ciarletto, A. M., Narick, C., Malchoff, C. D., Massoll, N. A., Labourier, E., Haugh, K., Mireskandari, A., Finkelstein, S. D., & Kumar, G. (2021). Analytical and clinical validation of pairwise microRNA expression analysis to identify medullary thyroid

cancer in thyroid fine-needle aspiration samples. *Cancer Cytopathol*, *129*(3), 239-249. https://doi.org/10.1002/cncy.22365

Cibas, E. S., & Ali, S. Z. (2009). The Bethesda System for Reporting Thyroid Cytopathology. *American Journal of Clinical Pathology*, *132*(5), 658-665. https://doi.org/10.1309/AJCPPHLWMI3JV4LA

Douglas, R. (2023, 01/24/2023). *Evaluation and management of thyroid nodules with indeterminate cytology*. https://www.uptodate.com/contents/evaluation-and-management-of-thyroid-nodules-with-indeterminate-cytology

Durante, C., Hegedüs, L., Czarniecka, A., Paschke, R., Russ, G., Schmitt, F., Soares, P., Solymosi, T., & Papini, E. (2023). 2023 European Thyroid Association Clinical Practice Guidelines for thyroid nodule management. *Eur Thyroid J*, *12*(5). https://doi.org/10.1530/etj-23-0067

Endo, M., Nabhan, F., Porter, K., Roll, K., Shirley, L. A., Azaryan, I., Tonkovich, D., Perlick, J., Ryan, L. E., Khawaja, R., Meng, S., Phay, J. E., Ringel, M. D., & Sipos, J. A. (2019). Afirma Gene Sequencing Classifier Compared with Gene Expression Classifier in Indeterminate Thyroid Nodules. *Thyroid*, *29*(8), 1115-1124. https://doi.org/10.1089/thy.2018.0733

Filetti, S., Durante, C., Hartl, D., Leboulleux, S., Locati, L. D., Newbold, K., Papotti, M. G., Berruti, A., & Committee, t. E. G. (2019). Thyroid cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. *Annals of Oncology*, *30*(12), 1856-1883. https://doi.org/10.1093/annonc/mdz400 %J Annals of Oncology

Francis, G. L., Waguespack, S. G., Bauer, A. J., Angelos, P., Benvenga, S., Cerutti, J. M., Dinauer, C. A., Hamilton, J., Hay, I. D., Luster, M., Parisi, M. T., Rachmiel, M., Thompson, G. B., & Yamashita, S. (2015). Management Guidelines for Children with Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid*, *25*(7), 716-759. https://doi.org/10.1089/thy.2014.0460

Gharib, H., Papini, E., Garber, J. R., Duick, D. S., Harrell, R. M., Hegedus, L., Paschke, R., Valcavi, R., Vitti, P., & Nodules, A. A. A. T. F. o. T. (2016). American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi Medical Guidelines for Clinical Practice for the Diagnosis and Management of Thyroid Nodules--2016 Update. *Endocr Pract*, *22*(5), 622-639. https://doi.org/10.4158/EP161208.GL

Haugen, B. R., Alexander, E. K., Bible, K. C., Doherty, G. M., Mandel, S. J., Nikiforov, Y. E., Pacini, F., Randolph, G. W., Sawka, A. M., Schlumberger, M., Schuff, K. G., Sherman, S. I., Sosa, J. A., Steward, D. L., Tuttle, R. M., & Wartofsky, L. (2016). 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid*, *26*(1), 1-133. https://doi.org/10.1089/thy.2015.0020

Hodak, S. P., & Rosenthal, D. S. (2013). Information for clinicians: commercially available molecular diagnosis testing in the evaluation of thyroid nodule fine-needle aspiration specimens. *Thyroid*, *23*(2), 131-134. https://doi.org/10.1089/thy.2012.0320

Interpace. (2023). https://thygenext-thyramir.com/

Jackson, S., Kumar, G., Banizs, A. B., Toney, N., Silverman, J. F., Narick, C. M., & Finkelstein, S. D. (2020). Incremental utility of expanded mutation panel when used in combination with microRNA classification in indeterminate thyroid nodules. *Diagnostic Cytopathology*, *48*(1), 43-52. https://doi.org/10.1002/dc.24328

Jug, R. C., Datto, M. B., & Jiang, X. S. (2018). Molecular testing for indeterminate thyroid nodules: Performance of the Afirma gene expression classifier and ThyroSeq panel. *Cancer Cytopathol*, *126*(7), 471-480. https://doi.org/10.1002/cncy.21993

Krane, J. F., Cibas, E. S., Endo, M., Marqusee, E., Hu, M. I., Nasr, C. E., Waguespack, S. G., Wirth, L. J., & Kloos, R. T. (2020). The Afirma Xpression Atlas for thyroid nodules and thyroid cancer metastases: Insights to inform clinical decision-making from a fine-needle aspiration sample. *Cancer Cytopathology*, *128*(7), 452-459. https://doi.org/10.1002/cncy.22300

Labourier, E., Shifrin, A., Busseniers, A. E., Lupo, M. A., Manganelli, M. L., Andruss, B., Wylie, D., & Beaudenon-Huibregtse, S. (2015). Molecular Testing for miRNA, mRNA, and DNA on Fine-Needle Aspiration Improves the Preoperative Diagnosis of Thyroid Nodules With Indeterminate Cytology. *J Clin Endocrinol Metab*, *100*(7), 2743-2750. https://doi.org/10.1210/jc.2015-1158

Lithwick-Yanai, G., Dromi, N., Shtabsky, A., Morgenstern, S., Strenov, Y., Feinmesser, M., Kravtsov, V., Leon, M. E., Hajdúch, M., Ali, S. Z., VandenBussche, C. J., Zhang, X., Leider-Trejo, L., Zubkov, A., Vorobyov, S., Kushnir, M., Goren, Y., Tabak, S., Kadosh, E., . . . Meiri, E. (2017). Multicentre validation of a microRNA-based assay for diagnosing indeterminate thyroid nodules utilising fine needle aspirate smears. *J Clin Pathol*, *70*(6), 500-507. https://doi.org/10.1136/jclinpath-2016-204089

Lupo, M. A., Walts, A. E., Sistrunk, J. W., Giordano, T. J., Sadow, P. M., Massoll, N., Campbell, R., Jackson, S. A., Toney, N., Narick, C. M., Kumar, G., Mireskandari, A., Finkelstein, S. D., & Bose, S. (2020). Multiplatform molecular test performance in indeterminate thyroid nodules. *Diagnostic Cytopathology*, *48*(12), 1254-1264. https://doi.org/10.1002/dc.24564

NCCN. (2023, August 16, 2023). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines): Thyroid Carcinoma Version 4.2023 — August 16, 2023*. https://www.nccn.org/professionals/physician_gls/pdf/thyroid.pdf

NeoGenomics. (2023). NeoTYPE® Thyroid Profile. https://neogenomics.com/test-menu/neotype-thyroid-profile

NICE. (2022, December 19, 2022). *Thyroid cancer: assessment and management*. NICE. Retrieved 2024 from

https://www.nice.org.uk/guidance/ng230/chapter/Recommendations#assessment-and-diagnosis
Nikiforov, Y. E., Carty, S. E., Chiosea, S. I., Coyne, C., Duvvuri, U., Ferris, R. L., Gooding, W. E., Hodak, S. P., LeBeau, S. O., Ohori, N. P., Seethala, R. R., Tublin, M. E., Yip, L., & Nikiforova, M. N. (2014). Highly accurate diagnosis of cancer in thyroid nodules with follicular neoplasm/suspicious for a follicular neoplasm cytology by ThyroSeq v2 next-generation sequencing assay. *Cancer*, *120*(23), 3627-3634. https://doi.org/10.1002/cncr.29038
Nikiforov, Y. E., Ohori, N. P., Hodak, S. P., Carty, S. E., LeBeau, S. O., Ferris, R. L., Yip, L., Seethala, R. R., Tublin, M. E., Stang, M. T., Coyne, C., Johnson, J. T., Stewart, A. F., & Nikiforova, M. N. (2011). Impact of mutational testing on the diagnosis and management of patients with cytologically indeterminate thyroid nodules: a prospective analysis of 1056 FNA samples. *J Clin Endocrinol Metab*, *96*(11), 3390-3397. https://doi.org/10.1210/jc.2011-1469
Nikiforov, Y. E., Yip, L., & Nikiforova, M. N. (2013). New strategies in diagnosing cancer in thyroid nodules: impact of molecular markers. *Clin Cancer Res*, *19*(9), 2283-2288. https://doi.org/10.1158/1078-0432.ccr-12-1253
Nikiforova, M. N., Mercurio, S., Wald, A. I., Barbi de Moura, M., Callenberg, K., Santana-Santos, L., Gooding, W. E., Yip, L., Ferris, R. L., & Nikiforov, Y. E. (2018). Analytical performance of the ThyroSeq v3 genomic classifier for cancer diagnosis in thyroid nodules. *Cancer*, *124*(8), 1682-1690. https://doi.org/10.1002/cncr.31245
Paschke, R., Cantara, S., Crescenzi, A., Jarzab, B., Musholt, T. J., & Sobrinho Simoes, M. (2017). European Thyroid Association Guidelines regarding Thyroid Nodule Molecular Fine-Needle Aspiration Cytology Diagnostics. *Eur Thyroid J*, *6*(3), 115-129. https://doi.org/10.1159/000468519
Patel, K. N., Angell, T. E., Babiarz, J., & et al. (2018). Performance of a genomic sequencing classifier for the preoperative diagnosis of cytologically indeterminate thyroid nodules. *JAMA Surgery*, *153*(9), 817-824. https://doi.org/10.1001/jamasurg.2018.1153
Randolph, G., Angell, Trevor, Barbiarz, Joshua, Barth, Neil. (2017). ABSTRACT: Clinical Validation of the Afirma Genomic Sequencing Classifier for Medullary Thyroid Cancer. 87th Annual Meeting of the American Thyroid Association,
Ross, D. (2023, 06/07/2022). *Diagnostic approach to and treatment of thyroid nodules*. https://www.uptodate.com/contents/diagnostic-approach-to-and-treatment-of-thyroid-nodules
Steward, D. L., Carty, S. E., Sippel, R. S., & et al. (2018). Performance of a multigene genomic classifier in thyroid nodules with indeterminate cytology: A prospective blinded multicenter study. *JAMA Oncology*. https://doi.org/10.1001/jamaoncol.2018.4616
ThyroSeq. (2024). https://thyroseq.com/physicians/test-details/test-description

Vuong, H. G., Nguyen, T. P. X., Hassell, L. A., & Jung, C. K. (2021). Diagnostic performances of the Afirma Gene Sequencing Classifier in comparison with the Gene Expression Classifier: A meta-analysis. *Cancer Cytopathol*, *129*(3), 182-189. https://doi.org/10.1002/cncy.22332

Wei, S., Veloski, C., Sharda, P., & Ehya, H. (2019). Performance of the Afirma genomic sequencing classifier versus gene expression classifier: An institutional experience. *Cancer Cytopathology*, *127*(11), 720-724. https://doi.org/10.1002/cncy.22188

Xing, M., Haugen, B. R., & Schlumberger, M. (2013). Progress in molecular-based management of differentiated thyroid cancer. *Lancet*, *381*(9871), 1058-1069. https://doi.org/10.1016/s0140-6736(13)60109-9

X. Revision History

Revision Date	Summary of Changes

Molecular Profiling for Cancers of Unknown Primary Origin

Policy Number: AHS – M2065 – Molecular Profiling for Cancers of Unknown Primary Origin	Prior Policy Name and Number, as applicable: • AHS-M2065-Gene Expression Based Assays for Cancers of Unknown Primary
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Cancers of unknown primary origin (CUPs) are defined as the four to five percent of invasive cancers for which no primary site can be identified despite an extensive diagnostic work-up (Fizazi et al., 2015; Varadhachary & Raber, 2014). The American Cancer Society (ACS) estimates that about 34,950 cases of cancer of unknown primary will be diagnosed in 2024 in the United States (ACS, 2024). CUPs are generally considered to represent metastases and are associated with a very poor prognosis (Vikeså et al., 2015).

Gene expression assays measure the number of specific messenger RNAs (mRNAs) being transcribed to assess the genes that are active in a particular cell or tissue. Analyses of gene expression can be clinically useful for disease classification, diagnosis, prognosis, and tailoring treatment to underlying genetic determinants of pharmacologic response (Steiling, 2023).

For guidance concerning Tumor Mutational Burden Testing (TMB) and/or Microsatellite instability (MSI) analysis, please refer to AHS-M2178-Microsatellite Instability and Tumor Mutational Burden Testing policy.

II. Related Policies

Policy Number	Policy Title

AHS-M2178	Microsatellite Instability and Tumor Mutational Burden Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

1) To evaluate the site of origin of a tumor of unknown primary, to distinguish a primary from a metastatic tumor, or to guide site-specific therapy, molecular cancer classifiers **and/or** gene expression profiling assays **DO NOT MEET COVERAGE CRITERIA.**

IV. Table of Terminology

Term	Definition
ACS	American Cancer Society
ACUP	Adeno carcinomas of unknown primary site
ARID1A	*AT-rich interactive domain-containing protein 1A*
CDKN2A	*Cyclin-dependent kinase inhibitor 2a*
CUP	Cancers of unknown primary origin
ESMO	European Society of Medical Oncology
FFPE	Formalin-fixed paraffin-embedded
Gas	Genomic alterations
GEP	Gene expression profiling
ICI	Immune checkpoint inhibitor
IHC	Immunohistochemistry
KRAS	*Kirsten rat sarcoma viral oncogene homolog*
MAKP	*Mitogen-Activated Protein Kinase*
MDM2	*Mouse double minute 2 homolog*
MSI	Microsatellite instability
mRNA	Messenger ribonucleic acid
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NICE	National Institute for Health and Clinical Excellence
NSCLC	Non-small cell lung cancer

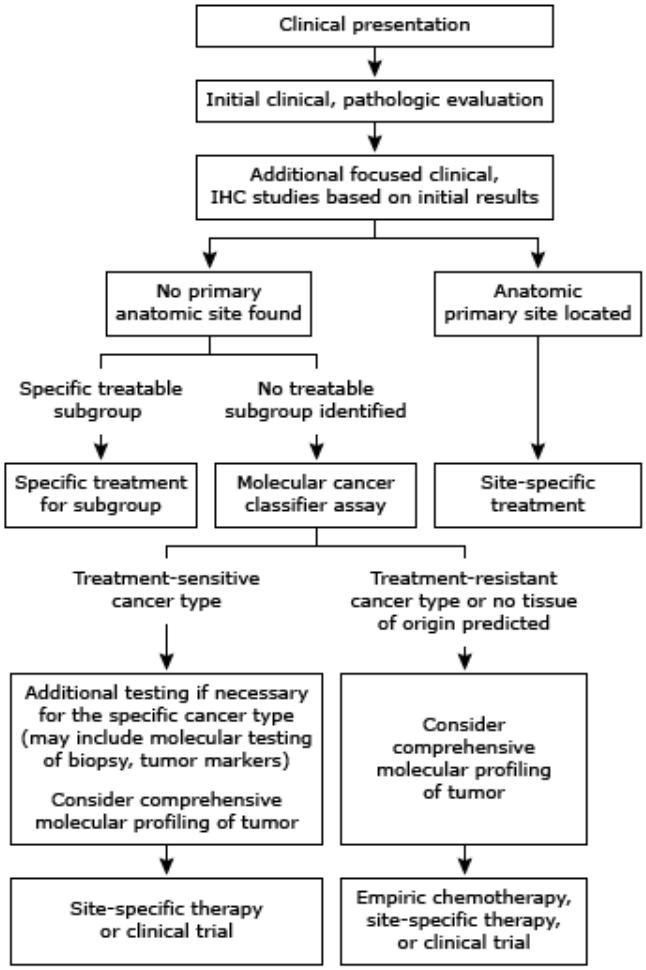
PD-L1	Programmed death-ligand 1
RTK	*Receptor tyrosine kinase*
RT-PCR	Reverse transcription polymerase chain reaction
SMAD4	*SMAD family member 4*
TML	Total mutational load
TNBC	Triple-negative breast cancer
TP53	*Tumor protein p53*

V. Scientific Background

Cancers of unknown primary origin (CUPs) typically present with symptoms attributable to metastases where subsequent work-up fails to identify the primary site (J. Hainsworth & F. Greco, 2023). Given their rapid progression and dissemination, it was assumed that regardless of the site of origin, the tumors in unknown primary cancers shared biologic properties common to their pathogenesis and that identification of the exact tissue of origin would not have a substantial effect on therapeutic approaches or survival. However, biologic events that allow development of metastases without a discernable tumor at the primary site have not yet been determined (Varadhachary & Raber, 2014).

Accurate prediction of the tissue of origin using immunohistochemical staining and/or gene expression profiling is now possible in certain CUP cases. Appropriate classification, based upon all available evidence, is essential to identify patients for whom a specific treatment may be particularly useful and site-specific therapy based on these predictions is replacing empiric chemotherapy as the new treatment standard (J. Hainsworth & F. Greco, 2023). Tumors in unknown primary cancer despite different degrees of loss of differentiation retain the signature of their primary origin, even after metastasis (Fizazi et al., 2015).

Presently, patients are initially placed into one of four categories (adenocarcinoma, squamous cell carcinoma, neuroendocrine carcinoma, poorly differentiated) based upon the light microscopic examination of the initial biopsy. This classification is then used to guide further evaluation as indicated below (J. D. Hainsworth & F. A. Greco, 2023):



Although the true tissue of origin may not be identifiable in any given case, recent evidence suggests it may be useful to provide "site-specific" treatment based on tumor type (J. Hainsworth & F. Greco, 2023). Certain characteristics of a tumor, such as its histology, may indicate more responsive cases and as such, may warrant specific treatments. For example, an individual with peritoneal carcinomatosis may benefit from therapies that are effective against advanced epithelial ovarian cancer, as the histology in both types may be similar. However, although several of these subgroups have been identified, most patients will not fall in these groups; approximately 70% of cancers of unknown primary are classified as adenocarcinomas, and 80-85% of these adenocarcinomas are not yet classified into these subgroups. In the absence of a targeted therapy, "empiric" chemotherapy with efficacy against a broad swath of cancer types is usually provided (J. Hainsworth & F. Greco, 2023; Hainsworth, 2024).

Proprietary Testing

Several proprietary tests exist for the assessment of the origin of cancer. One of these tests is "Tissue of Origin" from Cancer Genetics Inc. This test assesses the expression level of over 2000 genes and reports the likeliest tissue of origin from the most 15 common tumor types ("breast, non-small cell lung, pancreas, gastric, colorectal, liver, bladder, kidney, thyroid, non-Hodgkin lymphoma, melanoma, ovarian, sarcoma, testicular germ cell, and prostate"). An RNA "profile" is generated from the expression levels and compared to tissue profiles representative of the 15 tumors (Cancer Genetics Inc., 2018).

Tissue of Origin was validated by Pillai et al. (2011) where they created microarray data files for 462 "metastatic, poorly differentiated, or undifferentiated FFPE tumor specimens, all of which had a reference diagnosis" and analyzed these files with the Tissue of Origin model. Overall agreement with the reference diagnosis was 89%, and an average of 12 tissues could be ruled out with >99% probability (Pillai et al., 2011). Nystrom et al. (2012) examined the utility of this test by sending a survey to 65 physicians overseeing 107 patients. They found that, with the gene expression profile results, the diagnosis was changed for 50% of patients and management was changed for 65% of patients (Nystrom et al., 2012).

Hologic (2024) released CancerTYPE ID used to identify tumor origin in metastatic cancers. CancerTYPE ID is a gene expression assay that uses "real-time RT-PCR to measure the expression of 92-genes in the patient's tumor and classifies the tumor by matching the gene expression pattern to a database of over 2,000 known tumor types and subtypes. CancerTYPE ID can differentiate between 50 different tumor types and subtypes, covering >95% of all solid tumors based on incidence" (Hologic, 2024). CancerTYPE ID has been validated with 87% accuracy, 98% tumor type identified, and 37% improved survival.

Other than proprietary tests, The Jackson Laboratory developed CUP-AI-Dx, an RNA-based classifier, that uses RNA sequencing data from 817 genes to determine the metastatic cancer's primary tissue of origin and identify a tumor's molecular subtype. This machine was trained with the transcriptional profiles of 18,217 primary tumors and 32 cancer types. CUP-AI-Dx may be an important tool to help guide therapies for those who are limited to generalized treatment approaches (Zhao et al., 2020).

Analytical Validity

Kerr et al. (2012) conducted a large multi-institution validation study to examine the performance of a 92-gene molecular cancer classifier. The assay showed overall sensitivities of 87% for tumor type and 82% for subtype. No decrease in comparative

performance was observed when metastatic tumors, high-grade tumors or cases with limited tissue were analyzed. The authors concluded that the assay showed strong performance for accurate molecular classification for various tumor histologies. They further state that "results support potential use of the assay as a standardized molecular adjunct to routine clinicopathologic evaluation for tumor classification and primary site diagnosis" (Kerr et al., 2012).

Handorf et al. (2013) published the results of a prospectively conducted, blinded, multicenter study that compared the diagnostic accuracy of gene expression profiling (GEP) with IHC in identifying the primary site of metastatic tumors with known primaries. Overall, GEP accurately identified 89% of specimens, compared with 83% accuracy using IHC. In the subset of 33 poorly differentiated and undifferentiated carcinomas, GEP had higher accuracy (91%) compared to IHC (71%). The authors concluded that GEP "was significantly more accurate than IHC when used to identify the primary site of metastatic tumors" (Handorf et al., 2013).

In a similar study design, Handorf et al. (2013) compared the diagnostic accuracy of IHC analysis versus molecular classification using a 92-gene RT-PCR assay for determination of the primary tumor site. The authors reported 79% accuracy for gene expression profiling compared with 69% for immunohistochemistry. The authors concluded that the results "demonstrate superior accuracy with the 92-gene assay versus standard-of-care IHC analysis and strongly support the diagnostic utility of molecular classification in difficult-to-diagnose metastatic cancer" (Weiss et al., 2013).

Loffler et al. (2016) performed next-generation sequencing (of 50 genes) on 55 patients with adenocarcinoma or undifferentiated carcinoma. 46 cases harbored tumor-specific mutations and other alterations. *TP53, KRAS, CDKN2A* and *SMAD4* were the most mutated genes, and 8 cases were identified as having targetable mutations by currently approved drugs. The authors concluded that mutations of relevant driver genes were present in "vast majority" of CUP tumors and that these genes may carry impact on prognosis and targeted therapy" (Loffler et al., 2016).

Santos et al. (2017) aimed to develop and validate a gene-expression classifier to identify potential primary sites for metastatic cancers more accurately. "The gene-expression classifier correctly identified, by a cross-validation, 86.6% of the expected cancer superclasses of 4429 samples from the RefDB, with a specificity of 99.43%. Next, the performance of the algorithm for classifying the validation set of metastatic FFPE samples was 83.81%, with 99.04% specificity. The overall reproducibility of our gene-expression-classifier system was 97.22% of precision, with a coefficient of variation for inter-assays and intra-assays and intra-lots <4.1%" (Santos et al., 2017).

In a study by Zhao et al. (2020),the CUP-AI-DX was tested on 394 metastatic samples of unknown primary origin. The machine correctly identified the tissue of origin 96.7% of the time. The authors also compared the classification accuracy to the CancerTypeID GEP test. While the accuracy of CUP-AI-DX was 98.54% in cross-validation, while CancerType ID was 87% in cross-validation. The authors conclude that "The CUP-AI-Dx predicts tumour primary site and molecular subtype with high accuracy and therefore can be used to assist the diagnostic work-up of cancers of unknown primary origin" (Zhao et al., 2020).

Raghav et al. (2020) studied the use of CancerType ID GEP assay to identify cancers of unknown primary for immune checkpoint inhibitor (ICI) therapy. A total of 9,250 cases were studied, and the assay found that non-small cell lung cancer (NSCLC) accounted for 33% of the molecular diagnosis. The assay also frequently recognized urothelial carcinomas, gastric cancer, and head and neck squamous cell carcinoma, all of which could be treated with ICI therapy. This assay identified 40% of cases for which an FDA-approved ICI was available. Currently, ICIs are only indicated for CUP on rare occurrences; therefore, identifying ICI-eligible CUP patients with this assay is an important step towards improving treatments (Raghav et al., 2020).

Sun et al. (2022) studied the diagnostic utility of a 90-gene expression test for tumor classification. 1,417 samples were analyzed using the 90-gene expression test and the results were compared to histopathological diagnosis. Overall, the 90-gene expression test reached an accuracy of 94.4%. "Among different tumor types, sensitivities ranged from 74.2% (head & neck tumor) to 100% (adrenal carcinoma, mesothelioma, and prostate cancer). Sensitivities for the most prevalent cancers of lung, breast, colorectum, and gastroesophagus are 95.0%, 98.4%, 93.9%, and 90.6%, respectively. Moreover, specificities for all 21 tumor types are greater than 99%" (Sun et al., 2022). The authors conclude that this 90-gene expression test can be used as an adjunct for tumor classification in clinical practice.

Clinical Utility and Validity

Several studies have investigated the validity and diagnostic utility of gene expression profiling in addition to or in place of standard immunohistochemistry in the diagnosis and management of CUP.

Hainsworth et al. (2013) conducted a prospective trial testing the tumor biopsy specimens from previously untreated patients with CUP with a 92-gene reverse transcriptase polymerase chain reaction cancer classification assay. Molecular tumor profiling correctly identified tissue of origin in 85% of carcinomas of known primary origin. The study showed that molecular tumor profiling predicted a tissue of origin in 247 of 252 (98%) patients with CUP. The authors concluded that "molecular tumor

profiling contributes to the management of patients with CUP and should be a part of their standard evaluation" (Hainsworth et al., 2013).

Greco et al. (2013) demonstrated that 18 of 24 patients (75%) with latent primaries discovered months to years later were predicted by molecular tumor profiling. The authors concluded that molecular tumor profiling "complements standard pathologic evaluation in determining the tissue of origin in patients with CUP, particularly when IHC is inconclusive" (Greco et al., 2013).

Oien and Dennis (2012) concluded that "in already well worked-up poorly differentiated and/or metastatic tumours, including CUP, molecular profiling performs well, with sensitivities of 72%–95% and may outperform optimal IHC by 10%–20%" (Oien & Dennis, 2012). The authors conclude that molecular profiling could thus contribute to diagnosis of poorly differentiated and/or metastatic tumors.

Ross et al. (2015) conducted comprehensive genomic profiling on 200 CUP formalin-fixed paraffin-embedded specimens (mean, 756× coverage) using the hybrid-capture-based FoundationOne assay for presence of targetable genomic alterations (GAs) in CUP and responses to targeted therapies. They concluded that "Almost all CUP samples harbored at least 1 clinically relevant GA with potential to influence and personalize therapy. The ACUP [adenocarcinomas of unknown primary site] tumors were more frequently driven by GAs in the highly druggable RTK/Ras/mitogen-activated protein kinase (MAPK) signaling pathway than the non-ACUP tumors. Comprehensive genomic profiling can identify novel treatment paradigms to address the limited options and poor prognoses of patients with CUP" (Ross et al., 2015).

Groschel et al. (2016) investigated if their results from a difficult case could be extrapolated. The authors described an advanced-stage malignancy that mimicked a poorly differentiated soft-tissue sarcoma and did not respond to multiagent chemotherapy. Despite molecular profiling and histopathology analysis, the tissue of origin was not identified. However, the authors believed that immune checkpoint inhibitor therapy was warranted, and several differential diagnoses were theorized, including triple-negative breast cancer (TNBC). The authors assessed 157 TNBC cases from the Cancer Genome Atlas and found PD-L1 copy number gains (leading to excess PD-L1 mRNA expression) in 24% of cases. The authors concluded that their results "illustrate the impact of multidimensional tumor profiling in cases with nondescript histology and immunophenotype, show the predictive potential of PDL1 amplification for immune checkpoint inhibitors (ICIs), and suggest a targeted therapeutic strategy in Chromosome 9p24.1/PDL1-amplified cancers" (Groschel et al., 2016).

Zehir et al. (2017) attempted to characterize the mutational landscape of metastatic cancer. 10945 tumor samples from 10336 patients were included. Tumors were

sequenced with two panels, one of 341 genes, and another of 410 genes (with all 341 genes from the former panel included). Tumors were sequenced to an average of 718x coverage. Non-small cell lung cancer was the most common, with 1563 patients, followed by breast carcinoma at 1237 patients and colorectal cancer at 978 patients. Cancers of unknown primary comprised of a total of 160 patients. Overall, the authors identified 36.7% of patients as having actionable mutations (n = 3792). Gastrointestinal stromal tumors (76%), thyroid cancer (60%) and breast cancer (57%) were found to have the highest proportion of actionable mutations. However, the highest standard of actionable mutation met for cancers of unknown primary was "level 2B", or "standard of care biomarker for an FDA-approved drug in another indication". The authors concluded that their data "demonstrate [d] the feasibility and utility of large-scale prospective clinical sequencing of matched tumor-normal pairs to guide clinical management (Zehir et al., 2017).

Varghese et al. (2021) aimed to provide a "clinical and pathologic" description of patients with cancers of unknown primary. A total of 150 patients had targeted next-generation sequencing performed. There were 45 patients identified to have "potentially actionable" mutations, and 15 patients received targeted therapies. The authors remarked that CUP patients may benefit from targeted therapies (Varghese et al., 2021).

Gatalica et al. (2018) attempted to identify predictive biomarkers for immune checkpoint blockade therapy in cancers of unknown primary (CUP). A total of 389 cases were analyzed for 592 mutations and 52 gene fusions through next-generation sequencing. Microsatellite instability (MSI), total mutational load (TML), and PD-L1 expression were all evaluated. The authors identified "high" TML in 11.8% of tumors, high MSI in 7 tumors, and PD-L1 expression in 80 (of 362 tested cases) tumors. Other predictive biomarkers such as *MDM2* gene amplification were identified. *TP53* gene mutations were found in 54% of cases, followed by *KRAS* (22%) and *ARID1A* (13%). Overall, the authors identified 28% of CUP cases as carrying a predictive biomarker for immune checkpoint blockade therapy (Gatalica et al., 2018).

Clynick et al. (2018) attempted to identify actionable mutations in cancers of unknown primary. There were 21 cases included, and two gene panels were used to evaluate variants in 76 cancer-related genes. The authors found variants in 17 of 21 cases, with 11 considered "potentially actionable". The most common variants detected were *TP53* (47%), *KRAS* (12%), *MET* (12%) and *MYC* (12%). The authors also remarked that CUP adenocarcinomas and poorly differentiated carcinomas tended to harbor gene mutations involved in signal transduction pathways (eight of eleven cases harboring mutations such as *BRAF, HRAS,* and *KRAS*), whereas squamous cell carcinomas tended to harbor mutations in genes involved in cell cycle control and DNA repair genes (all

eight cases harboring mutations such as *TP53, MLH1*, and *CDKN2A*). Overall, the authors identified mutations in "biologically relevant" genes in the "vast majority" of CUP tumors, noting that half provided a "potentially novel treatment not generally considered in CUP" (Clynick et al., 2018).

Hayashi et al. (2019) compared two treatments for cancers of unknown primary site. Empirical chemotherapy was compared against site-directed therapy (directed by comprehensive microarray-based gene expression profiling). Efficacy analysis was performed for 50 patients in the site-specific arm and 51 patients in the empirical chemotherapy arm. One-year survival rate was found to be 44% for site-specific treatment and 54.9% for empirical treatment (p = .264). Median overall and progression-free survival was found to be 9.8 months and 5.1 months respectively for site-specific treatment and 12.5 months and 4.8 months respectively for empirical treatment (p=.896 and .550, respectively). Overall, the authors concluded that "Site-specific treatment that was based on microarray profiling did not result in a significant improvement in one-year survival compared with empirical PC [treatment]" (Hayashi et al., 2019).

Fizazi et al. (2019) evaluated the utility of "tailored treatment" for cancers of unknown primary. 243 patients with cancers of unknown primary were included and were randomized to Arm A ("Cisplatin 100 mg/m2 d1þ Gemcitabine 1250 mg/m2, day one and eight, q3w", empiric treatment) or Arm B ("gene expression test followed by a la carte treatment according to the suspected primary", tailored). The gene expression tests used were Pathwork's Tissue of Origin (n = 21) or CancerTYPE (n = 222), and the primary endpoint was defined as progression-free survival at a hazard ratio of 0.625. The four most common tissues of origin were "pancreatico-biliary cancer (19%), squamous cell carcinoma (eleven percent, kidney cancer (eight percent) and lung cancer (eight percent)". A total of 91 out of the 123 patients in Arm B were given tailored treatment. Progression-free survival in both arms were similar (both by central and local review), overall survival was similar in both arms (hazard ratio = 0.92). Overall, the authors concluded that "using a molecular test followed by tailored systemic treatment did not improve outcomes of pts [patients] with CUP" (Fizazi et al., 2019).

Cobain et al. (2021) studied which patients have the greatest clinical benefit from NGS profiling. NGS was performed in 1,015 patients and clinically actionable genomic alterations were found in 817 patients (80.5%). Of the 817 patients, 132 (16.2%) received sequencing-directed therapy, and 49 had clinical benefit (37.1%). "For 55 patients with carcinoma of unknown primary origin, NGS identified the primary site in 28 (50.9%), and sequencing-directed therapy in 13 patients resulted in clinical benefit in 7 instances (53.8%), including 5 exceptional responses" (Cobain et al., 2021). The

authors conclude directed germline testing and genomic profiling should be used as a standard of care for patients with cancer of unknown origin.

Saeed et al. (2022) studied the utility and impact of genomic profiling to determine tissue origin of CUPs. The study included tissue or cytology specimens from 22 CUPs, 15 of which were adequate for analysis. "Primary tumor site was suggested in 12 cases (80%), whereas it remained indeterminate in 3 (20%)." The doctors concluded that "genomic profiling helped confirm the original diagnosis and suggested primary sites in two third of our cases" (Saeed et al., 2022).

Posner et al. (2023) compared gene expression profiling (GEP) and DNA sequencing as tools for predicting primary tissue of origin in CUP. The study included 215 CUP patients, 82% of whom received both tests. Based on retrospective clinicopathological data, 77%, of cases had insufficient evidence to support a tissue of origin diagnosis. After DNA sequencing, "mutations and mutational signatures provided additional diagnostic evidence in 31% of cases." Alternatively, "GEP classification was useful in only 13% of cases and oncoviral detection in 4%." The authors conclude that DNA mutation profiling was "the more diagnostically informative assay" compared to GEP (Posner et al., 2023).

VI. Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

The NCCN lists two primary applications of molecular profiling in management of CUP; using gene expression profiling and molecular cancer classifier assays to determine tissue of origin for site-specific therapy, and identifying actionable mutations for targeted therapy (NCCN, 2024).

The 2023 NCCN guidelines for the workup of an occult primary malignancy address the use of molecular methods in the classification of tumors. The guidelines state "Gene sequencing to predict tissue of origin is not recommended." The guidelines also state that "molecular profiling of tumor tissue using NGS or other technique to identify gene fusions can be considered after an initial determination of histology has been made." Further, the guidelines note that "While there may be a diagnostic benefit to GEP, a clinical benefit has not been demonstrated." The guidelines further recommend that "until more robust outcomes and comparative effectiveness data are available, pathologists and oncologists must collaborate on the judicious use of IHC and GEP on a case-by-case basis, with the best possible individualized patient outcome in mind" (NCCN, 2024).

Overall, the NCCN states that "the clinical benefit of using molecular profiling to guide treatment decisions in CUP remains to be determined." The NCCN also states that "currently, there is no evidence of improved outcomes with the use of site-specific therapy guided by molecular testing results in patients with CUP" (NCCN, 2024).

National Institute for Health and Clinical Excellence (NICE)

A 2010 clinical guideline from NICE, which was reaffirmed in 2017, recommended against the use of gene expression-based profiling to identify primary tumors in patients with provisional CUPs. The guideline also states, "do not use gene-expression-based profiling when deciding which treatment to offer patients with confirmed CUP" (NICE, 2010).

In the April 2023 update of the Metastatic malignant disease of unknown primary origin in adults: diagnosis and management clinical guideline, NICE withdrew recommendations on gene-expression-based profiling (NICE, 2010).

European Society of Medical Oncology (ESMO)

In 2023, EMSO updated their clinical practice guidelines for the diagnosis, treatment, and follow-up of cancer of unknown primary. The guideline states that "The clinical utility of gene expression profiling to help elucidate the likely primary is not currently supported by high-level evidence. Consequently, it is not generally recommended outside of clinical research." Additionally, "there is currently no high-level evidence that gene expression profiling-directed therapy leads to an improvement in patient outcomes. Consequently, such strategies are not recommended outside of clinical trials" (Krämer et al., 2023).

National Cancer Institute (NCI)

The NCI acknowledges the possible utility of gene expression profiling and next generation sequencing to identify a potential site of origin in patients with CUP (NCI, 2023).

American Cancer Society (ACS)

The American Cancer Society acknowledges the possible use of gene expression profiling and molecule genetic testing for the diagnosis of cancers of unknown primary. However, they note that molecular genetic testing "is not needed in most cases, but it's sometimes helpful in classifying some cancers when other tests have not provided clues regarding their origin." Regarding gene expression profiling, the ACS

states that "these tests can sometimes help your doctor discover where the cancer may have started, but so far, they haven't been linked to better outcomes in patients" (ACS, 2018; Losa et al., 2018).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

No FDA-approved tests for the assessment of cancer from an unknown primary site were found. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81479	Unlisted molecular pathology procedure
81504	Oncology (tissue of origin), microarray gene expression profiling of > 2000 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as tissue similarity scores
81540	Oncology (tumor of unknown origin), mRNA, gene expression profiling by real-time RT-PCR of 92 genes (87 content and 5 housekeeping) to classify tumor into main cancer type and subtype, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a probability of a predicted main cancer type and subtype
81599	Unlisted multianalyte assay with algorithmic analysis

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

ACS. (2018). Tests for Cancer of Unknown Primary. https://www.cancer.org/cancer/cancer-unknown-primary/detection-diagnosis-staging/how-diagnosed.html

ACS. (2024, January 17, 2024). *Key Statistics for Cancers of Unknown Primary*. https://www.cancer.org/cancer/cancer-unknown-primary/about/key-statistics.html

Cancer Genetics Inc. (2018). Cancer Genetics' Unique Tissue Of Origin Test (TOO®) Receives Special FDA 510(K) Clearance. https://www.globenewswire.com/news-release/2018/04/16/1471915/0/en/Cancer-Genetics-Unique-Tissue-of-Origin-Test-TOO-Receives-Special-FDA-510-k-Clearance.html

Clynick, B., Dessauvagie, B., Sterrett, G., Harvey, N. T., Allcock, R. J. N., Saunders, C., Erber, W., & Meehan, K. (2018). Genetic characterisation of molecular targets in carcinoma of unknown primary. *J Transl Med*, *16*(1), 185. https://doi.org/10.1186/s12967-018-1564-x

Cobain, E. F., Wu, Y.-M., Vats, P., Chugh, R., Worden, F., Smith, D. C., Schuetze, S. M., Zalupski, M. M., Sahai, V., Alva, A., Schott, A. F., Caram, M. E. V., Hayes, D. F., Stoffel, E. M., Jacobs, M. F., Kumar-Sinha, C., Cao, X., Wang, R., Lucas, D., . . . Chinnaiyan, A. M. (2021). Assessment of Clinical Benefit of Integrative Genomic Profiling in Advanced Solid Tumors. *JAMA Oncol*, *7*(4), 525-533. https://doi.org/10.1001/jamaoncol.2020.7987

Fizazi, K., Greco, F. A., Pavlidis, N., Daugaard, G., Oien, K., & Pentheroudakis, G. (2015). Cancers of unknown primary site: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, *26 Suppl 5*, v133-138. https://doi.org/10.1093/annonc/mdv305

Fizazi, K., Maillard, A., Penel, N., Baciarello, G., Allouache, D., Daugaard, G., Van de Wouw, A., Soler, G., Vauleon, E., Chaigneau, L., Janssen, R., Losa Gaspa, F., Morales Barrera, R., Balana, C., Tosi, D., Chauffert, B., Schnabel, C. A., Martineau, G., Culine, S., & Borget, I. (2019). LBA15_PR - A phase III trial of empiric chemotherapy with cisplatin and gemcitabine or systemic treatment tailored by molecular gene expression analysis in patients with carcinomas of an unknown primary (CUP) site (GEFCAPI 04). *Annals of Oncology*, *30*, v851. https://doi.org/10.1093/annonc/mdz394

Gatalica, Z., Xiu, J., Swensen, J., & Vranic, S. (2018). Comprehensive analysis of cancers of unknown primary for the biomarkers of response to immune checkpoint blockade therapy. *Eur J Cancer*, *94*, 179-186. https://doi.org/10.1016/j.ejca.2018.02.021

Greco, F. A., Lennington, W. J., Spigel, D. R., & Hainsworth, J. D. (2013). Molecular profiling diagnosis in unknown primary cancer: accuracy and ability to complement

standard pathology. *J Natl Cancer Inst*, *105*(11), 782-790. https://doi.org/10.1093/jnci/djt099

Groschel, S., Bommer, M., Hutter, B., Budczies, J., Bonekamp, D., Heining, C., Horak, P., Frohlich, M., Uhrig, S., Hubschmann, D., Georg, C., Richter, D., Pfarr, N., Pfutze, K., Wolf, S., Schirmacher, P., Jager, D., von Kalle, C., Brors, B., . . . Frohling, S. (2016). Integration of genomics and histology revises diagnosis and enables effective therapy of refractory cancer of unknown primary with PDL1 amplification. *Cold Spring Harb Mol Case Stud*, *2*(6), a001180. https://doi.org/10.1101/mcs.a001180

Hainsworth, J., & Greco, F. (2023, 12/15/2023). *Overview of the classification and management of cancers of unknown primary site*. https://www.uptodate.com/contents/overview-of-the-classification-and-management-of-cancers-of-unknown-primary-site

Hainsworth, J. D., & Greco, F. A. (2023, 12/15/2023). *Treatment of patients with cancer of unknown primary site*. https://www.uptodate.com/contents/overview-of-the-classification-and-management-of-cancers-of-unknown-primary-site#topicGraphics

Hainsworth, J. D., Greco, Anthony. (2024, 01/31/2024). *Adenocarcinoma of unknown primary site*. https://www.uptodate.com/contents/adenocarcinoma-of-unknown-primary-site

Hainsworth, J. D., Rubin, M. S., Spigel, D. R., Boccia, R. V., Raby, S., Quinn, R., & Greco, F. A. (2013). Molecular gene expression profiling to predict the tissue of origin and direct site-specific therapy in patients with carcinoma of unknown primary site: a prospective trial of the Sarah Cannon research institute. *J Clin Oncol*, *31*(2), 217-223. https://doi.org/10.1200/jco.2012.43.3755

Handorf, C. R., Kulkarni, A., Grenert, J. P., Weiss, L. M., Rogers, W. M., Kim, O. S., Monzon, F. A., Halks-Miller, M., Anderson, G. G., Walker, M. G., Pillai, R., & Henner, W. D. (2013). A multicenter study directly comparing the diagnostic accuracy of gene expression profiling and immunohistochemistry for primary site identification in metastatic tumors. *Am J Surg Pathol*, *37*(7), 1067-1075. https://doi.org/10.1097/PAS.0b013e31828309c4

Hayashi, H., Kurata, T., Takiguchi, Y., Arai, M., Takeda, K., Akiyoshi, K., Matsumoto, K., Onoe, T., Mukai, H., Matsubara, N., Minami, H., Toyoda, M., Onozawa, Y., Ono, A., Fujita, Y., Sakai, K., Koh, Y., Takeuchi, A., Ohashi, Y., . . . Nakagawa, K. (2019). Randomized Phase II Trial Comparing Site-Specific Treatment Based on Gene Expression Profiling With Carboplatin and Paclitaxel for Patients With Cancer of Unknown Primary Site. *J Clin Oncol*, *37*(7), 570-579. https://doi.org/10.1200/jco.18.00771

Hologic. (2024). CancerTYPE ID. https://www.hologic.com/hologic-products/diagnostic-solutions/cancertype-id

Kerr, S. E., Schnabel, C. A., Sullivan, P. S., Zhang, Y., Singh, V., Carey, B., Erlander, M. G., Highsmith, W. E., Dry, S. M., & Brachtel, E. F. (2012). Multisite validation study to

determine performance characteristics of a 92-gene molecular cancer classifier. *Clin Cancer Res*, *18*(14), 3952-3960. https://doi.org/10.1158/1078-0432.ccr-12-0920

Krämer, A., Bochtler, T., Pauli, C., Baciarello, G., Delorme, S., Hemminki, K., Mileshkin, L., Moch, H., Oien, K., Olivier, T., Patrikidou, A., Wasan, H., Zarkavelis, G., Pentheroudakis, G., & Fizazi, K. (2023). Cancer of unknown primary: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol*, *34*(3), 228-246. https://doi.org/10.1016/j.annonc.2022.11.013

Loffler, H., Pfarr, N., Kriegsmann, M., Endris, V., Hielscher, T., Lohneis, P., Folprecht, G., Stenzinger, A., Dietel, M., Weichert, W., & Kramer, A. (2016). Molecular driver alterations and their clinical relevance in cancer of unknown primary site. *Oncotarget*, *7*(28), 44322-44329. https://doi.org/10.18632/oncotarget.10035

Losa, F., Soler, G., Casado, A., Estival, A., Fernández, I., Giménez, S., Longo, F., Pazo-Cid, R., Salgado, J., & Seguí, M. (2018). SEOM clinical guideline on unknown primary cancer (2017). *Clin Transl Oncol*, *20*(1), 89-96. https://doi.org/10.1007/s12094-017-1807-y

NCCN. (2024). *Occult Primary (Cancer of Unknown Primary [CUP]) Version 1.2024*. National Comprehensive Cancer Network. https://www.nccn.org/professionals/physician_gls/pdf/occult.pdf

NCI. (2023, December 22, 2023). *Carcinoma of Unknown Primary Treatment (PDQ®)–Health Professional Version*. https://www.cancer.gov/types/unknown-primary/hp/unknown-primary-treatment-pdq#_10

NICE. (2010, 04/26/2023). *Metastatic malignant disease of unknown primary origin in adults: diagnosis and management | Guidance and guidelines | NICE*. NICE. https://www.nice.org.uk/guidance/cg104

Nystrom, S. J., Hornberger, J. C., Varadhachary, G. R., Hornberger, R. J., Gutierrez, H. R., Henner, D. W., Becker, S. H., Amin, M. B., & Walker, M. G. (2012). Clinical utility of gene-expression profiling for tumor-site origin in patients with metastatic or poorly differentiated cancer: impact on diagnosis, treatment, and survival. *Oncotarget*, *3*(6), 620-628. https://doi.org/10.18632/oncotarget.521

Oien, K. A., & Dennis, J. L. (2012). Diagnostic work-up of carcinoma of unknown primary: from immunohistochemistry to molecular profiling. In *Ann Oncol* (Vol. 23 Suppl 10, pp. x271-277). https://doi.org/10.1093/annonc/mds357

Pillai, R., Deeter, R., Rigl, C. T., Nystrom, J. S., Miller, M. H., Buturovic, L., & Henner, W. D. (2011). Validation and reproducibility of a microarray-based gene expression test for tumor identification in formalin-fixed, paraffin-embedded specimens. *J Mol Diagn*, *13*(1), 48-56. https://doi.org/10.1016/j.jmoldx.2010.11.001

Posner, A., Prall, O. W., Sivakumaran, T., Etemadamoghadam, D., Thio, N., Pattison, A., Balachander, S., Fisher, K., Webb, S., Wood, C., DeFazio, A., Wilcken, N., Gao, B., Karapetis, C. S., Singh, M., Collins, I. M., Richardson, G., Steer, C., Warren, M., . . . Tothill, R. W. (2023). A comparison of DNA sequencing and gene expression profiling

to assist tissue of origin diagnosis in cancer of unknown primary. *J Pathol*, *259*(1), 81-92. https://doi.org/10.1002/path.6022

Raghav, K., Overman, M., Poage, G. M., Soifer, H. S., Schnabel, C. A., & Varadhachary, G. R. (2020). Defining a Distinct Immunotherapy Eligible Subset of Patients with Cancer of Unknown Primary Using Gene Expression Profiling with the 92-Gene Assay. *Oncologist*, *25*(11), e1807-e1811. https://doi.org/10.1634/theoncologist.2020-0234

Ross, J. S., Wang, K., Gay, L., Otto, G. A., White, E., Iwanik, K., Palmer, G., Yelensky, R., Lipson, D. M., Chmielecki, J., Erlich, R. L., Rankin, A. N., Ali, S. M., Elvin, J. A., Morosini, D., Miller, V. A., & Stephens, P. J. (2015). Comprehensive Genomic Profiling of Carcinoma of Unknown Primary Site: New Routes to Targeted Therapies. *JAMA Oncol*, *1*(1), 40-49. https://doi.org/10.1001/jamaoncol.2014.216

Saeed, O. A. M., Armutlu, A., Cheng, L., Longe, H. O., & Saxena, R. (2022). Tumor Genomic Profiling to Determine Tissue Origin of Cancers of Unknown Primary: A Single Institute Experience With its Utility and Impact on Patient Management. *Appl Immunohistochem Mol Morphol*, *30*(9), 592-599. https://doi.org/10.1097/pai.0000000000001057

Santos, M. T. D., Souza, B. F., Carcano, F. M., Vidal, R. O., Scapulatempo-Neto, C., Viana, C. R., & Carvalho, A. L. (2017). An integrated tool for determining the primary origin site of metastatic tumours. *J Clin Pathol*. https://doi.org/10.1136/jclinpath-2017-204887

Steiling, K., Christenson, Stephanie. (2023, 10/03/2023). *Tools for genetics and genomics: Gene expression profiling*. https://www.uptodate.com/contents/tools-for-genetics-and-genomics-gene-expression-profiling

Sun, W., Wu, W., Wang, Q., Yao, Q., Feng, Q., Wang, Y., Sun, Y., Liu, Y., Lai, Q., Zhang, G., Qi, P., Sun, Y., Qian, C., Ren, W., Luo, Z., Chen, J., Wang, H., Xu, Q., Zhou, X., . . . Lin, D. (2022). Clinical validation of a 90-gene expression test for tumor tissue of origin diagnosis: a large-scale multicenter study of 1417 patients. *Journal of Translational Medicine*, *20*(1), 114. https://doi.org/10.1186/s12967-022-03318-6

Varadhachary, G. R., & Raber, M. N. (2014). Cancer of unknown primary site. *N Engl J Med*, *371*(8), 757-765. https://doi.org/10.1056/NEJMra1303917

Varghese, A. M., Arora, A., Capanu, M., Camacho, N., Won, H. H., Zehir, A., Gao, J., Chakravarty, D., Schultz, N., Klimstra, D. S., Ladanyi, M., Hyman, D. M., Solit, D. B., Berger, M. F., & Saltz, L. B. (2021). Clinical and molecular characterization of patients with cancer of unknown primary in the modern era. *Ann Oncol*, *28*(12), 3015-3021. https://doi.org/10.1093/annonc/mdx545

Vikeså, J., Møller, A. K. H., Kaczkowski, B., Borup, R., Winther, O., Henao, R., Krogh, A., Perell, K., Jensen, F., Daugaard, G., & Nielsen, F. C. (2015). Cancers of unknown primary origin (CUP) are characterized by chromosomal instability (CIN) compared to metastasis of know origin. *BMC Cancer*, *15*. https://doi.org/10.1186/s12885-015-1128-x

Weiss, L. M., Chu, P., Schroeder, B. E., Singh, V., Zhang, Y., Erlander, M. G., & Schnabel, C. A. (2013). Blinded comparator study of immunohistochemical analysis versus a 92-gene cancer classifier in the diagnosis of the primary site in metastatic tumors. *J Mol Diagn*, *15*(2), 263-269. https://doi.org/10.1016/j.jmoldx.2012.10.001

Zehir, A., Benayed, R., Shah, R. H., Syed, A., Middha, S., Kim, H. R., Srinivasan, P., Gao, J., Chakravarty, D., Devlin, S. M., Hellmann, M. D., Barron, D. A., Schram, A. M., Hameed, M., Dogan, S., Ross, D. S., Hechtman, J. F., DeLair, D. F., Yao, J., . . . Berger, M. F. (2017). Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med*, *23*(6), 703-713. https://doi.org/10.1038/nm.4333

Zhao, Y., Pan, Z., Namburi, S., Pattison, A., Posner, A., Balachander, S., Paisie, C. A., Reddi, H. V., Rueter, J., Gill, A. J., Fox, S., Raghav, K. P. S., Flynn, W. F., Tothill, R. W., Li, S., Karuturi, R. K. M., & George, J. (2020). CUP-AI-Dx: A tool for inferring cancer tissue of origin and molecular subtype using RNA gene-expression data and artificial intelligence. *EBioMedicine*, *61*. https://doi.org/10.1016/j.ebiom.2020.103030

Molecular Testing for Cutaneous Melanoma

Policy Number: AHS – M2029 – Molecular Testing for Cutaneous Melanoma	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Cutaneous melanoma is a common and serious form of skin cancer. Diagnosis of this type of cancer often involves visual examination of the skin by a dermatologist; however, due to the various presentations of nodules, it can be difficult to properly recognize a melanoma case. Although several visual systems have been developed to assist in diagnosis (such as the ABCDE system for signature features), a biopsy is often performed to diagnose a case (Swetter, 2024).

Genetic testing (particularly gene expression panels) has been explored by researchers to assist in diagnosing cases without a biopsy (Bollard et al., 2021). Another application of genetic testing is "targeted testing"; certain genetic mutations show better patient response with targeted treatment, and it is therefore clinically useful to identify these mutations to help guide selection of the most appropriate therapy.

II. Related Policies

Policy Number	Policy Title
AHS-M2026	Molecular Testing for Cutaneous Melanoma
AHS-M2030	Testing for Targeted Therapy of Non-Small-Cell Lung Cancer

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals who have received genetic counseling and who have a diagnosis of cutaneous melanoma, multigene panel testing for cutaneous melanoma-associated likely pathogenic or pathogenic variants (see Note 1, Note 2) **MEETS COVERAGE CRITERIA** when **one** of the following conditions is met:
   a) When the individual has three or more invasive cutaneous melanomas.
   b) When the individual has invasive cutaneous melanoma and **one** of the following:
      i) A personal history of astrocytoma.
      ii) A personal or family history (first-degree relative; see Note 3) with pancreatic cancer.

2) For individuals without a diagnosis of melanoma who have received genetic counseling and who are in a family with a known deleterious familial likely pathogenic or pathogenic variant in a cutaneous melanoma-associated gene, the following genetic testing for inherited forms of melanoma **MEETS COVERAGE CRITERIA**:
   a) Testing restricted to the known familial likely pathogenic or pathogenic variant.
   b) Comprehensive genetic testing, including muti-gene panel testing (see Note 1, Note 2), when the specific familial likely pathogenic or pathogenic variant is unknown.

3) For individuals without a diagnosis of melanoma who have received genetic counseling, multi-gene panel testing for cutaneous melanoma-associated likely pathogenic or pathogenic variants (see Note 1, Note 2) **MEETS COVERAGE CRITERIA** only if a family likely pathogenic or pathogenic variant is unknown **and** the individual has a family history of **one** of the following:
   a) Three or more invasive cutaneous melanomas.
   b) Renal cancer.
   c) Astrocytoma.
   d) Uveal melanoma.
   e) Mesothelioma.

4) For individuals who are being considered for molecular-targeted therapy and who have stage III or stage IV cutaneous melanoma, testing of tumor tissue for *BRAF, KIT*, and *NRAS* gene mutations (single gene or multi-gene panel testing) **MEETS COVERAGE CRITERIA**.

5) For individuals with a suspicious primary melanocytic skin lesion, risk assessment of the lesion using the DermTech Pigmented Lesion Assay (PLA) (see Note 4) **MEETS COVERAGE CRITERIA** to help inform a biopsy decision when **all** of the following conditions are met:
   a) When lesion size is 5-19 mm;
   b) When the lesion meets one or more ABCDE criteria (Asymmetry, Border, Color, Diameter, Evolving);
   c) When the lesion is located where the skin is intact (i.e., non-ulcerated or non-bleeding lesions) and free of psoriasis, eczema, or similar skin conditions;
   d) When the lesion does not contain a scar;
   e) When the lesion has not been previously biopsied;
   f) When the lesion is not already clinically diagnosed as benign or melanoma;
   g) When the lesion is **NOT** located on the palms of hands, soles of feet, nails, mucous membranes, or hair covered areas that cannot be trimmed.

6) For individuals with a primary cutaneous melanocytic lesion that has not been re-excised, gene expression profiling (GEP) of the lesion with the myPath® Melanoma Assay **MEETS COVERAGE CRITERIA** when **all** of the following conditions are met:
   a) When the lesion has not already been definitively diagnosed as benign or malignant;
   b) When diagnostic GEP has not already been performed on the same lesion.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

7) For all other situations not described above, genetic testing for inherited forms of cutaneous melanoma **DOES NOT MEET COVERAGE CRITERIA**.

8) For all other situations not described above, genetic expression profiling tests for cutaneous melanoma **DO NOT MEET COVERAGE CRITERIA**.

9) For all situations not described above (e.g., individuals not being considered for targeted therapy, other forms or stages of melanoma), testing for *BRAF, KIT, NRAS*, and other mutations **DOES NOT MEET COVERAGE CRITERIA**.

10) Prognostic biomarker testing of a tumor biopsy to assess the risk of melanoma progression (e.g., AMBLor®) **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: When germline multi-gene panel testing is performed, the panel should at minimum include the following melanoma susceptibility genes: *BAP1, CDK4, CDKN2A, MC1R, MITF, PTEN,* and *TERT*.

Note 2: For two or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

Note 3: First-degree relatives include parents, full siblings, and children of the individual.

Note 4: While this is not considered a surgical procedure, it is recommended that individuals who are receiving this test should first undergo an informed consent process to discuss the benefits and risks of pursuing this test versus receiving a biopsy to rule out melanoma.

IV. Table of Terminology

Term	Definition
AAD	The American Academy of Dermatology
ABCDE	Asymmetry, border, color, diameter and evolving
ACD	Adrenocortical dysplasia protein homolog
ACMG	American College of Medical Genetics and Genomics
AMBRA1	Autophagy and beclin 1 regulator 1
AMLo	Loricrin
anti-CTLA-4	Anti-cytotoxic T-lymphocyte-associated protein 4
ARAF	Serine/threonine-protein kinase A-Raf
ASCO	American Society of Clinical Oncology
ATM	*Ataxia-telangiectasia mutated*
BAP1	*Breast cancer susceptibility gene-associated protein-1*
BRAF	*B-RAF Proto-Oncogene, serine/threonine kinase*
BRCA1	*Breast cancer susceptibility gene*
BRCA2	*Breast cancer susceptibility gene 2*
CAP	College of American Pathologists
CDK4	*Cyclin-dependent kinase 4*
CDKN2A	*Cyclin-dependent kinase inhibitor 2a*
CDx	Companion diagnostic

CGH	Comparative genomic hybridization
CHEK2	*Checkpoint kinase 2*
C-KIT	*Proto-oncogene c-KIT*
CLIA'88	Clinical Laboratory Improvement Amendments of 1988
CLND	Completion lymph node dissection
CM	Cutaneous melanoma
CMS	Centers for Medicare and Medicaid
COVID-19	Coronavirus disease 2019
CP-GEP	Clinicopathological and Gene Expression Profile
CRAF	RAF proto-oncogene serine/threonine-protein kinase
ddPCR	Droplet digital polymerase chain reaction
DFS	Disease-free survival
DMFS	Distant metastasis-free survival
DNA	Deoxyribonucleic acid
E	Glutamate
EADO	European Association of DermatoOncology
EDF	European Dermatology Forum
EORTC	European Organization of Research and Treatment of Cancer
ESMO	European Society for Medical Oncology
FAMMM	Familial atypical multiple mole-melanoma
FDA	Food and Drug Administration
FFPE	Formalin-fixed, paraffin-embedded
FISH	Fluorescence in situ hybridization
GEP	Gene expression profile
GIST	Gastrointestinal stromal tumor
HR	Hazard ratio
KIT	*KIT proto-oncogene, receptor tyrosine kinase*
LDTs	Laboratory developed tests
LINC00518	*Long intergenic non-protein coding ribonucleic acid 518*
MAS	Melanoma-astrocytoma syndrome
MC1R	*Melanocortin-1 receptor*
MDM2	*Mouse double minute 2*
MEK	Mitogen-activated protein kinase/extracellular signal-regulated kinase-1 kinase
MITF	*Microphthalmia-associated transcription factor*
MPWG	Melanoma Prevention Working Group
NBN	*Nibrin*
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute

NED	Without evidence of disease
NER	Nucleotide excision repair
NGS	Next-generation sequencing
NP	Nurse practitioners
NPV	Negative predictive value
NRAS	*Neuroblastoma RAS viral [v-ras] oncogene homolog*
NSGC	National Society of Genetic Counselors
OS	Overall survival
p14ARF	Adenosine pyrophosphate -ribosylation factor tumor suppressor
p16	Cyclin-dependent kinase inhibitor 2A
p53	Tumor protein P53
PA	Physician assistants
PCR	Polymerase chain reaction
PFS	Progression-free survival
PIK3CA	*Phosphoinositide 3-kinases catalytic subunit alpha*
PLA	Pigmented lesion assay
PLA(−)	Pigmented legion assay negative
POT1	*Protection of telomeres 1*
PRAME	*Preferentially expressed antigen in melanoma*
PTEN	*Phosphatase and tensin homolog*
qPCR	Quantitative polymerase chain reaction
RB1	*Retinoblastoma protein*
RCM	Reflectance confocal microscopy
RFS	Recurrence-free survival
RNA	Ribonucleic acid
ROC	Receiver operating characteristic
RTK	Receptor tyrosine kinase
RT-PCR	Real-time polymerase chain reaction
SIC	Satisfactory disease free
SIGN	Scottish Intercollegiate Guidelines
SLN	Sentinel lymph node
SLNB	Sentinel lymph node biopsy
SLNBx	Sentinel lymph node biopsy
SSE	Skin self-examination
SSO	Society of Surgical Oncology
TERT	*Telomerase reverse transcriptase*
TMB	Tumor mutation burden
TN	True-negative
TP	True-positive

UV	Ultraviolet
WRN	Werner's syndrome protein
WT	Wild type
XP	*Xeroderma pigmentosum*
XPC	*Xeroderma pigmentosum group c*
XPD	*Xeroderma pigmentosum group d*

V. Scientific Background

Skin cancer is the most common form of cancer, arising from the metaplastic transformation from any of the cell types of the skin (Linares et al., 2015). Melanomas, which develop from the pigment-producing melanocytes, although much less prevalent than non-melanoma skin cancer, are increasing in incidence (Holmes, 2014; Lee & Lian, 2018). Early and accurate diagnosis is essential, as late-stage melanoma is among the most fatal forms of skin cancer (Cockerell et al., 2017). This, however, presents a significant challenge due to the difficulty of interpreting the histopathology of melanoma and the resulting interobserver and intra-observer variability (Elmore et al., 2017; Gerami et al., 2014).

Genetic Testing for Familial Cutaneous Melanoma

A family history of melanoma is reported by about 10% of melanoma patients (Soura et al., 2016b), and inherited germline mutations reportedly "increase melanoma risk from 4- to >1000-fold" (Ransohoff et al., 2016). Determining the genetic origin, however, is complicated, as a portion of familial melanoma can be attributed to shared sun exposure experiences in family members with susceptible skin types (Goldstein & Tucker, 2001).

The majority of familial cases lack identifiable germ-line mutations in either known susceptibility genes or in genes commonly mutated in sporadic melanoma (Hawkes et al., 2016). Uncommon, but high-risk, alleles have been found to contribute to the hereditary cancer phenotype that includes unilateral lineage, multi-generational, multiple primary lesions, and early onset of disease (Soura et al., 2016b). Additional research has identified a relationship between telomere length and familial melanoma; patients with familial melanoma had longer telomeres compared to patients with sporadic melanoma (Menin et al., 2016). As such, genes such as *TERT*—which encodes for the catalytic subunit of telomerase—and proteins such as POT1—a shelterin complex protein—have been implicated in the presence of multiple primary melanomas and early-onset melanoma in a subset of high-density families (Rossi et al., 2019).

Cyclin-dependent kinase inhibitor 2A (*CDKN2A*) and cyclin-dependent kinase 4 (*CDK4*) are the most identified gene mutations in familial forms of melanoma, which can be defined as a family where either two first-degree relatives or three or more melanoma patients on the same side of the family are diagnosed with melanoma. Germline *CDKN2A* mutations have been identified in approximately 20-40% of familial melanoma cases where three or more family members are affected (Harland et al., 2016; Rossi et al., 2019). *CDKN2A* encodes several proteins involved in cell cycle regulation, including p16, which inhibits *CDK4* (Hussussian et al., 1994; Koh et al., 1995), and p14ARF, which inhibits *MDM2* from regulating p53 (Zhang et al., 1998). Germline *CDKN2A* mutations in melanoma families are usually missense or nonsense changes that impair the function of the p16 protein, allowing for unchecked cell cycle progression; however, rare mutations in the p14ARF protein have also been reported and result in proteasomal degradation of p53 with subsequent accumulation of DNA damage (Marzuka-Alcala et al., 2014). Overall survival is worse in those with a germline *CDKN2A* mutation than those with sporadic melanoma or familial melanoma with wild-type *CDKN2A* genes. Germline mutations also predisposed individuals to an increased number of malignancies, such as pancreatic and lung cancer (Tsao & McCormick, 2024).

Mutations in *CDKN2A*/p16 are associated with familial atypical multiple mole-melanoma (FAMMM syndrome), which is characterized by numerous nevi (some atypical), a family history of melanoma, and an increased risk of pancreatic cancer (Goldstein et al., 2007; Lynch & Krush, 1968). Carriers of a FAMMM mutation typically present with cancer at a younger age than non-carriers (Middlebrooks et al., 2019). Mutations in p14ARF are linked to Melanoma-Astrocytoma Syndrome (MAS), a variant of FAMMM characterized by both cutaneous melanomas and nervous system tumors (Randerson-Moor et al., 2001). Inheritance of *CDKN2A* mutations are autosomal dominant, but these mutations have variable penetrance based on sun exposure patterns and coinheritance of other melanoma-associated variants, conferring a 76% lifetime risk of developing melanoma in the US (Bishop et al., 2002; Cust et al., 2011). Mutations in *CDK4* are even less common, but were most often found affecting arginine 24, resulting in a CDK4 protein that is insensitive to inhibition by the p16 protein. No apparent differences exist in the phenotype (e.g., age at diagnosis, number of melanomas) of families carrying either *CDKN2A* or *CDK4* mutations. In aggregate, between 20-45% of familial melanomas are associated with germline mutations in *CDKN2A* or *CDK4* (Goldstein et al., 2007; Nelson & Tsao, 2009).

Other rare mutations have been associated with melanoma. Germline variations in the melanocortin-1 receptor (*MC1R*) gene alter the risk of melanoma in individuals with and without *CDKN2A* mutations (Marzuka-Alcala et al., 2014; Pasquali et al., 2015; Wendt et al., 2018). Germline variants in genes that encode for *BRCA1*-associated

protein-1 (*BAP1*), telomerase reverse transcriptase (*TERT*), and microphthalmia-associated transcription factor (*MITF*) have also been added to the list of genes harboring familial melanoma-predisposing mutations (Simone et al., 2017; Soura et al., 2016a). These are more often associated with "mixed cancer syndrome," where melanoma may appear in the context of a more general predisposition for malignancy. The *BAP1* tumor syndrome is associated with the appearance of cutaneous melanoma, uveal melanoma, and various internal malignancies (Wiesner et al., 2011). Mutations in the promoter region of *TERT*, the protein component of telomerase, and in various components of the shelterin complex have been associated with a higher incidence of melanoma and other internal malignancies (Burke et al., 2013; Horn et al., 2013). Mutations in *MITF* are associated with a higher nevus count, cutaneous malignant melanoma onset before 40 years of age, and non-blue eye color with no association to freckling, skin color, or hair color (Bertolotto et al., 2011; Yokoyama et al., 2011). Xeroderma pigmentosum (XP) is a rare disorder in which patients have a mutation in genes involved in nucleotide excision repair (NER). Patients with mutations in *XPC* and *XPD* have an increased risk of melanoma (Paszkowska-Szczur et al., 2013). Lastly, Cowden syndrome, a type of *PTEN* hamartoma tumor syndrome characterized by the appearance of trichilemmomas, papillomatous papules, mucosal lesions (papules) and palmar-plantar keratosis within the first three decades of life, is associated with a higher risk of melanoma (Bubien et al., 2013; Soura et al., 2016a).

Several proprietary gene panels exist for assessment of familial cutaneous melanoma. For example, the DermTech Pigmented Legion Assay (PLA) leverages their "Smart Sticker" technology to remove cellular material from the stratum corneum, the material of which is then analyzed for over-expression of *LINC00518* (*long intergenic non-coding RNA 518*) and *PRAME* (*preferentially expressed antigen in melanoma*), which may be incidental with genomic atypia associated with melanomas (Gerami et al., 2017). Moreover, Invitae offers a 12-gene panel (nine primary genes plus three genes with "preliminary evidence for melanoma"), Fulgent offers a 14-gene panel, and GeneDx offers a 9-gene panel (Fulgent, 2023; GeneDx, 2023; Invitae, 2024). These panels may include genes traditionally associated with familial melanoma itself (such as *CDKN2A*) as well as genes whose variants are not primarily associated with familial melanoma, but confer added risk regardless (Tsao & McCormick, 2024)

Clinical Utility and Validity of Genetic Testing for Familial Cutaneous Melanoma

The frequency of *CDKN2A* mutations in patients with a single primary melanoma or multiple primary melanoma were 1.2% and 2.9%, respectively (Berwick et al., 2006); however, depending on selection criteria, mutation frequency rates of *CDKN2A* can range from 5% to 72% (Delaunay et al., 2017) with a family history of melanoma considered the most important risk factor. The established rule of three is used when

proposing genetic testing for primary melanomas; it is generally understood that when three or more melanomas or genetically related cancers are identified in the same patient, or in first- and second-degree relatives, the pretest probability is increased above ten percent and the cost of genetic screening can be justified (Leachman et al., 2017).

In a study on *CDK2NA* genetic testing conducted in Sweden between 2015 and 2020, 913 members across 403 families were identified with cutaneous melanoma. Pissa et al. (2021) found that melanoma cases found in families testing positive for pathogenic variants of *CDK2NA* boasted significantly higher mortality rates, such that families with the variants had 37.6% melanoma cases that died from melanoma as compared to the 10.0% without ($p<0.001$), independent of age, sex, and tumor stage. This significant melanoma-specific mortality associated with families with *CDK2NA* variants is motivation to identify and enroll carrier families in a preventive surveillance program (Pissa et al., 2021). However, a potential confounding variable involves the diagnoses of pancreatic cancers in the subjects in the study; as pathogenic strains of CDK2NA are also associated with cancers other than melanomas, the 129 of the 913 members present a limitation to the specificity of the study as there did not appear to be a clear manner of segregating its influence.

Stolarova et al. (2020) analyzed 264 Czech melanoma patients with early onset, double primary tumors or family history by next generation sequencing NGS analysis of 217 genes, and they identified that "mutations in high-to-moderate melanoma risk genes and in other cancer syndrome genes were significantly associated with melanoma risk," with those genes including *CDKN2A, POT1,* and *ACD* for high-to-moderate melanoma risk, and *NBN, BRCA1/2, CHEK2, ATM, WRN,* and *RB1* for other cancer syndrome genes. An increased potential of carrying mutations was found in "patients with double primary melanoma, melanoma and other primary cancer, but not in patients with early age at onset" (Stolarova et al., 2020). *CDK2NA* was the most frequently mutated gene among those with high-to-moderate risk, and in other studies reviewed by Stolarova et al. (2020), there was an increased risk for pancreatic cancer among families with *CDK2NA* mutation, and a more established family history.

Leachman et al. (2017) published an updated algorithm for the identification, testing, and management of hereditary melanoma; the rule of three has been incorporated into this algorithm as an indication for genetic testing in multiple melanomas. The researchers state that "Any patient or family that meets the updated rule of threes should be considered a candidate for genetic testing. If melanoma is the only cancer in a pedigree, then to meet the threshold of genetic testing, a pedigree should have three primary melanomas in first- or second-degree relatives in areas with a high melanoma incidence or two primary melanomas in a low-incidence area. This

melanoma panel should include *BAP1*, *CDK4*, and *CDKN2A*. Genes for which risk has not been established but for which studies suggest an elevated risk include *MITF* and *POT1* and we recommend including these in the melanoma panel" (Leachman et al., 2017).

Genetic testing for commonly known cutaneous melanoma mutations can be utilized to determine prognosis and overall survival. Aoude et al. (2020) found that "germline mutation status was the most significant biomarker for OS [overall survival]" and "survival outcomes for germline carriers are poor with the current standards of care." When using BRAF status and tumor mutation burden (TMB) for prognosis of cutaneous melanoma patients, "BRAF V600 wild-type patients had significantly longer PFS [progression-free survival] than the V600 mutant group ($p = 0.0317$) … For stage III/IV resected patients, TMB was also significantly associated with longer PFS ($p = 0.0034$)" (Aoude et al., 2020). The greater the number of recognizable mutations, the more targeted attacks against cancerous cells can be made and the better the prognosis.

Genetic Testing for Acquired Cutaneous Melanoma

Melanoma is a particularly lethal and aggressive cancer with the ability to metastasize to any organ (Leong et al., 2011). Cutaneous tumors as little as one mm in thickness are capable of lymph node metastasis (Stage III), resulting in a significant decrease in the five-year survival rate from 90% to 56% (Yee et al., 2005). If the cancer has spread beyond the lymph nodes (Stage IV), an even more dramatic decrease in five-year survival to 15% will occur (Grossmann et al., 2012). Genetic testing for acquired melanoma can identify the activating genetic alterations.

Genetic testing is important to determine the most efficient treatment method for a melanoma patient. Despite the high frequency in nevi, the role of *BRAF* mutations in oncogenesis is well established (Davies & Gershenwald, 2011) and has been confirmed in clinical trials (Flaherty et al., 2010). BRAF is a member of the RAF family of protein serine/threonine kinases (ARAF, BRAF, CRAF) that is activated by Ras proteins during intracellular signaling cascades. Mutations in *BRAF* appear to be the most common genetic alteration in melanoma (Hocker & Tsao, 2007) and occur more frequently in melanoma than lung, colon, and ovarian carcinoma (Grossmann et al., 2012). The remarkable efficacy of *BRAF* inhibitors led to the accelerated approval of vemurafenib for unresectable and metastatic melanoma. Importantly, *BRAF* mutation testing is warranted for determining therapeutic eligibility as selective *BRAF* inhibitors pose significant risk of cutaneous squamous cell carcinoma and have the potential to increase disease progression in *BRAF* wild type (mutation negative) tumors (Grossmann et al., 2012).

The mutations, *BRAF, KIT, PIK3CA* and *NRAS,* are commonly seen in melanoma cases (Alrabadi et al., 2019; Lokhandwala et al., 2019) with an estimated 50% of melanomas exhibiting the *BRAF* V600E mutation (Burjanivova et al., 2019).

Table 1. Frequency of Melanoma Subtypes with Activating Genetic Alterations in *BRAF* and *KIT* (taken from (Grossmann et al., 2012))

Aberration	Cutaneous –CSD	Cutaneous + CSD	ALM	MM
***BRAF* mutation**	53%	8–11%	10%	15%
***KIT* mutation; amplification**	0	17%	11–38%; 19–27%	6–19%; 20–33%

A variety of methods are utilized for *BRAF* and *KIT* mutational analysis testing in melanoma, which has resulted in no standardized procedures for testing. Because numerous techniques are available and updated methods continue to be released, labs have been reluctant to switch *BRAF* platforms to accommodate one specific drug for one disease (Grossmann et al., 2012). Current *BRAF* genetic testing methods include *BRAF* V600E by real-time PCR, *BRAF* V600E mutation only by Sanger sequencing, *BRAF* full gene sequence analysis, and *BRAF* next generation sequencing (CMGL, 2014).

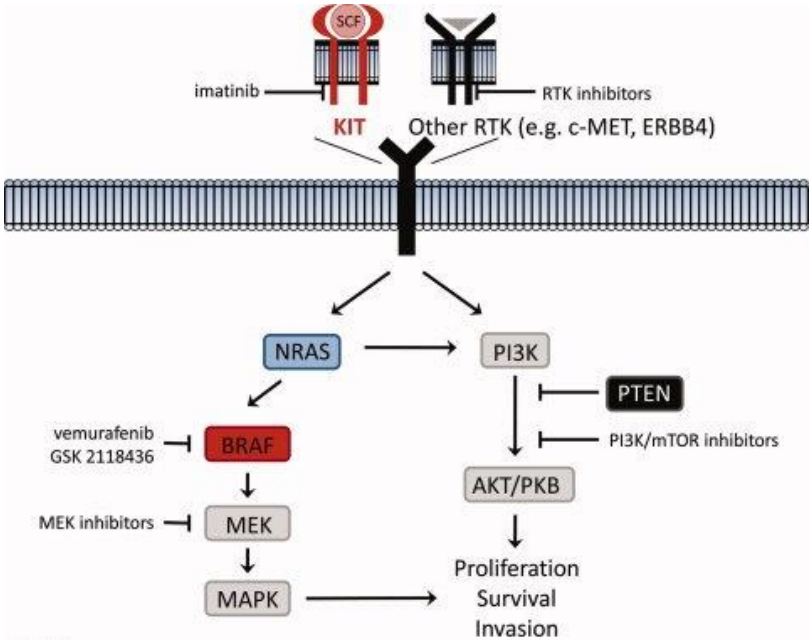
However, a recent study found good overall compliance of labs with the College of American Pathologists (Bellomo et al.) (Cree et al., 2014) and National Comprehensive Cancer Network (NCCN) guidelines for molecular diagnosis of tumors (Volmar et al., 2015) despite not using the specific FDA-approved test.

Table 2. Molecular Testing Adherence to NCCN Guidelines (taken from (Volmar et al., 2015))

	All Institutions Percentiles					
	n	**10th**	**25th**	**Median**	**75th**	**90th**
Retrospective study (lung, colorectal, melanoma)						
Percentage of tests that strictly meet the guideline	26	32.6	64.7	70.9	82.7	89.7
Percentage of tests that at least loosely meet the guideline	26	57.4	90.7	95.1	98.9	100.0
Prospective study (all case type)						
Percentage of tests that strictly meet the guideline	23	20.0	31.4	53.3	66.7	70.5
Percentage of tests that at least loosely meet the guideline	23	75.0	87.0	94.3	100.0	100.0

Historically, systemic therapy for metastatic melanoma provided very low response rates and little to no benefit in overall survival (Atkins et al., 2000; Tsao et al., 2004). However, this is beginning to change. For example, researchers have now identified that those with the *BRAF* V600 mutation are able to obtain both immune checkpoint inhibitor therapy and other targeted therapies for an improved overall treatment regimen (Ghate et al., 2019). Moreover, the immune-boosting anti-CTLA-4 antibody ipilimumab (Hodi et al., 2010) and testing and development of small molecule kinase (*KIT* and *BRAF*) inhibitors have yielded improvements in long-term survival for melanoma patients (Davies & Gershenwald, 2011; Ribas & Flaherty, 2011; Woodman & Davies, 2010).

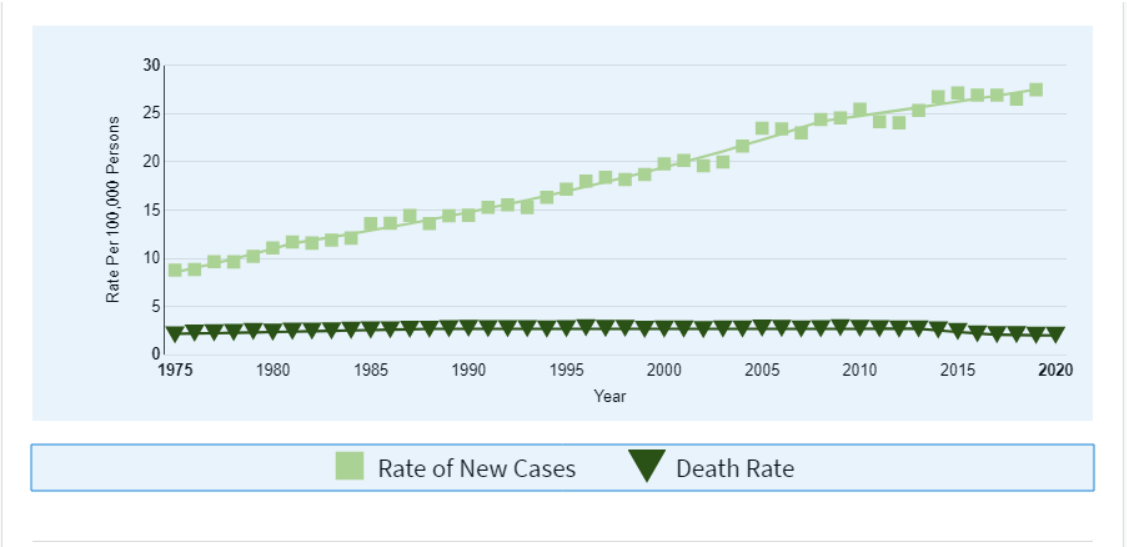
Figure 1: Imatinib and RTK Inhibitor Pathways for Melanoma Treatment (image taken from (Grossmann et al., 2012))



The lifetime risk for the general population of developing melanoma is one in 55 (NCI, 2023) and that risk has increased approximately two percent annually since 1960 (Rashid & Zager, 2015). National Cancer Institute now reports that melanoma of the skin represents 5.2% of all new cancer cases in the United States; approximately 99,780 Americans in total will be diagnosed with melanoma in 2022 (NCI, 2023).

In 2022, the number of new cases of melanoma of the skin per 100,000 people was 21.5. Further, the number of melanoma patient deaths in 2022 was 2.1 per 100,000 individuals, accounting for 1.3% of all cancer deaths in United States (NCI, 2023).

Figure 2. New Cases and Deaths of Melanoma of the Skin per 100,000 Persons (taken from (NCI, 2023))



Clinical Utility and Validity of Genetic Testing for Acquired Cutaneous Melonama

O'Brien et al. (2017) analyzed samples to determine if *BRAF* mutation identification by immunohistochemistry was a suitable alternative to PCR. The study included 132 patients, and the anti-*BRAF* V600E VE1 clone antibody was used for immunohistochemistry detection. A sensitivity of 86.1% and specificity of 96.9% was shown with the anti-*BRAF* V600E VE1 clone antibody; "The concordance rate between PCR and immunohistochemical BRAF status was 95.1% (116/122)" (O'Brien et al., 2017). As both methods were in high agreement, immunohistochemistry may be a viable alternative to PCR for *BRAF* mutation testing.

Corean et al. (2019) utilized several different techniques on metastatic melanoma samples, including bone marrow morphology, histology, immunophenotyping, molecular genetic testing and *BRAF* V600E immunohistochemistry. *BRAF* immunohistochemistry was detected in two patients, and molecular testing confirmed these results; researchers then stated that "*BRAF* V600E immunohistochemistry is useful as a surrogate marker of molecular results," once again highlighting the fact that immunohistochemistry may be a viable alternative for *BRAF* mutation testing (Corean et al., 2019).

Available *BRAF* tests and analytical sensitivities:

- "The *BRAF* V600E by real-time PCR test uses a TaqMan® Mutation Detection Assay to detect the V600E mutation in exon 15 of *BRAF* in tumor (somatic) cells.

The sensitivity of the TaqMan assay is ~0.1% mutant DNA in a wild-type background. Poor DNA quality, insufficient DNA quantity or the presence of PCR inhibitors can result in uninterpretable or (rarely) inaccurate results.

- The *BRAF* (V600E) mutation only by Sanger sequencing uses a DNA-based PCR-sequencing assay to detect the V600E in exon 15 of *BRAF*. The limit of detection for Sanger sequencing is >20% mutant DNA in a wild-type background.
- The *BRAF* full gene sequence analysis test uses a DNA-based PCR-sequencing assay to detect point mutations in the coding sequence and intron/exon boundaries of the *BRAF* gene. The sensitivity of DNA sequencing is over 99% for the detection of nucleotide base changes, small deletions and insertions in the regions analyzed. Rare variants at primer binding sites may lead to erroneous results. The limit of detection for Sanger sequencing is >20% mutant DNA in a wild-type background" (CMGL, 2014).
- The *BRAF* NGS with TruSeq had sensitivity of over 99% (Froyen et al., 2016).

Colombino et al. (2020) compared *BRAF* mutation testing performed with conventional nucleotide sequencing approaches (Sanger sequencing and pyrosequencing) with real-time polymerase chain reaction (RT-PCR) or next-generation sequencing (NGS) assays to assess the levels of concordance between these various techniques. There were 319 tissue samples analyzed and initially screened with conventional approaches. The initial screen found pathogenic *BRAF* mutations in 144 (45.1%) cases. RT-PCR (Idylla™ *BRAF* mutation assay) detected 11 (16.2%) and three (4.8%) additional *BRAF* mutations after Sanger sequencing and pyrosequencing, respectively. NGS detected one additional *BRAF*-mutated case (2.1%) among 48 wild-type cases previously tested with pyrosequencing and RT-PCR. According to the data, RT-PCR is more accurate than both Sanger sequencing and pyrosequencing in detecting *BRAF* mutations. Overall, RT-PCR had a good concordance with the other tests; "real-time PCR is a rapid method which achieves the same maximum level of sensitivity of NGS (up to 98%), without requiring particular skills" (Colombino et al., 2020). According to the author, "[RT-PCR and NGS] improved the diagnostic accuracy of *BRAF* testing via the detection of additional *BRAF* mutations in a subset of false-negative cases previously tested with Sanger sequencing or pyrosequencing. In attendance of further confirmations in larger prospectively designed studies, the use of two sensitive molecular methods may ensure the highest level of diagnostic accuracy" (Colombino et al., 2020).

The *BRAF* analysis is an accepted medical practice for patients with unresectable, metastatic stage IV melanoma (NCCN, 2024). However, recent randomized controlled trials have indicated the benefits of expanding *BRAF* analysis. A study published in The Lancet Oncology by Amaria et al. (2018) compared standard of care in patients with high-risk, surgically resectable melanoma (stage III or IV) to similar patients receiving a regimen of a neoadjuvant plus adjuvant dabrafenib and trametinib. All patients had to

be of confirmed *BRAF* V600E or *BRAF* V600K status to participate in either the control or experimental groups. In the follow-up (median of 18.6 months), 10/14 (or 71%) of patients in the experimental group remained event-free (i.e., alive without disease progression), whereas zero out of seven (0%) of the control group receiving standard of care remained event-free. The authors conclude that the "Neoadjuvant plus adjuvant dabrafenib and trametinib significantly improved event-free survival versus standard of care in patients with high-risk, surgically resectable, clinical stage III-IV melanoma" (Amaria et al., 2018).

Another study published by Zippel et al. (2017) researched the use of perioperative *BRAF* inhibitors on patients with stage III melanoma. All patients had to be confirmed *BRAF* V600E to participate in the study. Of the thirteen patients, twelve "patients showed a marked clinical responsiveness to medical treatment, enabling a macroscopically successful resection in all cases"; moreover, "at a median follow up of 20 months, 10 patients remain free of disease" (Zippel et al., 2017). One patient died prior to surgery in this study. The authors conclude that the "Perioperative treatment with *BRAF* inhibiting agents in *BRAF*V600E mutated Stage III melanoma patients facilitates surgical resection and affords satisfactory disease free (sic) survival" (Zippel et al., 2017).

Gene Expression Profiling and Biomarkers for Diagnosis and Prognosis

Currently, patients presenting with a suspicious pigmented lesion undergo excisional biopsy which is then subjected to histopathologic examination by a pathologist (NCCN, 2024). The majority of melanocytic neoplasms can be accurately classified by this approach; however, in some cases confidently differentiating benign melanocytic nevi from malignant melanoma can be extremely difficult or impossible despite additions to histopathologic assessment, such as the evaluation of Breslow depth (Chiaravalloti et al., 2018; Lee & Lian, 2018). In these cases, even diagnoses from expert pathologists can be discordant (Elmore et al., 2017; Farmer et al., 1996; Gerami et al., 2014) and subject to diagnostic drift (Bush et al., 2015).

A number of diagnostic and prognostic genetic tests for melanoma have been developed as ancillary tests to assist in this differentiation and resultant risk stratification (Lee & Lian, 2018), including microRNAs as biomarkers to distinguish between melanomas and nevi (Torres et al., 2019). Gene expression profiling is growing in popularity for the diagnosis and prognosis of cutaneous melanoma. Molecular tests based on gene expression profiling of cutaneous melanoma are commercially available. They include the myPath Melanoma (Castle Biosciences, 2024b), 2-GEP Pigmented Lesion Assay (DermTech, 2023a), DecisionDx-Melanoma

(Castle Biosciences, 2024a), and a clinicopathologic (CP)-GEP model by SkylineDx (SkylineDx, 2024).

Another risk stratification test is the melanoma prognostic test, AMBLor®, described by Avero Diagnostics as a test used to identify "early-stage melanomas at low risk of progression." Avero Diagnostics licensed this biotechnology from AMLo Biosciences Ltd. The AMBLor® test looks for the presence of two prognostic biomarker proteins, AMBRA1 and loricrin, both of which are found in the skin overlying the tumor. An absence of these biomarkers in AJCC stages I and II indicates melanoma at a normal or high risk of progression, while normal expression of the biomarkers is linked to a low risk of cancer progression(AMLo Biosciences, 2023).

Clinical Utility and Validity of Gene Expression Profiling and Biomarkers for Diagnosis and Prognosis

myPath Melanoma (Castle Biosciences)

A 23-gene expression profile and algorithm that assigns various weights and thresholds of expression for each gene was developed to differentiate benign melanocytic nevi from malignant melanoma; this gene expression profile and algorithm was determined to have a sensitivity of 89% and specificity of 93% (Clarke et al., 2015). Further, three experienced dermatopathologists validated this method against an independent histopathologic evaluation; a sensitivity of 91.5% and a specificity of 92.5% was determined (Clarke et al., 2017). This gene expression profile was developed into a commercial test known as myPath Melanoma, which generates a single numerical score along with a classification of likely malignant, likely benign, or indeterminate, for a given cutaneous lesion. Across various validation studies, myPath Melanoma has demonstrated a sensitivity in the range of 90-94% and a specificity in the range of 91-96% (Ko et al., 2017).

myPath Melanoma correlates closely with long-term clinical outcomes by adding valuable adjunctive information to aid in the diagnosis of melanoma. The test is intended for "the in vitro analysis of primary cutaneous melanocytic lesions for which malignant potential is uncertain", but has not been validated on "metastatic melanomas, recurrent tissue specimens, non-melanocytic neoplasms, or biopsies from a patient receiving immunosuppressant therapy or radiation treatment" (Castle Biosciences Inc., 2022). An examination of the utility of this test (Cockerell et al., 2017) found that the results of this gene expression signature have a significant clinical impact with 71.4% (55/77) of cases changing from pretest recommendations to actual treatment. Majority of changes were consistent with the test result. There was an 80.5% (33/41) reduction in the number of biopsy site re-excisions performed for cases with a benign test result. However, when more challenging samples were included (Minca et

al., 2016) with 39 histopathologically unequivocal lesions (15 malignant, 24 benign) and 78 challenging lesions interpreted by expert consensus (27 favor malignant, 30 favor benign, and 21 ambiguous), myPath Melanoma had a lower sensitivity and specificity than fluorescence in situ hybridization (Yokoyama et al.) (FISH: 69% sensitivity, 91% specificity; myPath; 55% sensitivity, 88% specificity).

In contrast, Clarke et al. (2020) completed another study that evaluated the accuracy of myPath melanoma in diagnostically uncertain neoplasms. Out of 125 diagnostically "uncertain" cases, myPath melanoma demonstrated a sensitivity of 90.4%. In this study, the test displayed no significant difference in sensitivity between the diagnostically uncertain (equivocal) and unequivocal lesion groups.

Pigmented Lesion Assay (PLA) (DermTech)

DermTech has developed a pre-diagnostic quantitative polymerase chain reaction (qPCR)-based pigmented lesion assay (PLA) that measures the expression of two genes in the stratum corneum to assist with diagnostic or prognostic information for potential melanoma cases (Varedi et al., 2019). DermTech's PLA identifies malignant changes on a genomic level that cannot be detected with the human eye; this assay can be used to support clinicians in their decision to biopsy suspicious nevi. This test has the potential to increase the number of early melanomas biopsied and reduce the number of benign lesions biopsied, thereby improving patient outcomes (Ferris et al., 2017). A recent study has given this pigmented lesion assay a sensitivity of 91-96%, a specificity of 69-91%, and a negative predictive value of approximately 99% (Ferris, Rigel, et al., 2019).

To help support clinicians in their decision to biopsy, this noninvasive two gene expression assay of the *LINC00518* and *PRAME* genes has been developed for use on adhesive patch biopsies. Skin sampling via an adhesive patch allows for DNA, RNA, skin tissue and microbiome samples to be safely obtained and transported cost effectively by mail at room temperature; skin cells, T-cells, dendritic cells, melanocytes and other types of cells can be analyzed by this method (Yao et al., 2017). The use of an adhesive patch also allows 100% of the lesion's surface to be sampled, compared to less than one to two percent of surgical biopsies (DermTech, 2023b). Further, this technique is a much more cost-effective option. Hornberger and Siegel (2018) report that PLA testing could save approximately $447 per lesion compared to traditional biopsies.

Gerami et al. (2017) tested the validity of the two-gene panel based on *LINC00518* and *PRAME* on differentiating melanoma from nonmelanoma in a multicenter study across 28 sites in the United States, Europe, and Australia. In a sample of 398 (87 melanomas and 311 nonmelanomas), it was found that this classification method was able to

accurately identify melanomas from nonmelanomas with a sensitivity of 91% and a specificity of 69%. An application study of 381 patients found that the estimated real-world sensitivity of the DermTech PLA was 95% and specificity was 91% (Ferris et al., 2018); overall, 93% of PLA results positive for both *LINC00518* and *PRAME* were diagnosed histopathologically as melanoma. Further, this study was also used to identify if the real-world clinical use of the DermTech PLA could change physician behavior and reduce the overall number of biopsies performed. The PLA identified 51 PLA(+) test results, and 100% of these pigmented skin lesions were biopsied (37% were melanomas). Furthermore, "Nearly all (99%) of 330 PLA(-) test results were clinically managed with surveillance. None of the three follow-up biopsies performed in the following three to six months, were diagnosed as melanoma histopathologically" (Ferris et al., 2018). The PLA test altered clinical management of pigmented lesions and shows high clinical performance. However, it is uncertain if the negative samples—as determined by the PLA—will remain so, as "we [the authors] cannot rule out that some PLA(−) lesions may not have been adequately reassessed in the follow-up period and we certainly recommend erring on the side of caution and surgically biopsying a lesion in question if additional risk factors, further clinical suspicion, or patient concern mandate such a step" (Ferris et al., 2018).

The PLA test has also been validated against driver mutations in melanoma, including *BRAF, NRAS,* and *TERT*. Ferris, Moy, et al. (2019) studied the mutation frequency of these genes using samples obtained from the PLA technique (e.g., adhesive patch) in both histopathologically confirmed melanomas (n = 30) and non-melanoma controls (n = 73). "The frequency of these hotspot mutations in samples of early melanoma was 77%, which is higher than the 14% found in nonmelanoma samples ($P < 0.0001$). *TERT* promoter mutations were the most prevalent mutation type in PLA-positive melanomas; 82% of PLA-negative lesions had no mutations, and 97% of histopathologically confirmed melanomas were PLA and/or mutation positive" (Ferris, Moy, et al., 2019). A total of 86% of the non-melanomas within this validation cohort contained no mutations. The authors next analyzed 519 real-world PLA samples for the same mutations. Similar to the previous validation cohort, 88% of this larger cohort also contained no mutations, indicating that the PLA test can rule out lesions with few mutational risk factors for melanoma.

further confirmed the "real-world" negative predictive value (NPV) and positive predictive value (PPV) of the PLA, "by following a cohort of 1,233 PLA-negative pigmented lesions for evidence of malignancy for up to 36 months and by re-testing a separate prospective cohort of 302 PLA-negative lesions up to two years after initial testing." Out of this total PLA-negative cohort, 1,233 lesions had a confirmed follow-up evaluation; of these, ten melanomas were subsequently detected by way of biopsy, resulting in an NPV of 99.2%. Out of 316 PLA-positive cases, 59 were diagnosed as

melanoma by histopathology, resulting in a PPV of 18.7% (Skelsey et al., 2021). The PPV achieved through use of the PLA may represent a five-fold improvement over the current standard according to the test's developers.

In a continuing medical education series, Skudalski et al. (2022) confirm that the PLA is to be used as a risk stratification aid in determining whether to biopsy a skin lesion. The authors recommend that in the case of a positive PLA result, "biopsy i*n toto* should be performed immediately." They further comment that after a negative PLA result, clinicians have chosen to follow up within 12 months to further evaluate the skin lesion; however, a biopsy *in toto* should be considered if a patient remains concerned about a lesion, or if the lesion has evolved (Skudalski et al., 2022).

A recent report by Robinson and Jansen (2020) outlined a proof-of-concept pilot program of remote physician-guided self-sampling (i.e. telehealth administration of the adhesive patch) during the Illinois stay-at-home order of the COVID-19 pandemic. The authors also surveyed skin self-examination (SSE) anxiety as well. Two cohorts were used in this pilot, an experimental group (n=7), and a randomly selected physician-sampled control case group (n=10). The authors report that SSE-induced anxiety has increased during the COVID-19 pandemic. It should be noted that surveys were administered to much larger groups than those administering self-sampling tests. There were 258 surveys about SSE anxiety conducted prior to the COVID-19 pandemic, and 211 surveys during the COVID-19 pandemic. The authors state, "Guided self-sampling led to molecular risk factor analyses in 7/7 (100%) of cases compared to 9/10 (90%) randomly selected physician-sampled control cases... Adhesive patch self-sampling under remote physician guidance is a viable specimen collection option" (Robinson & Jansen, 2020).

DecisionDx-Melanoma (Castle Biosciences)

After a melanoma case has been identified, several management approaches may be considered. However, best melanoma management practices are constantly evolving. A common technique to assess the spread of a tumor, such as melanoma, is a sentinel lymph node biopsy (SLNB). This procedure is used to evaluate whether the cancer has spread beyond the original tumor site and into the lymphatic system. The lymphatic or lymph system transports fluid known as lymph throughout the body; this fluid contains white blood cells which help to fight infections. The lymphatic system also aids in ridding the body of other waste and toxins. The SLNB technique essentially helps the physician to stage the tumor. However, melanoma-related lymph node spread is very complex and is associated with many factors, including age, location, thickness, ulceration, gender, and regression (Ribero et al., 2017).

The DecisionDx-Melanoma test is a gene expression profile (GEP) test that measures the expression of 31 different genes in a tumor tissue sample. This test was designed for cutaneous melanoma patients undergoing or considering SLNB and can help to identify the risk of cancer recurrence or metastasis in stage I-III melanoma (Castle Biosciences, 2024a). DecisionDx-Melanoma may help physicians guide treatment options, including whether to perform a SLNB in eligible patients, and what type of follow up treatment is necessary (Castle Biosciences, 2024a). After GEP analysis, the DecisionDx-Melanoma provides a score of either Class 1 (low risk) or Class 2 (high risk), as well as A or B scores with "A" reflecting a better outcome and "B" reflecting a worse outcome; this allows physicians to receive final scores of Class 1A, 1B, 2A, and 2B (Gastman et al., 2019).

The DecisionDx-Melanoma is performed on tumor tissue biopsies that have been preserved as formalin-fixed, paraffin-embedded (FFPE) samples (Castle Biosciences, 2024a; CastleBiosciences, 2024). Researchers have highlighted that the identification of tissue-based prognostic markers in melanoma has been a challenging obstacle for researchers, as "One major limitation is that most primary melanomas are preserved as formalin-fixed, paraffin-embedded (FFPE) samples rather than fresh-frozen tissues because of the small size of the specimens" (Weiss et al., 2015). High-quality genetic material is more challenging to extract from FFPE samples, and messenger RNA is inconsistent in FFPE samples (Weiss et al., 2015).

The prognostic utility of this test has been measured by Keller et al. (2019); a total of 159 patients participated in this study and were followed up with, on average, 44.9 months after initial testing. Gene expression profiling results helped to categorize patients into two groups: low-risk patients were placed in Class 1, and high-risk patients in Class 2; 117 patients were placed in Class 1 and 42 patients in Class 2 (Keller et al., 2019). Results showed that this gene expression profiling test had great prognostic abilities. "Gender, age, Breslow thickness, ulceration, SNB positivity, and AJCC stage were significantly associated with GEP classification ($P < 0.05$ for all). Recurrence and distant metastasis rates were five percent and one percent for Class 1 patients compared with 55% and 36% for Class 2 patients. Sensitivities of Class 2 and SNB for recurrence were 79% and 34%, respectively" (Keller et al., 2019).

Berman et al. (2019) gathered an expert panel of nine dermatologists/dermatologic surgeons/ dermatopathologists and completed 29 clinical scenarios in which gene expression tests could be used appropriately; several gene expression profiling (GEP) tests were used including a 2-GEP assay, 23-GEP assay and 31-GEP assay. "The 2-GEP assay for melanoma diagnosis received one B-strength and six C-strength recommendations. The 23-GEP diagnostic test received one A-strength, three B-strength, and four C-strength recommendations. The 31-GEP prognostic assay received

one A-strength, seven B-strength, and six C-strength recommendations"; this report shows that the 31-GEP assay received the highest overall recommendations by the expert panel (Berman et al., 2019).

Another study reported that the 31-GEP was validated in almost 1600 patients "as an independent predictor of risk of recurrence, distant metastasis and death in Stage I-III melanoma and can guide SLNB decisions in patient subgroups, as demonstrated in 1421 patients"; further, an appropriate 31-GEP testing population was identified and concluded that it is best used on patients with cutaneous melanoma tumors greater than or equal to 0.3 mm thick (Marks et al., 2019). However, this study reports several conflicts of interest that are important to note as multiple authors are employees at Castle Biosciences, Inc., and one author is a consultant and speaker for the company (Marks et al., 2019).

Greenhaw et al. (2020) completed a meta-analysis of the DecisionDx-Melanoma 31-GEP prognostic test in a total of 1,479 patients. This meta-analysis included participants from three different studies. The patient analysis showed that the five-year recurrence and distant metastasis-free survival rate for Class 1A patients was 91.4% and 94.1% respectively and were 43.6% and 55.5% for Class 2B patients. The 31-GEP was then used to estimate the likelihood of recurrence and distance metastasis. The GEP test exhibited a sensitivity of 76% for each endpoint, showing consistency and accuracy for the identification of at increased risk of metastasis (Greenhaw et al., 2020).

Moreover, Marchetti et al. (2020) completed an assessment of the prognostic accuracy of the 31-GEP in patients that had cancer labeled as AJCC state I or II disease. Patients with localized melanoma were included from external validation studies, and after exclusion criteria, there were seven studies analyzed. Five of these studies used the DecisionDx-Melanoma test. Authors looked at the results of the seven included studies (1450 study participants) and concluded that the performance of both GEP tests (Melagenix and DecisionDx-Melanoma) varied by AJCC stage. "For patients tested with DecisionDx-Melanoma, 623 had stage I disease (six true-positive ([TP], 15 false-negative, 61 false-positive, and 541 true-negative [TN] results) and 212 had stage II disease 959 TP, 13 FN, 78 FP, and 62 TN results). Among patients with recurrence, Decision Dx-Melanoma correctly classified 29% with stage I disease and 82% with stage II disease. Among patients without recurrence, the test correctly classified 90% with stage I disease, and 44% with stage II disease." The overall conclusion by authors was that the prognostic ability was "poor" in terms of accurately identifying recurrence in patients with stage I cancer (Marchetti et al., 2020).

Kangas-Dick et al. (2021) evaluated the DecisionDx-Melanoma assay in a retrospective chart review of patients at a large cancer institute. A total of 361 patients' data were

analyzed, and a follow-up period median of 15 months was established. First, sentinel node biopsy was performed for 75.9% of the patients, of which, 19.4% tested positive for recurrent cancer. Authors considered GEP class 2B status and noted that it was "significantly associated" with recurrence-free survival (RFS) and distant metastasis-free survival (MDFS) in a univariate analysis. The authors concluded that "genetic profiling of cutaneous melanoma can assist in predicting recurrence and help determine the need for close surveillance. However, traditional pathologic factors remain the strongest independent predictors of recurrence risk" (Kangas-Dick et al., 2021).

CP-GEP (Merlin Assay) (SkylineDx)

An eight gene expression profile test called CP [clinicopathologic]-GEP was developed to help identify patients who might safely avoid SLNB through reclassification of their risk of nodal metastasis to less than five percent. In considering certain CP variables, the developers determined that Breslow thickness and patient age were sufficiently predictive of the risk of SLN metastasis. Double-loop cross-validation [DLCV] and least absolute shrinkage and selection operator (LASSO) further identified several genes that distinguished between patients with positive and negative SLNB results. Ultimately, eight genes (*MLANA, GDF15, CXCL8, LOXL4, TGFBR1, ITGB3, PLAT, and SERPINE2*) along with the two CP variables (Breslow thickness and patient age) completed the CP-GEP model.

The CP-GEP model was tested in a cohort of 754 patients diagnosed with cutaneous melanoma, who underwent SLNB within 90 days of their diagnosis. The validity of the model changed based on different T categories of melanoma and demonstrated increasingly higher sensitivity with increasing T category. An inverse relationship was observed between specificity and T category. Theoretically, the model could have reduced the SNLB rate in T1b patients by 80%, though the rate reduction dropped as the T category increased across the patient population. The negative predictive value was 91% or higher across all T categories (Bellomo et al., 2020).

The CP-GEP model was independently validated by Yousaf et al. (2021) in a separate cohort of 208 adult patients with primary cutaneous melanoma and confirmed the trends originally published by (Bellomo et al., 2020). As the T category of melanomas increased, the sensitivity of the model sequentially increased, while the specificity decreased. The highest SLNB reduction rates were observed in lower T category patients (T1), and an NPV of at least 93.3% was observed across all T categories in the study. Importantly, the model was further validated focusing only on patients 65 years of age or older, as the incidence of melanoma is greater in older patient populations. Similar results were obtained compared to the full patient cohort, demonstrating the

applicability of the model across all age ranges included in the study (Yousaf et al., 2021).

11-GEP (Melagenix) (NeraCare)

Another GEP test was developed to better define the probability of patient survival at the time of first diagnosis. Eleven prognostically-relevant genes previously identified through whole-transcriptome analysis (Brunner et al., 2013) of fresh-frozen melanomas were evaluated (*KRT9, DCD, PIP, SCGB1D2, SCGB2A2, COL6A6, GBP4, KLHL41, ECRG2, HES6, and MUC7*), and eight of these were found to be statistically- significantly associated with melanoma-specific survival (all but *ECRG2, HES6, and MUC7*). Based on the coded expression data of the eight significantly associated genes, a binary GEP "score" of either zero or one was generated, representing "low risk" or "high risk", respectively.

To validate the GEP score, inter-assay variability was examined across four different laboratories. Concordance was high between replicates, with 93% of replicate determinations confirming the score. Next, the GEP score was challenged with a cohort of 211 melanomas whose prognostic assessment was erroneous by AJCC staging alone, where the test discriminated successfully between short-term and long-term survivors (Brunner et al., 2018).

An investigation by Amaral et al. (2020) supported the clinical validity of the Melagenix test (referred to in this study as the 11-gene expression profiling score [GEPS]). GEPS was calculated for 245 patients diagnosed with stage II cutaneous melanoma, and the scores were determined to be either "high" (median: 1.06) or "low" (median: –0.21). A significant difference in melanoma-specific survival, distant metastasis free survival, and relapse-free survival was confirmed between high-score and low-score patients. Similarly, Gambichler et al. (2021) used the 11-GEP test to determine whether a high or low score correlated with melanoma-specific survival. The authors documented decreasing probability of melanoma-specific survival with increasing 11-GEP scores. The authors further concluded that the 11-GEP is capable of identifying patients with ten year survival probabilities above 90% (Gambichler et al., 2021).

AMBLor® test (Avero Diagnostics)

Cosgarea et al. (2022) investigated the potential contribution of melanoma paracrine transforming growth factor (TGF)-β signaling in the loss of a protein called AMBRA1 in the skin overlying a primary tumor. The study included 109 all-stage melanoma samples, in which AMBRA1 and (TGF)-β were analyzed. Another cohort had 30 samples analyzed for (TGF)-β and 42 samples analyzed for claudin-1. Results showed "increased tumoral TGF-β2 was significantly associated with loss of peritumoral AMBRA1

(P < 0·05), ulceration (P < 0·001), AMLo high-risk status (P < 0·05) and metastasis (P < 0·01)." Although the American Joint Committee on Cancer (AJCC) staging system is an important tool, the authors noted, it leaves gaps in being unable to identify AJCC early-stage I/II tumors which are at risk of metastasis. The authors described an "urgent need" for credible prognostic information such as biomarkers in these early-stage tumors. "The combined loss of the autophagy regulatory protein autophagy-and-beclin-1 regulator one (AMBRA1) and epidermal differentiation marker loricrin (AMLo Biosciences) in the tumour microenvironment as a prognostic biomarker for early-stage primary melanoma [is] associated with significantly increased risk of metastasis" (Cosgarea et al., 2022).

Labus et al. (2020) investigated the use of the combined immunohistochemical (IHC) expression of AMBRA1 and Loricrin in the top layer of skin as a prognostic biomarker. The study was a retrospective analysis of AMBLor performed in three different cohorts of people with AJCC stage II melanomas. A clinically validated IHC assay and semi quantitative scoring method was used to define risk groups. The results showed a loss of AMBLor in the epidermis over high risk AJCC state IIA or B tumours- that was "correlated with significant reduction in disease free survival (DFS) at 12 years to 55% compared to 89% for patients with AMBLor low risk tumours (P = 0.015; HR 4.83, 95% CI: 2.29-10.14)." In addition, multivariate analysis of 80 of these tumors showed reduced disease-free survival at 12 years to 68% in the AMBLor high-risk cohort compared to 83% in AMBLor low-risk groups. The assay sensitivity was 97% and had a negative predictive value of 90%. The authors concluded that "these data indicate AMBlor as a novel prognostic biomarker for patients with non-ulcerated AJCC stage II melanomas as well as companion/stratifying biomarker for adjuvant immunotherapy" (Labus et al., 2020).

Ellis et al. (2020) sought to determine the utility of AMBRA1 and loricrin (AMLo Biosciences) expression as prognostic biomarkers for AJCC stage one cutaneous melanoma. This retrospective discovery study involved 76 AJCC stage I melanoma samples. Additionally, AMLo expression was correlated against clinical outcomes for up to 12 years in two independent and retrospective validation cohorts of 379 AJCC stage I melanomas. The outcome of decreased AMBRA1 expression was a seven year disease-free survival rate (DFS) of 81.5% vs. 100% when AMBRA1 expression was maintained (P < 0·081). A combined cohort analysis also revealed a DFS rate of 98.3% in the AMLo low-risk cohort vs. 85.4% in the AMLo high-risk group. The authors concluded that "loss of AMLo expression in the epidermis overlying primary AJCC stage I melanomas identifies high-risk tumour subsets independently of Breslow depth," and advocated for these biomarkers to be integrated into risk stratification: "The integration of peritumoral epidermal AMBRA1/loricrin biomarker expression into

melanoma care guidelines will facilitate more accurate, personalized risk stratification for patients with AJCC stage I melanomas" (Ellis et al., 2020).

VI. Guidelines and Recommendations

The National Comprehensive Cancer Network

The NCCN provides common follow-up recommendations for all patients:

- "Clinical and family history can identify patients in whom multigene testing might indicate an increased genetic risk for cutaneous and uveal melanoma, astrocytoma, mesothelioma, and cancers of the breast, pancreas, and kidney. This information can guide recommendations for surveillance and early detection in appropriate patients and their relatives.
  - Consider genetic counseling referral for *p16/CDKN2A* mutation testing in the presence of three or more invasive cutaneous melanomas, or a mix of invasive melanoma, pancreatic cancer, and/or astrocytoma diagnoses in an individual or family.
  - Multigene panel testing that includes *CDKN2A* is recommended for patients with invasive cutaneous melanoma who have a first-degree relative diagnosed with pancreatic cancer . . .
  - Testing for other genes that can harbor melanoma-predisposing mutations [see Genetic Predisposition below] may be warranted" (NCCN, 2024).

The NCCN includes the following as a risk factor for development of single or multiple primary melanomas:

- Genetic predisposition
  - Presence of germline mutations or polymorphisms predisposing to melanoma (eg, *CDKN2a, CDK4, MC1R, BAP1* [especially for uveal melanoma], *TERT, MITF, PTEN*, and potential other genes).
  - Family history of cutaneous melanoma (especially if multiple); pancreatic, renal, and/or breast cancer; astrocytoma; uveal melanoma; and/or mesothelioma" (NCCN, 2024).

Regarding indications for genetic testing using emerging molecular technologies for diagnosis and prognostication, the NCCN recommended the following:

- "The panel does not recommend *BRAF* or NGS [next generation sequencing] testing for resected stage I-II cutaneous melanoma unless it will inform clinical trial participation.
- The *BRAF* mutation testing is recommended for patients with stage III at high risk for recurrence for whom *BRAF*-directed therapy may be an option.

- For initial presentation with stage IV disease or clinical recurrence, obtain tissue to ascertain alterations in *BRAF*, and in the appropriate clinical setting, *KIT* from either biopsy of the metastasis (preferred) or archival material if the patient is being considered for targeted therapy. Broader genomic profiling (e.g., larger NGS panels, *BRAF* non-V600 mutation) is recommended if feasible, especially if the test results might guide future treatment decisions or eligibility for participation in a clinical trial.
- If *BRAF* single-gene testing was the initial test performed, and is negative, clinicians should strongly consider larger NGS panels to identify other potential genetic targets (e.g., *KIT*, *BRAF* non-V600)" (NCCN, 2024).

The NCCN also recognizes *NRAS* as a relevant mutation for melanoma, noting that *NRAS* mutations are present in "approximately 15% of melanomas with chronic and intermittent sun exposure, acral surfaces, and mucosal surfaces." Due to the low probability of overlapping targetable mutations (such as *BRAF* or *KIT*), the NCCN remarks that "the presence of an *NRAS* mutation may identify patients who will not benefit from additional molecular testing" (NCCN, 2024).

NCCN acknowledges that GEP tests may have clinical utility for diagnostic purposes, noting that "melanocytic neoplasms of uncertain biologic potential present a unique challenge to pathologists and treating clinicians. Ancillary tests to differentiate benign from malignant melanocytic neoplasms include immunohistochemistry (IHC) and molecular testing via comparative genomic hybridization (CGH), fluorescence in situ hybridization (FISH), gene expression profiling (GEP), single-nucleotide polymorphism (SNP) array, and next-generation sequencing (NGS). These tests may facilitate a more definitive diagnosis and guide therapy in cases that are diagnostically uncertain or controversial by histopathology. Ancillary tests should be used as adjuncts to clinical and expert dermatopathologic examination and therefore be interpreted within the context of these findings." Similarly, NCCN comments that "noninvasive patch testing" may also be helpful to guide biopsy decisions for melanocytic neoplasms that are clinically/dermoscopically suspicious for melanoma (NCCN, 2024).

For prognostic indications, however, NCCN guidelines are more cautionary:

- "Current GEP platforms do not provide clinically actionable prognostic information when combined or compared with known clinicopathologic (CP) factors (e.g., sex, age, primary tumor location, thickness, ulceration, mitotic rate, lymphovascular invasion, microsatellites, and/or SLNB status). Furthermore, the clinical utility of these tests to inform treatment recommendations and improve health outcomes by prompting an intervention has not been established.

- Various studies of prognostic GEP tests suggest their role as an independent predictor of worse outcome. However, GEP studies to date have not demonstrated added benefit beyond comprehensive CP variables, and it remains unclear whether available GEP tests are reliably predictive of outcome across the risk spectrum of cutaneous melanoma. Validation studies on prospectively collected, independent cohorts (similar to those performed in breast cancer) are necessary to define the clinical utility of molecular prognostic GEP testing as an adjunct to AJCC staging and other known prognostically significant CP variables or as part of the multidisciplinary decision-making process to guide surveillance imaging, SLBN, and adjuvant therapy" (NCCN, 2024).

The American Academy of Dermatology (AAD)

The AAD recently published guidelines for the care and management of primary cutaneous melanoma. Regarding skin biopsies, the AAD states that while many different molecular and imaging techniques have been developed, "skin biopsy remains the first step to establish a definitive diagnosis of CM [cutaneous melanoma]"; further, the guidelines also state that "Newer noninvasive techniques (eg, reflectance confocal microscopy [RCM], as well as electrical impedance spectroscopy, gene expression analysis, optical coherence tomography, and others [see the section Emerging Diagnostic Technologies]) can also be considered as these become more readily available" (Swetter et al., 2019).

The AAD notes that these guidelines highlight several gaps in research including "the clinical utility and prognostic significance of various biomarkers and molecular tests; optimal clinical situations in which to pursue multigene somatic and germline mutational analysis; and the value of ancillary molecular tests in comparison with well-established clinicopathologic predictors of outcome" (Swetter et al., 2019). It is then noted that "Efforts to standardize the histopathologic diagnosis and categorization of melanocytic neoplasms are under way to reduce the significant interobserver variability among pathologists. Ongoing advances in genomic medicine may make many of the aforementioned issues obsolete before the next AAD melanoma CPG is issued" (Swetter et al., 2019).

Regarding patients with a family history of invasive cutaneous melanoma (at least three affected members on one side of the family), "Cancer risk counseling by a qualified genetic counselor is recommended" (Swetter et al., 2019).

Finally, the AAD states "There is insufficient evidence to recommend routine molecular profiling assessment for baseline prognostication. Evidence is lacking that molecular classification should be used to alter patient management outside of current guidelines (eg. NCCN and AAD). The criteria for and the utility of prognostic molecular

testing, including GEP, in aiding clinical decision making (eg. SLNB eligibility, surveillance intensity, and/or therapeutic choice) needs to be evaluated in the context of clinical study or trial" (Swetter et al., 2019).

European Society for Medical Oncology (ESMO)

The ESMO has stated that "testing for actionable mutations is mandatory in patients with resectable or unresectable stage III or stage IV [melanoma] [I, A], and is highly recommended in high-risk resected disease stage IIC but not for stage I or stage IIA-IIB. *BRAF* testing is mandatory [I, A]. If the tumour is *BRAF* wild type (WT) at the V600 locus (Class I *BRAF* mutant) sequencing the loci of the other known minor *BRAF* mutations (Class II and Class III *BRAF* mutant) to confirm WT status and testing for *NRAS* and *c-kit* mutations are recommended [II, C]." Additionally, "patients with metastatic melanoma should have metastasis (preferably) or the primary tumour screened for detection of *BRAF* V600 mutation treatment options for the first- and second-line settings include anti-PD-1 antibodies (pembrolizumab, nivolumab), PD-1 and ipilimumab for all patients, and BRAFi/MEKi combination for patients with *BRAF*-mutated melanoma [II, B]" (ESMO, 2019).

American Society of Clinical Oncology (Cust et al.)

The ASCO expert panel recommends needle biopsy as the preferred diagnostic approach to clinically detected lymphadenopathy in any patient with known or suspected metastatic melanoma in regional nodes. Excisional biopsy, a more invasive approach, is not routinely required for diagnosis or characterization of melanoma. The Expert Panel recommends that *BRAF* mutation testing should be performed at time of diagnosis, but clinicians are not required to wait for the results of that testing if the decision has been made to initiate immunotherapy. If prior testing resulted in a false negative, the panel states that re-testing RAF status upon progression could be considered, but it would not be of value as the panel is not aware of data that supports re-testing. Regarding immunohistochemistry, the panel states "Immunohistochemistry for the *BRAF* V600E mutation has the most rapid turnaround time; however, less common mutations may be found with other methodologies such as *BRAF* gene sequencing." Beyond *BRAF* testing though, the Expert Panel did not make formal recommendations about other genetic testing (e.g., *NRAS* and *KIT*), given insufficient data regarding the efficacy of inhibitors targeting these pathways (Seth et al., 2020).

The ASCO notes that some cancer treatments most commonly used in metastatic melanoma patients (e.g., BRAF inhibitors) may increase the risk of secondary skin cancers; hence, clinicians should be aware of this potential adverse effect when considering such therapeutic agents (Alberg et al., 2020).

European Dermatology Forum (EDF), the European Association of DermatoOncology (EADO), and the European Organization of Research and Treatment of Cancer (EORTC)

A panel of experts from EDF, EADO, and EORTC recommend that *BRAF* mutation testing be required "in patients with distant metastasis or non-resectable regional metastasis to identify those who are eligible to receive treatment with combined BRAF and MEK inhibitors, and in resected high-risk stage III melanoma patients in the adjuvant setting" (Garbe et al., 2020). Mutational analysis should be performed on metastatic tissue, either distant or regional, or on the primary tumor if metastatic tissue is not feasible. Though there may be a discrepancy rate in the *BRAF* status between the primary versus metastatic melanoma lesions, the authors note that there is "a high concordance rate in the *BRAF* status…between primary and metastatic melanoma lesions" (Garbe et al., 2022). Regarding next generation sequencing, the panel claims that it may help in identifying genetic alterations and, when limited to currently actionable genes like *BRAF*, *NRAS*, and *KIT* in a single experiment, may be cost and time effective in the clinical setting (Garbe et al., 2022).

The guideline also commented on *NRAS*, stating that *NRAS* mutations are present in 15-20% of melanoma cases and are almost always mutually exclusive with *BRAF* mutations. The guideline remarked that *NRAS* status may inform clinicians regarding the *BRAF* status, although *NRAS*-based treatments are still under investigation.

Regarding c-*KIT*, the guideline states that acral and mucosal melanomas should initially be tested for *BRAF* and *NRAS* mutations; if both genes were found to be wild-type, the sample should be tested for c-*KIT* (Garbe et al., 2020).

Tumor mutational burden (TMB) is highlighted in the guideline as a predictive tool for response to immune checkpoint inhibitors in melanoma. The panel recognized a study that demonstrated the positive association of a high TMB value with response and overall survival of melanoma patients treated with a combination of ipilimumab and nivolumab (Forschner et al., 2019).

Finally, the guideline comments on GEP tests like DecisionDx-Melanoma™ and their increased use by clinicians. While a growing pool of literature describes the potential application of GEP tests in the management of various cancers, the authors state "additional data are required in melanoma to address if they provide independent prognostic information in addition to known clinicopathologic factors before they can be integrated into clinical decision-making" (Garbe et al., 2022).

Melanoma Prevention Working Group (MPWG)

This Working Group published a guideline regarding prognostic gene expression profile [GEP] testing for cutaneous melanoma. Although the Group is "optimistic" about the future use of prognostic GEP testing to "improve risk stratification and enhance clinical decision-making", it states that "more evidence is needed to support GEP testing to inform recommendations regarding SLNB [sentinel lymph node biopsy], intensity of follow-up or imaging surveillance, and postoperative adjuvant therapy." Overall, the Group remarks that "there are insufficient data to support routine use of currently available prognostic GEP tests to inform management of patients with CM [cutaneous melanoma]" (Grossman et al., 2020).

Skin Cancer Prevention Working Group (SCPWG)

An expert consensus on the appropriate use of prognostic gene expression profiling tests for the management of cutaneous melanoma was published in 2022. The SCPWG reviewed literature for DecisionDx-Melanoma, the 11-GEP (Melagenix) test by NeraCare, and the 8-GEP (CP-GEP; Merlin Assay) by SkylineDx. The eight-person consensus panel of dermatologists unanimously agreed that:

- "Gene expression profile (GEP) tests are validated, reproducible, and consistent across studies;
- Integrating GEPs into AJCC8 and NCCN models can improve prognostic accuracy;
- Prognostic GEP tests can inform clinical decision-making regarding sentinel lymph node biopsies;
- Incorporating GEPs into real-world clinical management has positively impacted patient outcomes"

Additionally, seven out of eight panelists agreed that "the AJCC8 and NCCN prognostic model, which does not account for genomic expression, may not optimize melanoma prognostic assessment" (Farberg et al., 2022).

The National Society for Cutaneous Medicine (NSCM)

An expert panel gathered to develop consensus-based guidelines on the appropriate use criteria for the integration of diagnostic and prognostic GEP assays into the management of cutaneous malignant melanoma. These guidelines include recommendations for the 2-GEP test (DermTech PLA), the 23-GEP test (myPath®), and the 31-GEP test (DecisionDx-Melanoma). Regarding use of the 31-GEP test, a total of 14 recommendations were considered:

A-strength recommendation:

- "Use of the 31-GEP test to aid in the management of patients who are SLNBx negative"

B-strength recommendation:

- "Integration of 31-GEP results into the decision to adjust follow-up frequency
- Integration of 31-GEP results into the decision to order adjunctive imaging studies
- Integration of 31-GEP results into management of patients with T1a tumors with Breslow depth <0.8 mm and other adverse prognostic factors
- Integration of 31-GEP results into management of patients with T1 or T2 tumors who are sentinel lymph node biopsy (SLNBx) eligible
- Integration of 31-GEP results into management of patients with T1b tumors
- Integration of 31-GEP results into management of patients with T2 tumors
- Integration of 31-GEP results into management of patients with a low-risk category based on traditional AJCC factors"

C-strength recommendation:

- "Integration of 31-GEP results into the assessment of prognosis and management options for patients with T1a tumors with a positive deep margin
- Integration of 31-GEP results into the assessment of prognosis and management options for patients with T1b tumors with a positive deep margin
- Integration of 31-GEP results to for risk-stratification of patients in clinical trials
- Use of 31-GEP results as a criterion for eligibility for a chemotherapy regimen
- T4 disease as a contraindication for use of the 31-GEP test
- Melanoma in situ as a contraindication for use of the 31-GEP test"

Seven recommendations for use of the 2-GEP test were considered as follows:

B-strength recommendation:

- "Cases in which patients present with atypical lesions requiring additional assessment beyond inspection in order to aid in the biopsy decision"

C-strength recommendations:

- Cases in which patients refuse surgical biopsy
- Cases in which suspicious lesions present in cosmetically sensitive areas
- Cases in which patients have undergone numerous biopsies in the past and wish to avoid additional biopsy procedures
- Scenarios in which patients have a relative contraindication to surgical biopsy
- Patients with increased risk of infection (e.g., immunosuppressed patients)

- Patients with heightened risk of poor wound healing

Expert consensus was reached on eight recommendations for use of the 23-GEP test:

A-strength recommendation:

- "Differentiation of a nevus from melanoma in an adult patient when the morphologic findings are ambiguous by light microscopic parameters"

B-strength recommendations:

- "Cases with pathology suggestive or suspicious for nevoid melanoma vs. benign melanocytic nevus
- Cases with pathology suggestive or suspicious for atypical Spitz tumor vs. Spitzoid melanoma
- Instances in which pathology is suggestive of or suspicious for severely atypical compound melanocytic proliferation vs melanoma on cosmetically sensitive areas"

C-strength recommendations:

- "Instances of pathology suggestive or suspicious for melanoma arising from within a severely dysplastic nevus
- Cases of pathology suggestive or suspicious for benign vs. malignant blue nevus
- To differentiate suspicious lesions in low-risk populations
- Upon request from the referring dermatologist following an ambiguous pathology report" (Berman et al., 2019)

In a separate editorial published by the NSCM discussing the management of pigmented lesions, the authors conclude that "[u]se of the 2-GEP [DermTech PLA] improves patient care and improves health outcomes by using genomic assessments to minimize avoidable surgical procedures on benign lesions, enriching the population of melanomas within biopsied lesions, and decreasing downstream costs of late-stage melanoma diagnoses. For all of these reasons, creating broad access and coverage to transformative diagnostic technologies such as the 2-GEP is in the best interest of our colleagues, the healthcare system, and most importantly, our patients" (Cockerell et al., 2022).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations

(LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

The FDA-approvals for all the BRAF-targeted therapies include the requirement that *BRAF* mutation testing be performed by an FDA-approved test.

On August 17, 2011, the U.S. Food and Drug Administration (FDA) announced the approval of Zelboraf (vemurafenib) for unresectable or metastatic melanoma with oncogenic *BRAF* mutation (V600E). The Cobas® 4800 *BRAF* V600 Mutation Test was approved as the companion diagnostic for vemurafenib (Bollag et al., 2012).

Dabrafenib was FDA-approved in May 2013 for the treatment of patients with unresectable or metastatic melanoma with *BRAF* V600E mutation, as detected by an FDA-approved test. Dabrafenib is specifically not indicated for the treatment of patients with wild-type *BRAF* melanoma (Tafinlar (dabrafenib), Jan 2014).

Trametinib was FDA-approved in May 2013 for the treatment of patients with unresectable or metastatic melanoma with *BRAF* V600E or V600K mutations, as detected by an FDA-approved test. Trametinib is specifically not indicated for the treatment of patients previously treated with *BRAF* inhibitor therapy (GlaxoSmithKline. Mekinist Aug 2014).

The companion diagnostic test coapproved for both dabrafenib and trametinib is the THxID™ *BRAF* Kit manufactured by bioMérieux. The kit is intended "as an aid in selecting melanoma patients whose tumors carry the *BRAF* V600E mutation for treatment with dabrafenib and as an aid in selecting melanoma patients whose tumors carry the *BRAF* V600E or V600K mutation for treatment with trametinib" (Genentech, Inc. Zelboraf® March 2014).

The FDA approved the use of the Oncomine Dx target test NGS panel for somatic or germline variants, which includes the *BRAF* V600E mutation for consideration with dabrafenib therapy as one of the gene variants (Life Technologies Corporation, approved in June 2017).

The FDA approved the FoundationOne CDx NGS panel in November 2017, which does include both the V600E and V600K mutation for possible dabrafenib or vemurafenib therapy (Foundation Medicine, Inc.).

The FDA approved the use of Therascreen BRAF V600E RGQ PCR Kit in April 2020, a real time PCR test that detects *BRAF V600E* mutations. The Therascreen BRAF V600E RGQ PCR Kit is for use on the Rotor-Gene Q MDx (US) instrument.

New therapies for melanoma continue to receive FDA approval, and the National Cancer Institute summarizes them here: https://www.cancer.gov/about-cancer/treatment/drugs/melanoma (NCI, 2024).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81210	BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer, melanoma), gene analysis, V600 variant(s)
81272	KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) (eg, gastrointestinal stromal tumor [GIST], acute myeloid leukemia, melanoma), gene analysis, targeted sequence analysis (eg, exons 8, 11, 13, 17, 18)
81273	KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) (eg, mastocytosis), gene analysis, D816 variant(s)
81311	NRAS (neuroblastoma RAS viral [v-ras] oncogene homolog) (eg, colorectal carcinoma), gene analysis, variants in exon 2 (eg, codons 12 and 13) and exon 3 (eg, codon 61)
81345	TERT (telomerase reverse transcriptase) (eg, thyroid carcinoma, glioblastoma multiforme) gene analysis, targeted sequence analysis (eg, promoter region)
81404	Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)
81479	Unlisted molecular pathology procedure
81529	Oncology (cutaneous melanoma), mRNA, gene expression profiling by real-time RT-PCR of 31 genes (28 content and 3 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as recurrence risk, including likelihood of sentinel lymph node metastasis

	Proprietary test: DecisionDx® Melanoma Lab/Manufacturer: Castle Biosciences, Inc
0089U	Oncology (melanoma), gene expression profiling by RTqPCR, PRAME and LINC00518, superficial collection using adhesive patch(es) Proprietary test: Pigmented Lesion Assay (PLA) Lab/Manufacturer: DermTech
0090U	Oncology (cutaneous melanoma), mRNA gene expression profiling by RT-PCR of 23 genes (14 content and 9 housekeeping), utilizing formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as a categorical result (i.e., benign, intermediate, malignant) Proprietary test: myPath® Melanoma Lab/Manufacturer: Myriad Genetic Laboratories
0314U	Oncology (cutaneous melanoma), mRNA gene expression profiling by RT-PCR of 35 genes (32 content and 3 housekeeping), utilizing formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as a categorical result (i.e., benign, intermediate, malignant) Proprietary test: DecisionDx® DiffDx™- Melanoma Lab/Manufacturer: Castle Biosciences, Inc
0387U	Oncology (melanoma), autophagy and beclin 1 regulator 1 (AMBRA1) and loricrin A by immunohistochemistry, formalin-fixed paraffin-embedded (FFPE) tissue, report for risk of progression Proprietary test: AMBLor® melanoma prognostic test Lab/Manufacturer: Avero® Diagnostics
0474U	Hereditary pan-cancer (eg, hereditary sarcomas, hereditary endocrine tumors, hereditary neuroendocrine tumors, hereditary cutaneous melanoma), genomic sequence analysis panel of 88 genes with 20 duplications/deletions using next-generation sequencing (NGS), Sanger sequencing, blood or saliva, reported as positive or negative for germline variants, each gene Proprietary test: GeneticsNow® Comprehensive Germline Panel Lab/Manufacturer: GoPath Diagnostics, Inc

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Alberg, A. J., LoConte, N. K., Foxhall, L., Weinstock, M. A., Gomez, S. L., Francisco, M., Moushey, E. A., & Gershenwald, J. E. (2020). American Society of Clinical Oncology

Policy Statement on Skin Cancer Prevention. *JCO Oncol Pract*, *16*(8), 490-499. https://doi.org/10.1200/JOP.19.00585

Alrabadi, N., Gibson, N., Curless, K., Cheng, L., Kuhar, M., Chen, S., Warren, S. J. P., & Alomari, A. K. (2019). Detection of driver mutations in BRAF can aid in diagnosis and early treatment of dedifferentiated metastatic melanoma. *Mod Pathol*, *32*(3), 330-337. https://doi.org/10.1038/s41379-018-0161-0

Amaral, T. M. S., Hoffmann, M. C., Sinnberg, T., Niessner, H., Sülberg, H., Eigentler, T. K., & Garbe, C. (2020). Clinical validation of a prognostic 11-gene expression profiling score in prospectively collected FFPE tissue of patients with AJCC v8 stage II cutaneous melanoma. *Eur J Cancer*, *125*, 38-45. https://doi.org/10.1016/j.ejca.2019.10.027

Amaria, R. N., Prieto, P. A., Tetzlaff, M. T., Reuben, A., Andrews, M. C., Ross, M. I., Glitza, I. C., Cormier, J., Hwu, W. J., Tawbi, H. A., Patel, S. P., Lee, J. E., Gershenwald, J. E., Spencer, C. N., Gopalakrishnan, V., Bassett, R., Simpson, L., Mouton, R., Hudgens, C. W., . . . Wargo, J. A. (2018). Neoadjuvant plus adjuvant dabrafenib and trametinib versus standard of care in patients with high-risk, surgically resectable melanoma: a single-centre, open-label, randomised, phase 2 trial. *Lancet Oncol*, *19*(2), 181-193. https://doi.org/10.1016/S1470-2045(18)30015-9

AMLo Biosciences. (2023). *Avero Diagnostics launches melanoma progression test with AMLo*. Retrieved April 24, 2023 from https://usa.amlo-biosciences.com/avero-diagnostics-launches-melanoma-progression-test-with-amlo/

Aoude, L. G., Bonazzi, V. F., Brosda, S., Patel, K., Koufariotis, L. T., Oey, H., Nones, K., Wood, S., Pearson, J. V., Lonie, J. M., Arneil, M., Atkinson, V., Smithers, B. M., Waddell, N., Barbour, A. P., Aoude, L. G., Bonazzi, V. F., Brosda, S., Patel, K., . . . Barbour, A. P. (2020). Pathogenic germline variants are associated with poor survival in stage III/IV melanoma patients. *Sci Rep*, *10*(1), 17687. https://doi.org/10.1038/s41598-020-74956-3

Atkins, M. B., Kunkel, L., Sznol, M., & Rosenberg, S. A. (2000). High-dose recombinant interleukin-2 therapy in patients with metastatic melanoma: long-term survival update. *Cancer J Sci Am*, *6 Suppl 1*, S11-14. https://pubmed.ncbi.nlm.nih.gov/10685652/

Bellomo, D., Arias-Mejias, S. M., Ramana, C., Heim, J. B., Quattrocchi, E., Sominidi-Damodaran, S., Bridges, A. G., Lehman, J. S., Hieken, T. J., Jakub, J. W., Pittelkow, M. R., DiCaudo, D. J., Pockaj, B. A., Sluzevich, J. C., Cappel, M. A., Bagaria, S. P., Perniciaro, C., Tjien-Fooh, F. J., van Vliet, M. H., . . . Meves, A. (2020). Model Combining Tumor Molecular and Clinicopathologic Risk Factors Predicts Sentinel Lymph Node Metastasis in Primary Cutaneous Melanoma. *JCO Precis Oncol*, *4*, 319-334. https://doi.org/10.1200/po.19.00206

Berman, B., Ceilley, R., COckerell, C., Ferria, L., High, W., Lebwohl, M., Nestor, M., Rosen, T., Litchman, G., Prado, G., Svoboda, R., & Rigel, D. (2019). Appropriate Use Criteria

for the Integration of Diagnostic and Prognostic Gene Expression Profile Assays into the Management of Cutaneous Malignant Melanoma: An Expert Panel Consensus-Based Modified Delphi Process Assessment. *SKIN The Journal of Cutaneous Medicine*, *3*(5). https://skin.dermsquared.com/skin/article/view/663

Bertolotto, C., Lesueur, F., Giuliano, S., Strub, T., de Lichy, M., Bille, K., Dessen, P., d'Hayer, B., Mohamdi, H., Remenieras, A., Maubec, E., de la Fouchardiere, A., Molinie, V., Vabres, P., Dalle, S., Poulalhon, N., Martin-Denavit, T., Thomas, L., Andry-Benzaquen, P., . . . Bressac-de Paillerets, B. (2011). A SUMOylation-defective MITF germline mutation predisposes to melanoma and renal carcinoma. *Nature*, *480*(7375), 94-98. https://doi.org/10.1038/nature10539

Berwick, M., Orlow, I., Hummer, A. J., Armstrong, B. K., Kricker, A., Marrett, L. D., Millikan, R. C., Gruber, S. B., Anton-Culver, H., Zanetti, R., Gallagher, R. P., Dwyer, T., Rebbeck, T. R., Kanetsky, P. A., Busam, K., From, L., Mujumdar, U., Wilcox, H., Begg, C. B., & Group, G. E. M. S. (2006). The prevalence of CDKN2A germ-line mutations and relative risk for cutaneous malignant melanoma: an international population-based study. *Cancer Epidemiol Biomarkers Prev*, *15*(8), 1520-1525. https://doi.org/10.1158/1055-9965.EPI-06-0270

Bishop, D. T., Demenais, F., Goldstein, A. M., Bergman, W., Bishop, J. N., Bressac-de Paillerets, B., Chompret, A., Ghiorzo, P., Gruis, N., Hansson, J., Harland, M., Hayward, N., Holland, E. A., Mann, G. J., Mantelli, M., Nancarrow, D., Platz, A., Tucker, M. A., & Melanoma Genetics, C. (2002). Geographical variation in the penetrance of CDKN2A mutations for melanoma. *J Natl Cancer Inst*, *94*(12), 894-903. https://www.ncbi.nlm.nih.gov/pubmed/12072543

Bollag, G., Tsai, J., Zhang, J., Zhang, C., Ibrahim, P., Nolop, K., & Hirth, P. (2012). Vemurafenib: the first drug approved for BRAF-mutant cancer. *Nat Rev Drug Discov*, *11*(11), 873-886. https://doi.org/10.1038/nrd3847

Bollard, S. M., Casalou, C., & Potter, S. M. (2021). Gene expression profiling in melanoma: A view from the clinic. *Cancer Treat Res Commun*, *29*, 100447. https://doi.org/10.1016/j.ctarc.2021.100447

Brunner, G., Heinecke, A., Falk, T. M., Ertas, B., Blodorn-Schlicht, N., Schulze, H. J., Suter, L., Atzpodien, J., & Berking, C. (2018). A Prognostic Gene Signature Expressed in Primary Cutaneous Melanoma: Synergism With Conventional Staging. *JNCI Cancer Spectr*, *2*(3), pky032. https://doi.org/10.1093/jncics/pky032

Brunner, G., Reitz, M., Heinecke, A., Lippold, A., Berking, C., Suter, L., & Atzpodien, J. (2013). A nine-gene signature predicting clinical outcome in cutaneous melanoma. *J Cancer Res Clin Oncol*, *139*(2), 249-258. https://doi.org/10.1007/s00432-012-1322-z

Bubien, V., Bonnet, F., Brouste, V., Hoppe, S., Barouk-Simonet, E., David, A., Edery, P., Bottani, A., Layet, V., Caron, O., Gilbert-Dussardier, B., Delnatte, C., Dugast, C., Fricker, J. P., Bonneau, D., Sevenet, N., Longy, M., Caux, F., & French Cowden Disease, N. (2013). High cumulative risks of cancer in patients with PTEN hamartoma tumour

syndrome. *J Med Genet*, *50*(4), 255-263. https://doi.org/10.1136/jmedgenet-2012-101339
Burjanivova, T., Malicherova, B., Grendar, M., Minarikova, E., Dusenka, R., Vanova, B., Bobrovska, M., Pecova, T., Homola, I., Lasabova, Z., & Plank, L. (2019). Detection of BRAFV600E Mutation in Melanoma Patients by Digital PCR of Circulating DNA. *Genet Test Mol Biomarkers*, *23*(4), 241-245. https://doi.org/10.1089/gtmb.2018.0193
Burke, L. S., Hyland, P. L., Pfeiffer, R. M., Prescott, J., Wheeler, W., Mirabello, L., Savage, S. A., Burdette, L., Yeager, M., Chanock, S., De Vivo, I., Tucker, M. A., Goldstein, A. M., & Yang, X. R. (2013). Telomere length and the risk of cutaneous malignant melanoma in melanoma-prone families with and without CDKN2A mutations. *PLoS One*, *8*(8), e71121. https://doi.org/10.1371/journal.pone.0071121
Bush, J. W., Hunt, E. L., & Fraga, G. R. (2015). BAM! Utilizing the frequency of Benign, Atypical and Malignant diagnoses for quality improvement in the histopathologic diagnosis of melanocytic neoplasms. *J Cutan Pathol*, *42*(10), 712-716. https://doi.org/10.1111/cup.12566
Castle Biosciences. (2024a). DecisionDx®-Melanoma Overview. https://castlebiosciences.com/products/decisiondx-melanoma/
Castle Biosciences. (2024b). myPath® Melanoma. https://castlebiosciences.com/products/mypath-melanoma-and-diffdx-melanoma/
Castle Biosciences Inc. (2022). myPath Melanoma Final Report. https://castlebiosciences.com/Sample%20Report%20and%20Order%20Form/Bengin_MPM-Final-Report-Template_v1.4.pdf
CastleBiosciences. (2024). *DecisionDx®-Melanoma Overview*. https://castlebiosciences.com/products/decisiondx-melanoma/
Chiaravalloti, A. J., Jinna, S., Kerr, P. E., Whalen, J., & Grant-Kels, J. M. (2018). A deep look into thin melanomas: What's new for the clinician and the impact on the patient. *Int J Womens Dermatol*, *4*(3), 119-121. https://doi.org/10.1016/j.ijwd.2018.01.003
Clarke, L. E., Flake, D. D., 2nd, Busam, K., Cockerell, C., Helm, K., McNiff, J., Reed, J., Tschen, J., Kim, J., Barnhill, R., Elenitsas, R., Prieto, V. G., Nelson, J., Kimbrell, H., Kolquist, K. A., Brown, K. L., Warf, M. B., Roa, B. B., & Wenstrup, R. J. (2017). An independent validation of a gene expression signature to differentiate malignant melanoma from benign melanocytic nevi. *Cancer*, *123*(4), 617-628. https://doi.org/10.1002/cncr.30385
Clarke, L. E., Mabey, B., Flake Ii, D. D., Meek, S., Cassarino, D. S., Duncan, L. M., High, W. A., Napekoski, K. M., Prieto, V. G., Tetzlaff, M. T., Vitale, P., & Elder, D. E. (2020). Clinical validity of a gene expression signature in diagnostically uncertain neoplasms. *Per Med*, *17*(5), 361-371. https://doi.org/10.2217/pme-2020-0048
Clarke, L. E., Warf, M. B., Flake, D. D., 2nd, Hartman, A. R., Tahan, S., Shea, C. R., Gerami, P., Messina, J., Florell, S. R., Wenstrup, R. J., Rushton, K., Roundy, K. M., Rock, C., Roa, B., Kolquist, K. A., Gutin, A., Billings, S., & Leachman, S. (2015). Clinical validation of a

gene expression signature that differentiates benign nevi from malignant melanoma. *J Cutan Pathol*, *42*(4), 244-252. https://doi.org/10.1111/cup.12475

CMGL. (2014). *Molecular Genetic Testing For BRAF Mutations*.

Cockerell, C., Ceilley, R., Lebwohl, M., & Rigel, D. (2022). Using Genomics to Improve Pigmented Lesion Management & Health Outcomes. *SKIN The Journal of Cutaneous Medicine*, *6*(2). https://pdfs.semanticscholar.org/583d/a6a748efb80acb4165b68a272e82ce433fd1.pdf

Cockerell, C., Tschen, J., Billings, S. D., Evans, B., Brown, K., Rock, C., & Clarke, L. E. (2017). The influence of a gene-expression signature on the treatment of diagnostically challenging melanocytic lesions. *Per Med*, *14*(2), 123-130. https://doi.org/10.2217/pme-2016-0097

Colombino, M., Rozzo, C., Paliogiannis, P., Casula, M., Manca, A., Doneddu, V., Fedeli, M. A., Sini, M. C., Palomba, G., Pisano, M., Ascierto, P. A., Caracò, C., Lissia, A., Cossu, A., & Palmieri, G. (2020). Comparison of BRAF Mutation Screening Strategies in a Large Real-Life Series of Advanced Melanoma Patients. *J Clin Med*, *9*(8). https://doi.org/10.3390/jcm9082430

Corean, J. L. E., George, T. I., Patel, J. L., & Li, K. D. (2019). Bone marrow findings in metastatic melanoma, including role of BRAF immunohistochemistry. *Int J Lab Hematol*, *41*(4), 550-560. https://doi.org/10.1111/ijlh.13051

Cosgarea, I., McConnell, A. T., Ewen, T., Tang, D., Hill, D. S., Anagnostou, M., Elias, M., Ellis, R. A., Murray, A., Spender, L. C., Giglio, P., Gagliardi, M., Greenwood, A., Piacentini, M., Inman, G. J., Fimia, G. M., Corazzari, M., Armstrong, J. L., & Lovat, P. E. (2022). Melanoma secretion of transforming growth factor-β2 leads to loss of epidermal AMBRA1 threatening epidermal integrity and facilitating tumour ulceration*. *British Journal of Dermatology*, *186*(4), 694-704. https://doi.org/10.1111/bjd.20889

Cree, I. A., Deans, Z., Ligtenberg, M. J., Normanno, N., Edsjö, A., Rouleau, E., Solé, F., Thunnissen, E., Timens, W., Schuuring, E., Dequeker, E., Murray, S., Dietel, M., Groenen, P., & Van Krieken, J. H. (2014). Guidance for laboratories performing molecular pathology for cancer patients. *J Clin Pathol*, *67*(11), 923-931. https://doi.org/10.1136/jclinpath-2014-202404

Cust, A. E., Harland, M., Makalic, E., Schmidt, D., Dowty, J. G., Aitken, J. F., Agha-Hamilton, C., Armstrong, B. K., Barrett, J. H., Chan, M., Chang, Y. M., Gascoyne, J., Giles, G. G., Holland, E. A., Kefford, R. F., Kukalizch, K., Lowery, J., Randerson-Moor, J. A., Schmid, H., . . . Jenkins, M. A. (2011). Melanoma risk for CDKN2A mutation carriers who are relatives of population-based case carriers in Australia and the UK. *J Med Genet*, *48*(4), 266-272. https://doi.org/10.1136/jmg.2010.086538

Davies, M. A., & Gershenwald, J. E. (2011). Targeted therapy for melanoma: a primer. *Surg Oncol Clin N Am*, *20*(1), 165-180. https://pubmed.ncbi.nlm.nih.gov/21111965/

Delaunay, J., Martin, L., Bressac-de Paillerets, B., Duru, G., Ingster, O., & Thomas, L. (2017). Improvement of Genetic Testing for Cutaneous Melanoma in Countries With Low to Moderate Incidence: The Rule of 2 vs the Rule of 3. *JAMA Dermatol*, *153*(11), 1122-1129. https://doi.org/10.1001/jamadermatol.2017.2926

DermTech. (2023a). The DermTech Melanoma Test. https://dermtech.com/how-it-works/

DermTech. (2023b). *PLA Pigmented Lesion Assay*. https://dermtech.com/physicians/

Ellis, R., Tang, D., Nasr, B., Greenwood, A., McConnell, A., Anagnostou, M. E., Elias, M., Verykiou, S., Bajwa, D., Ewen, T., Reynolds, N. J., Barrett, P., Carling, E., Watson, G., Armstrong, J., Allen, A. J., Horswell, S., Labus, M., & Lovat, P. E. (2020). Epidermal autophagy and beclin 1 regulator 1 and loricrin: a paradigm shift in the prognostication and stratification of the American Joint Committee on Cancer stage I melanomas. *Br J Dermatol*, *182*(1), 156-165. https://doi.org/10.1111/bjd.18086

Elmore, J. G., Barnhill, R. L., Elder, D. E., Longton, G. M., Pepe, M. S., Reisch, L. M., Carney, P. A., Titus, L. J., Nelson, H. D., Onega, T., Tosteson, A. N. A., Weinstock, M. A., Knezevich, S. R., & Piepkorn, M. W. (2017). Pathologists' diagnosis of invasive melanoma and melanocytic proliferations: observer accuracy and reproducibility study. *BMJ*, *357*, j2813. https://doi.org/10.1136/bmj.j2813

ESMO. (2019). Cutaneous melanoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*. https://www.esmo.org/Guidelines/Melanoma/Cutaneous-Melanoma

Farberg, A. S., Marson, J. W., Glazer, A., Litchman, G. H., Svoboda, R., Winkelmann, R. R., Brownstone, N., Rigel, D. S., & Skin Cancer Prevention Working, G. (2022). Expert Consensus on the Use of Prognostic Gene Expression Profiling Tests for the Management of Cutaneous Melanoma: Consensus from the Skin Cancer Prevention Working Group. *Dermatol Ther (Heidelb)*, *12*(4), 807-823. https://doi.org/10.1007/s13555-022-00709-x

Farmer, E. R., Gonin, R., & Hanna, M. P. (1996). Discordance in the histopathologic diagnosis of melanoma and melanocytic nevi between expert pathologists. *Hum Pathol*, *27*(6), 528-531. https://doi.org/10.1016/S0046-8177(96)90157-4

Ferris, L. K., Gerami, P., Skelsey, M. K., Peck, G., Hren, C., Gorman, C., Frumento, T., & Siegel, D. M. (2018). Real-world performance and utility of a noninvasive gene expression assay to evaluate melanoma risk in pigmented lesions. *Melanoma Res*, *28*(5), 478-482. https://doi.org/10.1097/cmr.0000000000000478

Ferris, L. K., Jansen, B., Ho, J., Busam, K. J., Gross, K., Hansen, D. D., Alsobrook, J. P., 2nd, Yao, Z., Peck, G. L., & Gerami, P. (2017). Utility of a Noninvasive 2-Gene Molecular Assay for Cutaneous Melanoma and Effect on the Decision to Biopsy. *JAMA Dermatol*, *153*(7), 675-680. https://doi.org/10.1001/jamadermatol.2017.0473

Ferris, L. K., Moy, R. L., Gerami, P., Sligh, J. E., Jansen, B., Yao, Z., & Cockerell, C. J. (2019). Noninvasive Analysis of High-Risk Driver Mutations and Gene Expression Profiles in

Primary Cutaneous Melanoma. *J Invest Dermatol*, *139*(5), 1127-1134. https://doi.org/10.1016/j.jid.2018.10.041

Ferris, L. K., Rigel, D. S., Siegel, D. M., Skelsey, M. K., Peck, G. L., Hren, C., Gorman, C., Frumento, T., Jansen, B., Yao, Z., Rock, J., Knezevich, S. R., & Cockerell, C. J. (2019). Impact on clinical practice of a non-invasive gene expression melanoma rule-out test: 12-month follow-up of negative test results and utility data from a large US registry study. *Dermatol Online J*, *25*(5). https://escholarship.org/uc/item/61w6h7mn

Flaherty, K. T., Puzanov, I., Kim, K. B., Ribas, A., McArthur, G. A., Sosman, J. A., O'Dwyer, P. J., Lee, R. J., Grippo, J. F., Nolop, K., & Chapman, P. B. (2010). Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med*, *363*(9), 809-819. https://doi.org/10.1056/NEJMoa1002011

Forschner, A., Battke, F., Hadaschik, D., Schulze, M., Weissgraeber, S., Han, C. T., Kopp, M., Frick, M., Klumpp, B., Tietze, N., Amaral, T., Martus, P., Sinnberg, T., Eigentler, T., Keim, U., Garbe, C., Docker, D., & Biskup, S. (2019). Tumor mutation burden and circulating tumor DNA in combined CTLA-4 and PD-1 antibody therapy in metastatic melanoma - results of a prospective biomarker study. *J Immunother Cancer*, *7*(1), 180. https://doi.org/10.1186/s40425-019-0659-0

Froyen, G., Broekmans, A., Hillen, F., Pat, K., Achten, R., Mebis, J., Rummens, J. L., Willemse, J., & Maes, B. (2016). Validation and Application of a Custom-Designed Targeted Next-Generation Sequencing Panel for the Diagnostic Mutational Profiling of Solid Tumors. In *PLoS One* (Vol. 11). https://doi.org/10.1371/journal.pone.0154038

Fulgent. (2023). *Melanoma Comprehensive Panel*. https://www.fulgentgenetics.com/comprehensivecancer-melanoma

Gambichler, T., Tsagoudis, K., Kiecker, F., Reinhold, U., Stockfleth, E., Hamscho, R., Egberts, F., Hauschild, A., Amaral, T., & Garbe, C. (2021). Prognostic significance of an 11-gene RNA assay in archival tissue of cutaneous melanoma stage I-III patients. *Eur J Cancer*, *143*, 11-18. https://doi.org/10.1016/j.ejca.2020.10.016

Garbe, C., Amaral, T., Peris, K., Hauschild, A., Arenberger, P., Basset-Seguin, N., Bastholt, L., Bataille, V., Del Marmol, V., Dreno, B., Fargnoli, M. C., Forsea, A. M., Grob, J. J., Holler, C., Kaufmann, R., Kelleners-Smeets, N., Lallas, A., Lebbe, C., Lytvynenko, B., . . . Treatment of, C. (2022). European consensus-based interdisciplinary guideline for melanoma. Part 1: Diagnostics: Update 2022. *Eur J Cancer*, *170*, 236-255. https://doi.org/10.1016/j.ejca.2022.03.008

Garbe, C., Amaral, T., Peris, K., Hauschild, A., Arenberger, P., Bastholt, L., Bataille, V., Del Marmol, V., Dréno, B., Fargnoli, M. C., Grob, J. J., Höller, C., Kaufmann, R., Lallas, A., Lebbé, C., Malvehy, J., Middleton, M., Moreno-Ramirez, D., Pellacani, G., . . . Eggermont, A. M. M. (2020). European consensus-based interdisciplinary guideline for melanoma. Part 1: Diagnostics - Update 2019. *Eur J Cancer*, *126*, 141-158. https://doi.org/10.1016/j.ejca.2019.11.014

Gastman, B. R., Zager, J. S., Messina, J. L., Cook, R. W., Covington, K. R., Middlebrook, B., Gerami, P., Wayne, J. D., Leachman, S., & Vetto, J. T. (2019). Performance of a 31-gene expression profile test in cutaneous melanomas of the head and neck. *Head Neck*, *41*(4), 871-879. https://doi.org/10.1002/hed.25473

GeneDx. (2023). *Melanoma Panel*. https://www.genedx.com/tests/detail/melanoma-panel-831

Gerami, P., Busam, K., Cochran, A., Cook, M. G., Duncan, L. M., Elder, D. E., Fullen, D. R., Guitart, J., LeBoit, P. E., Mihm, M. C., Jr., Prieto, V. G., Rabkin, M. S., Scolyer, R. A., Xu, X., Yun, S. J., Obregon, R., Yazdan, P., Cooper, C., Weitner, B. B., . . . Barnhill, R. L. (2014). Histomorphologic assessment and interobserver diagnostic reproducibility of atypical spitzoid melanocytic neoplasms with long-term follow-up. *Am J Surg Pathol*, *38*(7), 934-940. https://doi.org/10.1097/PAS.0000000000000198

Gerami, P., Yao, Z., Polsky, D., Jansen, B., Busam, K., Ho, J., Martini, M., & Ferris, L. K. (2017). Development and validation of a noninvasive 2-gene molecular assay for cutaneous melanoma. *J Am Acad Dermatol*, *76*(1), 114-120.e112. https://doi.org/10.1016/j.jaad.2016.07.038

Ghate, S., Ionescu-Ittu, R., Burne, R., Ndife, B., Laliberte, F., Nakasato, A., & Duh, M. S. (2019). Patterns of treatment and BRAF testing with immune checkpoint inhibitors and targeted therapy in patients with metastatic melanoma presumed to be BRAF positive. *Melanoma Res*, *29*(3), 301-310. https://doi.org/10.1097/cmr.0000000000000504

Goldstein, A. M., Chan, M., Harland, M., Hayward, N. K., Demenais, F., Bishop, D. T., Azizi, E., Bergman, W., Bianchi-Scarra, G., Bruno, W., Calista, D., Albright, L. A., Chaudru, V., Chompret, A., Cuellar, F., Elder, D. E., Ghiorzo, P., Gillanders, E. M., Gruis, N. A., . . . Melanoma Genetics, C. (2007). Features associated with germline CDKN2A mutations: a GenoMEL study of melanoma-prone families from three continents. *J Med Genet*, *44*(2), 99-106. https://doi.org/10.1136/jmg.2006.043802

Goldstein, A. M., & Tucker, M. A. (2001). Genetic epidemiology of cutaneous melanoma: a global perspective. *Arch Dermatol*, *137*(11), 1493-1496. https://doi.org/10.1001/archderm.137.11.1493

Greenhaw, B. N., Covington, K. R., Kurley, S. J., Yeniay, Y., Cao, N. A., Plasseraud, K. M., Cook, R. W., Hsueh, E. C., Gastman, B. R., & Wei, M. L. (2020). Molecular risk prediction in cutaneous melanoma: a meta-analysis of the 31-gene expression profile prognostic test in 1,479 patients. *J Am Acad Dermatol*. https://doi.org/10.1016/j.jaad.2020.03.053

Grossman, D., Okwundu, N., Bartlett, E. K., Marchetti, M. A., Othus, M., Coit, D. G., Hartman, R. I., Leachman, S. A., Berry, E. G., Korde, L., Lee, S. J., Bar-Eli, M., Berwick, M., Bowles, T., Buchbinder, E. I., Burton, E. M., Chu, E. Y., Curiel-Lewandrowski, C., Curtis, J. A., . . . Swetter, S. M. (2020). Prognostic Gene Expression Profiling in Cutaneous

Melanoma: Identifying the Knowledge Gaps and Assessing the Clinical Benefit. *JAMA Dermatology*, *156*(9), 1004-1011. https://doi.org/10.1001/jamadermatol.2020.1729

Grossmann, A. H., Grossmann, K. F., & Wallander, M. L. (2012). Molecular testing in malignant melanoma. *Diagn Cytopathol*, *40*(6), 503-510. https://doi.org/10.1002/dc.22810

Harland, M., Petljak, M., Robles-Espinoza, C. D., Ding, Z., Gruis, N. A., van Doorn, R., Pooley, K. A., Dunning, A. M., Aoude, L. G., Wadt, K. A., Gerdes, A. M., Brown, K. M., Hayward, N. K., Newton-Bishop, J. A., Adams, D. J., & Bishop, D. T. (2016). Germline TERT promoter mutations are rare in familial melanoma. *Fam Cancer*, *15*(1), 139-144. https://doi.org/10.1007/s10689-015-9841-9

Hawkes, J. E., Truong, A., & Meyer, L. J. (2016). Genetic predisposition to melanoma. *Semin Oncol*, *43*(5), 591-597. https://doi.org/10.1053/j.seminoncol.2016.08.003

Hocker, T., & Tsao, H. (2007). Ultraviolet radiation and melanoma: a systematic review and analysis of reported sequence variants. *Hum Mutat*, *28*(6), 578-588. https://doi.org/10.1002/humu.20481

Hodi, F. S., O'Day, S. J., McDermott, D. F., Weber, R. W., Sosman, J. A., Haanen, J. B., Gonzalez, R., Robert, C., Schadendorf, D., Hassel, J. C., Akerley, W., van den Eertwegh, A. J., Lutzky, J., Lorigan, P., Vaubel, J. M., Linette, G. P., Hogg, D., Ottensmeier, C. H., Lebbe, C., . . . Urba, W. J. (2010). Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*, *363*(8), 711-723. https://doi.org/10.1056/NEJMoa1003466

Holmes, D. (2014). The cancer that rises with the sun. *Nature*, *515*(7527), S110-111. https://doi.org/10.1038/515S110a

Horn, S., Figl, A., Rachakonda, P. S., Fischer, C., Sucker, A., Gast, A., Kadel, S., Moll, I., Nagore, E., Hemminki, K., Schadendorf, D., & Kumar, R. (2013). TERT promoter mutations in familial and sporadic melanoma. *Science*, *339*(6122), 959-961. https://doi.org/10.1126/science.1230062

Hornberger, J., & Siegel, D. M. (2018). Economic Analysis of a Noninvasive Molecular Pathologic Assay for Pigmented Skin Lesions. *JAMA Dermatol*, *154*(9), 1025-1031. https://doi.org/10.1001/jamadermatol.2018.1764

Hussussian, C. J., Struewing, J. P., Goldstein, A. M., Higgins, P. A., Ally, D. S., Sheahan, M. D., Clark, W. H., Jr., Tucker, M. A., & Dracopoli, N. C. (1994). Germline p16 mutations in familial melanoma. *Nat Genet*, *8*(1), 15-21. https://doi.org/10.1038/ng0994-15

Invitae. (2024). *Invitae Melanoma Panel*. https://www.invitae.com/en/physician/tests/01561/

Kangas-Dick, A. W., Greenbaum, A., Gall, V., Groisberg, R., Mehnert, J., Chen, C., Moore, D. F., Berger, A. C., & Koshenkov, V. (2021). Evaluation of a Gene Expression Profiling Assay in Primary Cutaneous Melanoma. *Ann Surg Oncol*. https://doi.org/10.1245/s10434-020-09563-7

Keller, J., Schwartz, T. L., Lizalek, J. M., Chang, E. S., Patel, A. D., Hurley, M. Y., & Hsueh, E. C. (2019). Prospective validation of the prognostic 31-gene expression profiling test in primary cutaneous melanoma. *Cancer Med*, *8*(5), 2205-2212. https://doi.org/10.1002/cam4.2128

Ko, J. S., Matharoo-Ball, B., Billings, S. D., Thomson, B. J., Tang, J. Y., Sarin, K. Y., Cai, E., Kim, J., Rock, C., Kimbrell, H. Z., Flake, D. D., 2nd, Warf, M. B., Nelson, J., Davis, T., Miller, C., Rushton, K., Hartman, A. R., Wenstrup, R. J., & Clarke, L. E. (2017). Diagnostic Distinction of Malignant Melanoma and Benign Nevi by a Gene Expression Signature and Correlation to Clinical Outcomes. *Cancer Epidemiol Biomarkers Prev*, *26*(7), 1107-1113. https://doi.org/10.1158/1055-9965.EPI-16-0958

Koh, J., Enders, G. H., Dynlacht, B. D., & Harlow, E. (1995). Tumour-derived p16 alleles encoding proteins defective in cell-cycle inhibition. *Nature*, *375*(6531), 506-510. https://doi.org/10.1038/375506a0

Labus, M., Ellis, R., Ewen, T., Podlipnik, S., Greenwood, A., McConnell, A., Long, A., Ness, T., Jones, C., Stefanos, N., Sloan, P., Husain, A., Barrett, P., Puig, S., McArthur, G. A., & Lovat, P. (2020). AMBLor: A prognostic and stratifying biomarker for adjuvant immunotherapy of AJCC stage II melanomas. *Journal of Clinical Oncology*, *38*(15_suppl), e22094-e22094. https://doi.org/10.1200/JCO.2020.38.15_suppl.e22094

Leachman, S. A., Lucero, O. M., Sampson, J. E., Cassidy, P., Bruno, W., Queirolo, P., & Ghiorzo, P. (2017). Identification, genetic testing, and management of hereditary melanoma. *Cancer Metastasis Rev*, *36*(1), 77-90. https://doi.org/10.1007/s10555-017-9661-5

Lee, J. J., & Lian, C. G. (2018). Molecular Testing for Cutaneous Melanoma: An Update and Review. *Arch Pathol Lab Med*. https://doi.org/10.5858/arpa.2018-0038-RA

Leong, S. P., Gershenwald, J. E., Soong, S. J., Schadendorf, D., Tarhini, A. A., Agarwala, S., Hauschild, A., Soon, C. W., Daud, A., & Kashani-Sabet, M. (2011). Cutaneous melanoma: a model to study cancer metastasis. *J Surg Oncol*, *103*(6), 538-549. https://doi.org/10.1002/jso.21816

Linares, M. A., Zakaria, A., & Nizran, P. (2015). Skin Cancer. *Prim Care*, *42*(4), 645-659. https://doi.org/10.1016/j.pop.2015.07.006

Lokhandwala, P. M., Tseng, L. H., Rodriguez, E., Zheng, G., Pallavajjalla, A., Gocke, C. D., Eshleman, J. R., & Lin, M. T. (2019). Clinical mutational profiling and categorization of BRAF mutations in melanomas using next generation sequencing. *BMC Cancer*, *19*(1), 665. https://doi.org/10.1186/s12885-019-5864-1

Lynch, H. T., & Krush, A. J. (1968). Heredity and malignant melanoma: implications for early cancer detection. *Can Med Assoc J*, *99*(1), 17-21. https://www.ncbi.nlm.nih.gov/pubmed/5663000

Marchetti, M. A., Coit, D. G., Dusza, S. W., Yu, A., McLean, L., Hu, Y., Nanda, J. K., Matsoukas, K., Mancebo, S. E., & Bartlett, E. K. (2020). Performance of Gene Expression Profile Tests for Prognosis in Patients With Localized Cutaneous

Melanoma: A Systematic Review and Meta-analysis. *JAMA Dermatol*, *156*(9), 953-962. https://doi.org/10.1001/jamadermatol.2020.1731

Marks, E., Caruso, H., Kurley, S., Ibad, S., Plasseraud, K., Monzon, F., & Cockerell, C. (2019). Establishing an evidence-based decision point for clinical use of the 31-gene expression profile test in cutaneous melanoma. *SKIN The Journal of Cutaneous Medicine*, *3*(4). https://www.jofskin.org/index.php/skin/article/view/628

Marzuka-Alcala, A., Gabree, M. J., & Tsao, H. (2014). Melanoma susceptibility genes and risk assessment. *Methods Mol Biol*, *1102*, 381-393. https://doi.org/10.1007/978-1-62703-727-3_20

Menin, C., Bojnik, E., Del Bianco, P., Elefanti, L., Gianesin, K., Keppel, S., Stagni, C., Mocellin, S., Vecchiato, A., & De Rossi, A. (2016). Differences in telomere length between sporadic and familial cutaneous melanoma. *Br J Dermatol*, *175*(5), 937-943. https://doi.org/10.1111/bjd.14652

Middlebrooks, C. D., Stacey, M. L., Li, Q., Snyder, C., Shaw, T. G., Richardson-Nelson, T., Rendell, M., Ferguson, C., Silberstein, P., Casey, M. J., Bailey-Wilson, J. E., & Lynch, H. T. (2019). Analysis of the CDKN2A Gene in FAMMM Syndrome Families Reveals Early Age of Onset for Additional Syndromic Cancers. *Cancer Res*, *79*(11), 2992-3000. https://doi.org/10.1158/0008-5472.Can-18-1580

Minca, E. C., Al-Rohil, R. N., Wang, M., Harms, P. W., Ko, J. S., Collie, A. M., Kovalyshyn, I., Prieto, V. G., Tetzlaff, M. T., Billings, S. D., & Andea, A. A. (2016). Comparison between melanoma gene expression score and fluorescence in situ hybridization for the classification of melanocytic lesions. *Mod Pathol*, *29*(8), 832-843. https://doi.org/10.1038/modpathol.2016.84

NCCN. (2024). *NCCN Clinical Practice Guidelines in Oncology; Melanoma: Cutaneous*. https://www.nccn.org/professionals/physician_gls/pdf/cutaneous_melanoma.pdf

NCI. (2023). *Surveillance, epidemiology, and end results program.* Retrieved 10/20/2021 from https://seer.cancer.gov/statfacts/html/melan.html

NCI. (2024). *Drugs Approved for Melanoma*. https://www.cancer.gov/about-cancer/treatment/drugs/melanoma

Nelson, A. A., & Tsao, H. (2009). Melanoma and genetics. *Clin Dermatol*, *27*(1), 46-52. https://doi.org/10.1016/j.clindermatol.2008.09.005

O'Brien, O., Lyons, T., Murphy, S., Feeley, L., Power, D., & Heffron, C. (2017). BRAF V600 mutation detection in melanoma: a comparison of two laboratory testing methods. *J Clin Pathol*, *70*(11), 935-940. https://doi.org/10.1136/jclinpath-2017-204367

Pasquali, E., Garcia-Borron, J. C., Fargnoli, M. C., Gandini, S., Maisonneuve, P., Bagnardi, V., Specchia, C., Liu, F., Kayser, M., Nijsten, T., Nagore, E., Kumar, R., Hansson, J., Kanetsky, P. A., Ghiorzo, P., Debniak, T., Branicki, W., Gruis, N. A., Han, J., . . . Group, M. S. S. (2015). MC1R variants increased the risk of sporadic cutaneous melanoma in darker-pigmented Caucasians: a pooled-analysis from the M-SKIP project. *Int J Cancer*, *136*(3), 618-631. https://doi.org/10.1002/ijc.29018

Paszkowska-Szczur, K., Scott, R. J., Serrano-Fernandez, P., Mirecka, A., Gapska, P., Gorski, B., Cybulski, C., Maleszka, R., Sulikowski, M., Nagay, L., Lubinski, J., & Debniak, T. (2013). Xeroderma pigmentosum genes and melanoma risk. *Int J Cancer*, *133*(5), 1094-1100. https://doi.org/10.1002/ijc.28123

Pissa, M., Helkkula, T., Appelqvist, F., Silander, G., Borg, Å., Pettersson, J., Lapins, J., Nielsen, K., Höiom, V., & Helgadottir, H. (2021). CDKN2A genetic testing in melanoma-prone families in Sweden in the years 2015-2020: implications for novel national recommendations. *Acta Oncol*, *60*(7), 888-896. https://doi.org/10.1080/0284186x.2021.1914346

Randerson-Moor, J. A., Harland, M., Williams, S., Cuthbert-Heavens, D., Sheridan, E., Aveyard, J., Sibley, K., Whitaker, L., Knowles, M., Bishop, J. N., & Bishop, D. T. (2001). A germline deletion of p14(ARF) but not CDKN2A in a melanoma-neural system tumour syndrome family. *Hum Mol Genet*, *10*(1), 55-62. https://www.ncbi.nlm.nih.gov/pubmed/11136714

Ransohoff, K. J., Jaju, P. D., Tang, J. Y., Carbone, M., Leachman, S., & Sarin, K. Y. (2016). Familial skin cancer syndromes: Increased melanoma risk. *J Am Acad Dermatol*, *74*(3), 423-434; quiz 435-426. https://doi.org/10.1016/j.jaad.2015.09.070

Rashid, O. M., & Zager, J. S. (2015). Genetic Testing in the Multidisciplinary Management of Melanoma. *Surg Oncol Clin N Am*, *24*(4), 779-793. https://pubmed.ncbi.nlm.nih.gov/26363541/

Ribas, A., & Flaherty, K. T. (2011). BRAF targeted therapy changes the treatment paradigm in melanoma. *Nat Rev Clin Oncol*, *8*(7), 426-433. https://doi.org/10.1038/nrclinonc.2011.69

Ribero, S., Sportoletti Baduel, E., Osella-Abate, S., Dika, E., Quaglino, P., Picciotto, F., Macripo, G., & Bataille, V. (2017). Sentinel lymph node biopsy in cutaneous melanoma. *G Ital Dermatol Venereol*, *152*(4), 355-359. https://doi.org/10.23736/s0392-0488.16.05358-x

Robinson, J. K., & Jansen, B. (2020). Caring for Melanoma Survivors with Self-Detected Concerning Moles During COVID-19 Restricted Physician Access: a Cohort Study. *SKIN The Journal of Cutaneous Medicine*, *4*(3), 248-251. https://doi.org/10.25251/skin.4.3.5

Rossi, M., Pellegrini, C., Cardelli, L., Ciciarelli, V., Di Nardo, L., & Fargnoli, M. C. (2019). Familial Melanoma: Diagnostic and Management Implications. *Dermatol Pract Concept*, *9*(1), 10-16. https://doi.org/10.5826/dpc.0901a03

Seth, R., Messersmith, H., Kaur, V., Kirkwood, J. M., Kudchadkar, R., McQuade, J. L., Provenzano, A., Swami, U., Weber, J., Alluri, K. C., Agarwala, S., Ascierto, P. A., Atkins, M. B., Davis, N., Ernstoff, M. S., Faries, M. B., Gold, J. S., Guild, S., Gyorki, D. E., . . . Funchain, P. (2020). Systemic Therapy for Melanoma: ASCO Guideline. *Journal of Clinical Oncology*, *0*(0), JCO.20.00198. https://doi.org/10.1200/jco.20.00198

Simone, Valiante, M., & Silipo, V. (2017). Familial melanoma and multiple primary melanoma. *G Ital Dermatol Venereol*, *152*(3), 262-265. https://doi.org/10.23736/s0392-0488.17.05554-7

Skelsey, M. K., Brouha, B., Rock, J., Howell, M. D., Jansen, B., Clarke, L. E., & Peck, G. (2021). Non-Invasive Detection of Genomic Atypia Increases Real-World NPV and PPV of the Melanoma Diagnostic Pathway and Reduces Biopsy Burden. *SKIN*, *5*(5), 512-523. https://pdfs.semanticscholar.org/05c8/81134b3206b251f8f7b90ae2aca4017191fd.pdf

Skudalski, L., Waldman, R., Kerr, P. E., & Grant-Kels, J. M. (2022). Melanoma: How and when to consider clinical diagnostic technologies. *J Am Acad Dermatol*, *86*(3), 503-512. https://doi.org/10.1016/j.jaad.2021.06.901

SkylineDx. (2024). Melanoma. https://www.skylinedx.com/what/our-pipeline

Soura, E., Eliades, P. J., Shannon, K., Stratigos, A. J., & Tsao, H. (2016a). Hereditary melanoma: Update on syndromes and management: Emerging melanoma cancer complexes and genetic counseling. *J Am Acad Dermatol*, *74*(3), 411-420; quiz 421-412. https://doi.org/10.1016/j.jaad.2015.08.037

Soura, E., Eliades, P. J., Shannon, K., Stratigos, A. J., & Tsao, H. (2016b). Hereditary melanoma: Update on syndromes and management: Genetics of familial atypical multiple mole melanoma syndrome. *J Am Acad Dermatol*, *74*(3), 395-407; quiz 408-310. https://doi.org/10.1016/j.jaad.2015.08.038

Stolarova, L., Jelinkova, S., Storchova, R., Machackova, E., Zemankova, P., Vocka, M., Kodet, O., Kral, J., Cerna, M., Volkova, Z., Janatova, M., Soukupova, J., Stranecky, V., Dundr, P., Foretova, L., Macurek, L., Kleiblova, P., & Kleibl, Z. (2020). Identification of Germline Mutations in Melanoma Patients with Early Onset, Double Primary Tumors, or Family Cancer History by NGS Analysis of 217 Genes. *Biomedicines*, *8*(10). https://doi.org/10.3390/biomedicines8100404

Swetter, S. M. (2024). *Melanoma: Clinical features and diagnosis*. Retrieved 10/19 from https://www.uptodate.com/contents/melanoma-clinical-features-and-diagnosis

Swetter, S. M., Tsao, H., Bichakjian, C. K., Curiel-Lewandrowski, C., Elder, D. E., Gershenwald, J. E., Guild, V., Grant-Kels, J. M., Halpern, A. C., Johnson, T. M., Sober, A. J., Thompson, J. A., Wisco, O. J., Wyatt, S., Hu, S., & Lamina, T. (2019). Guidelines of care for the management of primary cutaneous melanoma. *J Am Acad Dermatol*, *80*(1), 208-250. https://doi.org/10.1016/j.jaad.2018.08.055

Torres, R., Lang, U. E., Hejna, M., Shelton, S. J., Joseph, N. M., Shain, A. H., Yeh, I., Wei, M. L., Oldham, M. C., Bastian, B. C., & Judson-Torres, R. L. (2019). MicroRNA ratios distinguish melanomas from nevi. *J Invest Dermatol*. https://doi.org/10.1016/j.jid.2019.06.126

Tsao, H., Atkins, M. B., & Sober, A. J. (2004). Management of cutaneous melanoma. *N Engl J Med*, *351*(10), 998-1012. https://doi.org/10.1056/NEJMra041245

Tsao, H., & McCormick, S. R. (2024, May 22). *Inherited susceptibility to melanoma*. UpToDate. Retrieved October 30 from https://www.uptodate.com/contents/inherited-susceptibility-to-melanoma

Varedi, A., Gardner, L. J., Kim, C. C., Chu, E. Y., Ming, M. E., Leachman, S. A., Curiel-Lewandrowski, C., Swetter, S. M., & Grossman, D. (2019). Use of new molecular tests for melanoma by pigmented-lesion experts. *J Am Acad Dermatol*. https://doi.org/10.1016/j.jaad.2019.08.022

Volmar, K. E., Idowu, M. O., Souers, R. J., & Nakhleh, R. E. (2015). Molecular Testing in Anatomic Pathology and Adherence to Guidelines: A College of American Pathologists Q-Probes Study of 2230 Testing Events Reported by 26 Institutions. *Arch Pathol Lab Med*, *139*(9), 1115-1124. https://doi.org/10.5858/arpa.2014-0513-CP

Weiss, S. A., Hanniford, D., Hernando, E., & Osman, I. (2015). Revisiting determinants of prognosis in cutaneous melanoma. *Cancer*, *121*(23), 4108-4123. https://doi.org/10.1002/cncr.29634

Wendt, J., Mueller, C., Rauscher, S., Fae, I., Fischer, G., & Okamoto, I. (2018). Contributions by MC1R Variants to Melanoma Risk in Males and Females. *JAMA Dermatol*, *154*(7), 789-795. https://doi.org/10.1001/jamadermatol.2018.1252

Wiesner, T., Obenauf, A. C., Murali, R., Fried, I., Griewank, K. G., Ulz, P., Windpassinger, C., Wackernagel, W., Loy, S., Wolf, I., Viale, A., Lash, A. E., Pirun, M., Socci, N. D., Rutten, A., Palmedo, G., Abramson, D., Offit, K., Ott, A., . . . Speicher, M. R. (2011). Germline mutations in BAP1 predispose to melanocytic tumors. *Nat Genet*, *43*(10), 1018-1021. https://doi.org/10.1038/ng.910

Woodman, S. E., & Davies, M. A. (2010). Targeting KIT in melanoma: a paradigm of molecular medicine and targeted therapeutics. *Biochem Pharmacol*, *80*(5), 568-574. https://doi.org/10.1016/j.bcp.2010.04.032

Yao, Z., Moy, R., Allen, T., & Jansen, B. (2017). An Adhesive Patch-Based Skin Biopsy Device for Molecular Diagnostics and Skin Microbiome Studies. *J Drugs Dermatol*, *16*(10), 979-986. https://www.ncbi.nlm.nih.gov/pubmed/29036251

Yee, V. S., Thompson, J. F., McKinnon, J. G., Scolyer, R. A., Li, L. X., McCarthy, W. H., O'Brien, C. J., Quinn, M. J., Saw, R. P., Shannon, K. F., Stretch, J. R., & Uren, R. F. (2005). Outcome in 846 cutaneous melanoma patients from a single center after a negative sentinel node biopsy. *Ann Surg Oncol*, *12*(6), 429-439. https://doi.org/10.1245/aso.2005.03.074

Yokoyama, S., Woods, S. L., Boyle, G. M., Aoude, L. G., MacGregor, S., Zismann, V., Gartside, M., Cust, A. E., Haq, R., Harland, M., Taylor, J. C., Duffy, D. L., Holohan, K., Dutton-Regester, K., Palmer, J. M., Bonazzi, V., Stark, M. S., Symmons, J., Law, M. H., . . . Brown, K. M. (2011). A novel recurrent mutation in MITF predisposes to familial and sporadic melanoma. *Nature*, *480*(7375), 99-103. https://doi.org/10.1038/nature10630

Yousaf, A., Tjien-Fooh, F. J., Rentroia-Pacheco, B., Quattrocchi, E., Kobic, A., Tempel, D., Kolodney, M., & Meves, A. (2021). Validation of CP-GEP (Merlin Assay) for predicting

sentinel lymph node metastasis in primary cutaneous melanoma patients: A U.S. cohort study. *Int J Dermatol*, *60*(7), 851-856. https://doi.org/10.1111/ijd.15594

Zhang, Y., Xiong, Y., & Yarbrough, W. G. (1998). ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. *Cell*, *92*(6), 725-734. https://doi.org/10.1016/S0092-8674(00)81401-4

Zippel, D., Markel, G., Shapira-Frommer, R., Ben-Betzalel, G., Goitein, D., Ben-Ami, E., Nissan, A., Schachter, J., & Schneebaum, S. (2017). Perioperative BRAF inhibitors in locally advanced stage III melanoma. *J Surg Oncol*, *116*(7), 856-861. https://doi.org/10.1002/jso.24744

Molecular Testing for Pulmonary Disease

Policy Number: AHS –M2160 – Molecular Testing for Pulmonary Disease	Prior Policy Name and Number, as applicable: AHS-M2160- Molecular Testing of Pulmonary Specimens AHS-M2151 Molecular Testing of Bronchial Brushings AHS-M2160-Molecular Testing of Bronchial Brushings
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Pulmonary nodules are well-defined lesions found in lung tissue. These nodules are found on cross-sectional imaging and are frequently "incidental" (i.e., found on imaging not originally performed to identify the nodules). Assessment of malignancy risk is critical to managing pulmonary nodules, and a variety of tests have been used to accurately evaluate them. Some of these tests use gene expression profiling (GEP) on cells obtained from bronchoscopies, as these cells are purported to contain molecular markers indicative of malignancy (Islam, 2024; Weinberger, 2024). Other tests employ liquid biopsy and proteomic analysis to assign malignancy risk.

Idiopathic pulmonary fibrosis (IPF) is a disease of unknown etiology that causes irreversible scarring (fibrosis) of the lung. Disease progression and increasing fibrosis often result in breathing difficulties that may subsequently lead to respiratory failure. IPF is a diagnosis of exclusion, and molecular tests are emerging as potentially useful tools that may help differentiate between IPF and other interstitial lung diseases (ILD).

II. Related Policies

Policy Number	Policy Title
AHS-G2054	Liquid Biopsy
AHS-G2124	Serum Tumor Markers for Malignancies

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with a suspicious pulmonary nodule (see Note 1), cancer risk assessment using Nodify XL2 proteomic analysis **MEETS COVERAGE CRITERIA** when **all** of the following conditions are met:
   a) The pulmonary nodule size is 8-30 mm.
   b) The patient is 40 years of age or older.
   c) The pre-test risk of cancer is less than 50% based on the Solitary Pulmonary Nodule Malignancy Risk Score (Mayo Clinic Model).

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

2) For all other indications, molecular testing for pulmonary disease **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: While this is not considered a surgical procedure, it is recommended that individuals who are receiving this test should first undergo an informed consent process to discuss the benefits and risks of pursuing this test versus receiving a biopsy to rule lung cancer.

IV. Table of Terminology

Term	Definition
4MP	Four-marker protein panel
ACCP	American College of Chest Physicians
AEGIS	Airway Epithelial Gene Expression in the Diagnosis of Lung Cancer
ALAT	Latin American Thoracic Society
ALK	*Anaplastic lymphoma kinase*
ATS	American Thoracic Society

AUC	Area under curve
BGC	Bronchial Genomic Classifier
BRAF	*B-Raf proto-oncogene*
CA-125	Cancer antigen 125
CEA	Carcinoembryonic antigen
CLIA	Clinical Laboratory Improvement Amendments
CMS	Centers for Medicare and Medicaid Services
CT	Computed tomography
CYFRA 21–1	Cytokeratin-19 soluble fragment
ddMSP	methylation-specific droplet digital PCR
EGFR	*Epidermal growth factor receptor*
ERS	European Respiratory Society
ESMO	European Society for Medical Oncology
FDA	Food and Drug Administration
GSC	Genomic Sequencing Classifier
HRCT	High resolution computed tomography
ILD	Interstitial lung disease
IPF	Idiopathic pulmonary fibrosis
JRS	Japanese Respiratory Society
LCD	Local coverage determinations
LDCT	Low-dose computed tomography
LCDT1	Lung cancer detector test
LDT	Laboratory developed tests
LG3BP	Galectin-3-binding protein
MRM	Multiple reaction monitoring
mRNA	Messenger ribonucleic acid
NCCN	National Comprehensive Cancer Network
NLST	National Lung Screening Trial
NPV	Negative predictive value
NY-ESO-1	New York esophageal cancer-1 antibody
PANOPTIC	Pulmonary Nodule Plasma Proteomic Classifier
PCR	Polymerase chain reaction
PLCO	Prostate lung colorectal ovarian
PPF	Progressive pulmonary fibrosis
QALY	Quality-adjusted life-years
RNA	Ribonucleic acid

ROS1	*ROS proto-oncogene 1, receptor tyrosine kinase*
SLB	Surgical lung biopsy
TBB	Transbronchial biopsy
TBBx	Transbronchial lung cryobiopsy
TBNA	Transbronchial needle aspirates
TTNA/B	Transthoracic needle aspiration or biopsy
UIP	Usual interstitial pneumonia
USPSTF	United States Preventive Services Task Force

V. Scientific Background

In the United States, over 1.5 million lung nodules are detected annually (Kearney et al., 2017). These pulmonary nodules occur due to a variety of conditions, some malignant (i.e., cancer), and some benign (such as an infection). Since treatment varies widely between malignant and benign nodules, it is crucial to have well-validated and accurate methods to assess risk of malignancy. Traditionally, malignancy has been evaluated using a combination of factors, such as clinical, histological, and radiographic features. Once an initial assessment of malignancy has been performed, further management such as computed tomography (CT) surveillance or biopsy may follow. Low-dose computed tomography (LDCT) is the current standard for lung cancer screening. However, a limitation of the screening is that LDCT shows indeterminate pulmonary nodules which are not clearly defined as benign or cancerous. Assessment of a potentially malignant nodule typically involves invasive biopsy whereas benign nodules may be only placed under surveillance. Clinicians must often weigh the risk of a missed malignant diagnosis against performing an invasive procedure that may ultimately be unnecessary (Weinberger, 2024).

Biomarkers are emerging as clinically useful tools in the management of pulmonary disorders. Validated biomarkers can potentially help improve risk-stratification, facilitate appropriate diagnosis, and may also reduce the number of invasive procedures that patients receive, and the morbidity associated with them.

Proprietary Testing

To assist in the classification and management of indeterminate pulmonary nodules, several proprietary tests have been developed, such as Veracyte's Bronchial Genomic Classifier (Percepta BGC). This test focuses on molecular analysis of the nodules, rather than clinical or radiographic analysis. The Percepta BGC uses cells collected during bronchoscopy to detect genomic changes indicative of a cancerous nodule by analyzing the expression of twenty-three lung cancer associated genes in addition to patient age. Percepta BGC "is designed to reduce the number of invasive biopsies and

other procedures that can follow when suspicious lung nodules are found on computerized tomography (CT) scans" (BU, 2015), and may improve the diagnostic performance of bronchoscopy in detecting lung cancer.

A second-generation risk stratification algorithm called Percepta Genomic Sequencing Classifier (GSC) was described by Choi et al. (2020), and suggested to have broadened utility beyond the BGC for pulmonary nodule management. The GSC considers 1232 gene transcripts coupled with specific clinical factors (gender, age, pack-year, inhaled medication, and specimen collection timing) to calculate the risk of primary lung cancer, and is indicated for use in patients with an inconclusive bronchoscopy result, who are current or former smokers, and have no prior history of cancer (Veracyte, 2024c). A strength of the test is that it may be used in patients across several different pre-test risk categories of cancer, though the clinical validity measurements vary.

Veracyte has developed other tests that are undergoing validation to aid in the management of pulmonary nodules. These include the Percepta Nasal Swab, which is a genomic test that uses a sample from beyond the inferior nasal turbinate to evaluate a lung nodule's risk of malignancy, and the Percepta Genomic Atlas; an NGS-based assay that uses a tissue sample collected through bronchoscopy to identify genomic alterations in 54 genes, to help inform targeted therapy for confirmed malignancies (Babiarz et al., 2021; Veracyte, 2023).

Another condition that is sometimes associated with pulmonary nodules is idiopathic pulmonary fibrosis (IPF). Although the cause of IPF is unknown, by definition, clinical management of this condition may involve assessment of any lung nodules that are present, and further biopsy. Evaluation of nodules associated with IPF includes several of the same procedures discussed above, such as clinical assessment, imaging, and pulmonary function tests. Diagnosis of IPF typically requires "exclusion of other known causes of interstitial lung disease (ILD) and either definite features of usual interstitial pneumonia (UIP) on high resolution computed tomography (HRCT) or certain combinations of HRCT and histopathologic features of UIP." Much debate exists around the role of the lung biopsy in diagnosis of IPF; authorities are conflicted on its importance in IPF assessment (King, 2024).

Veracyte has developed a genomic test named Envisia intended to aid physicians in differentiating between "idiopathic pulmonary fibrosis (IPF) from other interstitial lung diseases (ILD), without the need for invasive, risky, and costly surgery" (Veracyte, 2024b). Envisia uses tissue samples obtained from a transbronchial biopsy and evaluates RNA of 190 genes purported to have common associations with fibrosis and inflammation. The results then report either "positive" or "negative" for usual interstitial

pneumonia, considered to be the signature histopathologic pattern for IPF (Raghu, 2023; Veracyte, 2024a).

There are plasma-based proteomic tests that can be used to screen pulmonary nodules and estimate their risk of malignancy. Nodify XL2 (also known as Xpresys Lung®, Xpresys Lung 2®, and BDX-XL2) is a plasma-based proteomic screening test that measures two proteins (LG3BP and C163A) thought to be important in the development of lung cancer. The test considers their abundance along with five additional factors (age, nodule size, smoking status, edge, and location. It is intended for use after the diagnosis of a pulmonary nodule in patients whose risk category is indeterminate by current risk calculators and may help reclassify a nodule as lower risk (<5%) for malignancy (Biodesix, 2024).

Other proteomic tests include REVEAL Lung Nodule Characterization, which is a proteomic test for classification of pulmonary nodules in current smokers that calculates a risk score between 0 and 100 based on three clinical factors (smoking history, patient age, nodule size) and three blood proteins. REVEAL Lung Nodule Characterization is reported to have a sensitivity of 94% and a negative predictive value of 94% (Arfoosh et al., 2019). Lung Cancer Detector Test (LCDT1) is a proteomic test being developed for stage 1 non-small cell lung cancer detection. LCDT1 is expected to have 95.6% accuracy, 89.1% sensitivity, and 97.7% specificity (Goebel et al., 2020). EarlyCDT-Lung is a serum-based test that measures seven autoantibodies associated with lung cancer to estimate the risk of malignancy in small cell lung cancer and non-small cell lung cancer. EarlyCDT-Lung is reported to have 41% sensitivity and 87% specificity (EarlyCDT, 2024; Ostrin et al., 2020).

Analytical Validity

Hu et al. (2016) conducted studies to evaluate analytical performance of a gene expression profiling test (Percepta) using bronchial brushing specimens. The authors found that "analytical sensitivity studies demonstrated tolerance to variation in RNA input (157 ng to 243 ng). Analytical specificity studies utilizing cancer positive and cancer negative samples mixed with either blood (up to 10 % input mass) or genomic DNA (up to 10 % input mass) demonstrated no assay interference." The authors concluded that "analytical sensitivity, analytical specificity and robustness of the Percepta test were successfully verified, supporting its suitability for clinical use" (Hu et al., 2016).

Pankratz et al. (2017) aimed to develop a genomic classifier to distinguish usual interstitial pneumonia (UIP) from non-UIP in tissue samples obtained by transbronchial biopsy (TBB). The authors stated that this study was performed because UIP was the hallmark symptom of idiopathic pulmonary fibrosis (IPF), and imaging to identify UIP

was frequently inconclusive. A total of 283 samples from TBB were taken from 84 subjects, and "exome-enriched RNA sequencing" was performed on these samples. Then, a machine learning algorithm was created from 53 of these samples. This algorithm was then validated in the remaining 31 samples. The authors found that this algorithm distinguished UIP from non-UIP conditions with an area under curve (AUC) of 0.86 with a single sample. The sensitivity was found to be 63%, and the specificity was found to be 86%. The AUC improved to 0.92 when three to five TBB samples were included. The authors concluded that "genomic analysis and machine learning improves the utility of TBB for the diagnosis of UIP" but acknowledged that "this approach requires validation in an independent cohort of subjects before application in the clinic" (Pankratz et al., 2017).

Roncarati et al. (2020) evaluated the suitability of molecular testing for lung cancer assessment on bronchial washings. A novel droplet digital methylation-specific PCR (ddMSP) test was run on bronchial washings taken during fiber-optic bronchoscopy from 91 lung cancer patients and 31 control patients. The ddMSP assessed the aberrant methylation status of four genes that "display aberrant methylation in more than 50% of cancer samples and no aberrant methylation in normal tissue." The authors confirm that their "methodological approaches, based on either ddMSP or NGS, exhibited an analytical sensitivity of 0.1% or lower, which is adequate to recognize the traces of nucleic acids originating from cancer cells." When used on bronchial washings obtained from patients during fibre-optic bronchoscopy, the ddMSP demonstrated a 97% sensitivity and 74% specificity. Additionally, DNA and RNA analysis of bronchial washings taken from 73 cancer patients and 14 noncancer patients found commonalities among mutations. The authors state that there is predictive value in mutation analysis but "frequent mutation detection in noncancer patients revealed the low specificity of this approach for diagnostic purposes." The authors concluded that molecular testing on bronchial washings "could be performed to support and complete the current clinical diagnostic/predictive strategies" (Roncarati et al., 2020).

Johnson et al. (2021) analyzed the performance of the Percepta Genomic Sequencing Classifier (GSC) in realistic conditions. Bronchial brushing samples were tested from bronchoscopy of patients with "suspicious lung nodules." The authors found no significant difference in Percepta GSC results with varying amounts of RNA input, 10% DNA contamination, and up to 11% blood RNA contamination. Additionally, results were reproducible between runs, within runs, and between laboratories. The authors concluded that "the analytical sensitivity, analytical specificity, and reproducibility of Percepta GSC laboratory results were successfully demonstrated under conditions of expected day to day variation in testing. Percepta GSC test results are analytically robust and suitable for routine clinical use" (Johnson et al., 2021).

Li et al. (2013) first reported on the analytical validity of Nodify XL2. A 371-protein multiplexed multiple reaction monitoring (MRM) assay was developed to identify lung cancer biomarker candidates in blood samples. The authors initially determined that a total of 190 MRM assays were able to detect their target proteins in plasma, reflecting an overall success rate of 51%. This multiplexed MRM assay was then applied to 143 discovery samples and subsequently refined to a 13-protein classifier. The authors established the performance of Nodify XL2 using a set of plasma samples. For this test, negative predictive value (NPV) is the likelihood that a prediction of a benign tumor by the classifier is true. Specificity is defined as "the fraction of the benign nodules that the classifier is able to detect with high confidence." With an assumed cancer prevalence of 15%, the classifier demonstrated a sensitivity of 82%, a specificity of 66%, and an NPV of 95% when run on an initial batch of "discovery" samples. In a follow up analysis on "validation" samples, the classifier demonstrated a sensitivity of 71%, a specificity of 44%, and an NPV of 90% (Li et al., 2013).

Clinical Utility and Validity

Whitney et al. (2015) collected bronchial epithelial cells of 223 cancer-positive and 76 cancer-free subjects undergoing bronchoscopy for suspected lung cancer in a prospective, multi-center study. RNA from these samples was run on gene expression microarrays for training a gene-expression classifier. Out of the 232 genes whose expression levels in the bronchial airway were found to be associated with lung cancer, the authors built a classifier based on the combination of 17 cancer genes, gene expression predictors of smoking status, smoking history, and gender, plus patient age. The authors concluded that their gene classifier "is able to detect lung cancer in current and former smokers who have undergone bronchoscopy for suspicion of lung cancer. Due to the high NPV of the classifier, it could potentially inform clinical decisions regarding the need for further invasive testing in patients whose bronchoscopy is non-diagnostic" (Whitney et al., 2015).

Silvestri et al. (2015) reported on the diagnostic performance of a gene-expression classifier. A total of 639 current or former smokers undergoing bronchoscopy for suspected lung cancer enrolled in two multicenter prospective studies (AEGIS-1 and AEGIS-2) were evaluated. A gene-expression classifier was measured in epithelial cells to assess the probability of lung cancer. In AEGIS-1, the classifier had a sensitivity of 88% and a specificity of 47%. In AEGIS-2, the classifier had a sensitivity of 89% and a specificity of 47%. The combination of the classifier plus bronchoscopy had a sensitivity of 96% in AEGIS-1 and 98% in AEGIS-2. The authors concluded that "the gene-expression classifier improved the diagnostic performance of bronchoscopy for the detection of lung cancer. In intermediate-risk patients with a nondiagnostic

bronchoscopic examination, a negative classifier score provides support for a more conservative diagnostic approach" (Silvestri et al., 2015).

Ferguson et al. (2016) conducted a randomized, prospective decision impact survey study to evaluate pulmonologist recommendations in patients undergoing workup for lung cancer who had an inconclusive bronchoscopy. The authors' goal was to examine if a negative genomic classifier result that down-classifies a patient from intermediate risk to low risk (<10 %) for lung cancer would reduce the rate that physicians recommend more invasive testing among patients with an inconclusive bronchoscopy. The authors found that "invasive procedure recommendations were reduced from 57 % without the classifier result to 18 % with a negative (low risk) classifier result. Invasive procedure recommendations increased from 50 to 65 % with a positive (intermediate risk) classifier result." The authors concluded that their results "support the potential clinical utility of the classifier to improve management of patients undergoing bronchoscopy for suspect lung cancer by reducing additional invasive procedures in the setting of benign disease" (Ferguson et al., 2016).

Lee et al. (2017) published interim results from a large prospective registry of 665 patients undergoing diagnostic bronchoscopy. In a subset of 209 patients with an intermediate pretest risk of malignancy, Advanced bronchoscopic techniques were used in in 68% of cases. The BGC test results reclassified 74 patients as low risk. At 10 months post follow up the patients reclassified as low risk had a 40% relative reduction in the use of invasive procedures. The authors concluded that the BGC improves the sensitivity of diagnostic bronchoscopy for patients undergoing evaluation for lung cancer and can reduce the number of unnecessary invasive procedures (Lee et al., 2017).

The Percepta GSC was validated in a study by Mazzone et al. (2021), in patients with low and intermediate pre-test risk of cancer who were down-classified with Percepta GSC, the test demonstrated a > 99% and 91% NPV in these groups, respectively. The GSC may also up-classify cancer risk; patients with intermediate and high pre-test risk of cancer were up-classified with a 65.4% and 91.5% PPV, respectively. Sensitivity of the classifier was reported as 92.3% among patients with low and intermediate pre-test risk of cancer; however, this value increased to 95.5% when the classifier result was combined with data from bronchoscopy. The authors further concluded that if Percepta GSC were employed in the management of lung nodules, "50% of patients with benign lesions and 29% of patients with malignant lesions undergoing additional invasive procedures could have avoided these procedures" (Mazzone et al., 2021).

The prospective PANOPTIC Trial investigated the accuracy of Nodify XL2 and included a subgroup of 178 patients with a physician-assessed probability for malignancy (pCA)

≤50%. From this study, Silvestri et al. (2018) concluded that Nodify XL2 demonstrated a sensitivity of 97%, specificity of 44%, and an NPV of 98%, in its ability to distinguish benign and malignant nodules. The authors state that 40% fewer procedures would have been performed on benign nodules if the test were used to direct care. Notably however, use of the test would have misclassified three percent of malignant nodules, underscoring the importance of ensuring that all patients reclassified as low-risk using Nodify XL2 receive appropriate follow up care, to continually rule out malignancy (Silvestri et al., 2018).

Tanner et al. (2021) published a follow up to the PANOPTIC trial, observing patients for up to two years. In a cohort of 132 patients with nodules categorized as benign at year one, all remained benign at year two. The authors conclude that the "performance characteristics [of the XL2 classifier] were maintained at year 2." Nodify XL2 was also found to perform equally well in patients with solitary versus multiple nodules (Tanner et al., 2021). This is important because it is common for patients to present with more than one nodule. The authors further conclude that Nodify XL2 was shown to outperform the physician pCA, validated Mayo, Veterans Administration, and Brock models, based on area under curve (AUC) analysis (Tanner et al., 2021).

Feller-Kopman et al. (2017) assessed the cost effectiveness of bronchoscopy plus a genomic classifier versus bronchoscopy alone in the diagnostic work-up of patients at intermediate risk for lung cancer. They found that "use of the genomic classifier reduced invasive procedures by 28% at one month and 18% at two years, respectively. Total costs and QALY gain were similar with classifier use ($27,221 versus $27,183 and 1.512 versus 1.509, respectively), resulting in an incremental cost-effectiveness ratio of $15,052 per QALY." The authors concluded that use of a genomic classifier was associated with meaningful cost reduction in invasive procedures (Feller-Kopman et al., 2017).

Raghu et al. (2019) evaluated the prospective findings for the clinical validity and utility of a machine-learning based molecular test (Envisia). Findings from 90 patients were used to train the classifier, and then the authors attempted to validate the classifier in a set of 49 patients. The authors found that the classifier identified "usual interstitial pneumonia in transbronchial lung biopsy samples" in these 49 patients at 70% sensitivity and 88% specificity. A total of 42 patients were noted to show "possible or inconsistent usual interstitial pneumonia on HRCT", and the classifier identified "underlying biopsy-proven usual interstitial pneumonia" at 81% positive predictive value. Clinical diagnoses based on histopathology data agreed with diagnoses based on classifier results at an 86% rate. The authors also found that diagnostic confidence was improved with addition of classifier results in 18 cases of idiopathic pulmonary fibrosis and all 48 patients with "non-diagnostic pathology or non-classifiable fibrosis

histopathology" (63% vs 42%). The authors concluded that "The molecular test provided an objective method to aid clinicians and multidisciplinary teams in ascertaining a diagnosis of IPF, particularly for patients without a clear radiological diagnosis in samples that can be obtained by a less invasive method", noting that further studies were planned (Raghu et al., 2019).

D'Andrea et al. (2020) evaluated the cost-effectiveness of introducing a bronchial gene-expression classifier (BGC) to "improve the performance of bronchoscopy and the overall diagnostic process for early detection of lung cancer." The authors evaluated a cohort of former and current smokers with indeterminate pulmonary nodules and compared two different strategies: "(i) location-based strategy—integrated the BGC to the bronchoscopy indication; (ii) simplified strategy—extended use of bronchoscopy plus BGC also on small and peripheral lesions." The authors modeled the following outcomes: "rate of invasive procedures, quality adjusted-life-years (QALYs), costs and incremental cost-effectiveness ratios." Both strategies were compared to the standard practice (defined as "bronchoscopy, transthoracic needle aspiration or biopsy (TTNA/B) or surgery, consistent with the current recommendations). " The location-based strategy reduced absolute rate of invasive procedures by 3.3% without increasing costs and resulted in savings when the classifier price was less than $3000. The simplified strategy reduced the absolute rate of invasive procedures by 10% and created an incremental cost-effectiveness ratio of $10109 per QALY. The authors concluded that both strategies reduced "unnecessary invasive procedures at high risk of adverse events" and that "the simplified use of BGC for central and peripheral lesions resulted in larger QALYs gains at acceptable cost." Finally, the authors noted that the location-based strategy is cost-saving if the classifier price declines (D'Andrea et al., 2020).

Lee et al. (2021) assessed the impact that Percepta results has on clinical management decisions. The authors conducted a prospective study on 283 patients with low- and intermediate-risk lung nodules across 35 centers in the US. In 35% of cases with a negative Percepta result, the risk of malignancy was down-classified. A total of 79% of the down-classified cases changed their management plan to avoid an invasive procedure. Percepta down-classification did not significantly delay the time to diagnosis for patients with confirmed lung cancer. The authors concluded that "down-classification of nodule malignancy risk with the Percepta test decreased additional invasive procedures without a delay in time to diagnosis among those with lung cancer" (Lee et al., 2021).

Babiarz et al. (2021) tested the use of Percepta Genomic Atlas for identifying key molecular markers in surgical lung biopsy (SLB) specimens, transbronchial needle aspirates (TBNA), and bronchial brush specimens from an initial bronchoscopy at the

time of diagnosis. DNA and RNA were extracted from Stage I, Stage II, and Stage III lung cancer SLB tissue. "Genomic alterations were observed in 65% of Stage I, 64% of Stage II and 73% of Stage III samples." TBNA and bronchial brush specimens were taken from 25 patients; multiple molecular alterations were detected in all patients. The authors concluded that "Percepta Genomic Atlas detects clinically actionable alterations in both SLB of early stage lung cancer tumors and in specimens collected at the time of diagnostic bronchoscopy or needle aspiration prior to surgery" (Babiarz et al., 2021).

Sethi et al. (2022) performed a study on the impact of the Percepta Genomic Sequencing Classifier (GSC) on decision-making in patients with high-risk lung nodules. In the study, 101 survey participants evaluated 37 cases, resulting in 1341 assessments across three cohorts. The results demonstrated that using Percepta GSC, which up-classes patients from a high (>60%) to a very high risk of malignancy, significantly increased the likelihood of recommending a surgical resection. Specifically, in the independent cohort with a GSC result, the recommendation for surgical resection was 45%, compared to 17% in the cohort without a GSC result ($p<0.001$) after reviewing the GSC result. The authors concluded that the classifier not only heightened the rate of referral for potentially curative therapies but also boosted pulmonologists' confidence in their decision-making following nondiagnostic bronchoscopy (Sethi et al., 2022).

There is a growing body of research investigating the utility of serum biomarker testing for the identification of lung cancer. However, the reported sensitivity of these panels is low and more work will likely be needed before they may be applied clinically. Doseeva et al. (2015) found that a panel of three tumor antigens (CEA, CA-125, and CYFRA 21–1) and one autoantibody marker (NY-ESO-1) discriminated between non-small cell lung cancer and controls with a sensitivity of 74%. P. J. Mazzone et al. (2018) further validated this panel, reporting a sensitivity and specificity of 49% and 96%, respectively. More recently, Fahrmann et al. (2022) developed a blood-based biomarker panel for personalized lung cancer risk assessment called 4MP (four-marker protein panel), that consisted of the precursor form of surfactant protein B, CA-125, CEA, and cytokeratin-19 fragment. In combination with a lung cancer risk prediction model ($PLCO_{m2012}$), the 4MP demonstrated a sensitivity of 88.4% and specificity of 56.2%, at a "≥ 1.0% 6-year risk threshold corresponding to the USPSTF 2021 criteria."

VI. Guidelines and Recommendations

American College of Chest Physicians (ACCP)

In 2013, the ACCP published evidence-based clinical practice guidelines for diagnosis and management of lung cancer (Detterbeck et al., 2013). The guidelines did not mention gene expression profiling as a potential diagnostic or screening tool.

In 2018, the ACCP published guidelines for screening of lung cancer. The guidelines state that "despite their potential promise, evidence that using such biomarkers would improve the efficiency of lung cancer screening is lacking. No applicable studies comparing molecular biomarkers vs NLST or USPSTF criteria were found that could be included in the systematic review for this guideline" (Peter J. Mazzone et al., 2018). The ACCP updated the guidelines for screening of lung cancer in 2021 but did not change the recommendations on the use of biomarkers in lung cancer screening (Mazzone et al., 2021). More generally however, the ACCP does acknowledge the importance that biomarkers will likely play in the evaluation of individuals with pulmonary nodules. They state that "research priorities include developing and validating risk assessment models to estimate the probability of cancer among individuals with small nodules or subsolid nodules, performing studies that compare the benefits and harms of alternative management strategies among individuals stratified by cancer risk, determining the safety of CT scan surveillance by examining outcomes among individuals who choose this strategy, and developing and validating novel noninvasive biomarkers to facilitate diagnosis and determine prognosis" (Gould et al., 2013).

National Comprehensive Cancer Network

The NCCN guidelines for non-small cell lung cancer address the utility of molecular profiling after a cancer diagnosis has been made, "with the goal of identifying rare driver mutations for which effective.
drugs may already be available, or to appropriately counsel patients regarding the availability of clinical trials" (NCCN, 2024b).

The NCCN Guidelines for small cell lung cancer state that "comprehensive molecular profiling can be considered in rare cases –particularly for patients with extensive-stage/relapses SCLC who do not smoke tobacco, lightly smoke, have remote smoking history, or have diagnostic or therapeutic dilemma, or at a time of relapse –if not previously done, because this may change management" (NCCN, 2024c).

The NCCN Guidelines for lung cancer screening did not mention gene expression as a potential diagnostic or screening tool but note that any nodules with the highest risk of lung cancer "should be sufficient to enable histology and molecular testing" (NCCN, 2024a).

European Society for Medical Oncology (ESMO)

The ESMO does not mention gene expression profiling in its guideline on the diagnosis, treatment and follow-up for early and locally advanced non-small cell lung cancer (Postmus et al., 2017). In 2021, they updated a few of the recommendations but did not mention GEP profiling (ESMO, 2021).

The ESMO Guidelines for metastatic non-small cell lung cancer recommend therapy-predictive biomarker testing after morphological diagnosis. Biomarker testing includes testing for *EGFR* mutation, *ALK* rearrangement, *ROS1* rearrangement, *BRAF* mutation, and PD-L1 expression. The guideline states that "this practice will be driven by the availability of treatments and will vary widely between different geopolitical health systems" (Planchard et al., 2018). The guidelines also comment on the utility of blood monitoring for oncogenic driver mutations, noting that the ability to detect such alterations or other factors associated with disease resistance to treatment through blood sampling, improves disease monitoring (Planchard et al., 2018).

An updated ESMO guideline for oncogene-addicted metastatic non-small lung cancer was published in 2023 (Hendriks et al., 2023). In a discussion, they note how essential biomarker testing is to identifying subgroups of NSCLC with oncogenic drivers that can be therapeutically targeted. These drivers are primarily found in lung adenocarcinomas (LUADs). Testing for the specific molecular alteration is vital to being able to predict and utilize the matching therapy (Hendriks et al., 2023).

American Thoracic Society, European Respiratory Society, Japanese Respiratory Society, and Latin American Thoracic Society (ATS/ERS/JRS/ALAT)

This set of joint guidelines remarks that "machine learning using molecular signatures is being developed to make a molecular diagnosis of UIP [usual interstitial pneumonia] in TBBx [transbronchial lung cryobiopsy] specimens but is not yet available in routine clinical practice. The guideline panel acknowledges that recent studies about the utility of molecular diagnostic tools that involve machine learning using TBBx samples are promising." The guidelines also note that further validation studies are pending (Raghu et al., 2018).

A 2022 updated guideline was published that also includes recommendations for progressive pulmonary fibrosis (PPF). In a patient with interstitial lung disease of known or unknown etiology other than IPF, PPF was defined as a patient exhibiting two out of three of the following criteria occurring within the past year with no alternative explanation: worsening primary symptoms, physiological evidence of disease

progression, and radiological evidence of disease progression. The committee made "no recommendation for or against the addition of [Envisia] genomic classifier testing for the purpose of diagnosing UIP in patients with ILD of undetermined type who are undergoing transbronchial forceps biopsy," concluding that "research is also needed to improve the technique's sensitivity, assess the downstream consequences of false-negative results (i.e., incorrectly categorizing a patient with the UIP pattern as not having the UIP pattern), and determine the ability of genomic classifier testing to differentiate UIP related to IPF and UIP related to other types of ILD" (Raghu et al., 2022).

European Paediatric Soft Tissue Sarcoma Study Group

This study group published a report on the clinical significance of indeterminate pulmonary nodules in rhabdomyosarcoma. The group included 316 patients with non-metastatic rhabdomyosarcoma, 67 of which had indeterminate pulmonary nodules, 249 of which didn't have nodules. The authors found event-free survival and overall survival rates to be 77% and 82% respectively for patients with indeterminate nodules, and 73.2% and 80.8% respectively for patients without nodules. The authors concluded that their study "demonstrated that indeterminate pulmonary nodules at diagnosis do not affect outcome in patients with otherwise localized RMS. There is no need to biopsy or upstage patients with RMS who have indeterminate pulmonary nodules at diagnosis" (Vaarwerk et al., 2019).

Another study group convened in 2023 to assess the clinical impact of indeterminate pulmonary nodules in those with diagnosed adult-type non-rhabdomyosarcoma soft tissue sarcoma (NRSTS). The authors examined 206 children/adolescent patients; 109 of these patients (52.9%) were without any nodules, 78 (38%) had at least one indeterminate nodule, and 19 (9.2%) had nodules that met the definition of metastases (considered to misclassified and then excluded from analyses). In the results, "Five-year event-free survival (EFS) was 78.5% (95% CI, 69.4%–85.1%) for patients without nodules and 69.6% (95% CI, 57.9%–78.7%) for patients with indeterminate nodules (p = .135); 5-year overall survival was 87.4% (95% CI, 79.3%–92.5%) and 79.0% (95% CI, 67.5%–86.8%), respectively (p = .086)." The authors concluded that survival did not differ in nonmetastatic patients with indeterminate pulmonary nodules compared to nonmetastatic patients without pulmonary nodules (Giraudo et al., 2024).

Fleischner Society White Paper, Diagnostic Criteria for Idiopathic Pulmonary Fibrosis

This guideline focused on diagnostic criteria for IPF, including discussion on traditional features such as clinical, histopathological, and imaging factors. Under the "Areas of uncertainty" subheading, the Society comments that "we anticipate that molecular diagnosis with machine learning will play an increasing role in the diagnosis of IPF, particularly when integrated with clinical and imaging features" and emphasizes the importance of identifying molecular predictors of IPF (Lynch et al., 2018).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81479	Unlisted molecular pathology procedure
81554	Pulmonary disease (idiopathic pulmonary fibrosis [IPF]), mRNA, gene expression analysis of 190 genes, utilizing transbronchial biopsies, diagnostic algorithm reported as categorical result (eg, positive or negative for high probability of usual interstitial pneumonia [UIP]) Proprietary test: Envisia® Genomic Classifier Lab/Manufacturer: Veracyte, Inc
0080U	Oncology (lung), mass spectrometric analysis of galectin-3-binding protein and scavenger receptor cysteine-rich type 1 protein M130, with five clinical risk factors (age, smoking status, nodule diameter, nodule-spiculation status and nodule location), utilizing plasma, algorithm reported as a categorical probability of malignancy

CPT	Code Description
	Proprietary test: BDX-XL2 Lab/Manufacturer: Biodesix®, Inc
0360U	Oncology (lung), enzyme-linked immunosorbent assay (ELISA) of 7 autoantibodies (p53, NY-ESO-1, CAGE, GBU4-5, SOX2, MAGE A4, and HuD), plasma, algorithm reported as a categorical result for risk of malignancy Proprietary test: Nodify CDT® Lab/Manufacturer: Biodesix, Inc
0406U	Oncology (lung), flow cytometry, sputum, 5 markers (meso-tetra [4-carboxyphenyl] porphyrin [TCPP], CD206, CD66b, CD3, CD19), algorithm reported as likelihood of lung cancer Proprietary test: CyPath® Lung Lab/Manufacturer: Precision Pathology Services

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Arfoosh, R., Nguyen, K., Fish, A. L., Wells, V., Rebeor, D., Seger, A., Beggs, M., Allen, G. G., El-Bizri, R., & Torricelli, R. (2019). Risk assessment of indeterminate lung nodule characterization by a novel plasma-protein multiplexed assay in current smokers: Results of a clinical experience program. *Biomed Res*, *3*, 1-4. https://doi.org/10.15761/BRR.1000128

Babiarz, J., Hao, Y., Cao, M., Griscom, B., Wilson, D. S., Krimsky, W., Sarkar, S., Bernstein, M., Lee, H., & Wahidi, M. M. (2021). Detection of actionable molecular alterations through combined DNA/RNA molecular profiling of biopsies collected in early-stage lung cancer at time of diagnosis. https://doi.org/10.1200/JCO.2021.39.15_suppl.e20546

Biodesix. (2024). *Nodify Lung*. https://www.biodesix.com/our-tests/nodify-lung

BU. (2015, 04/30/2015). *Veracyte Initiates Launch of Percepta™ Bronchial Genomic Classifier*. Boston University. http://www.bumc.bu.edu/compbiomed/2015/04/30/veracyte-initiates-launch-of-percepta-bronchial-genomic-classifier/

Choi, Y., Qu, J., Wu, S., Hao, Y., Zhang, J., Ning, J., Yang, X., Lofaro, L., Pankratz, D. G., Babiarz, J., Walsh, P. S., Billatos, E., Lenburg, M. E., Kennedy, G. C., McAuliffe, J., & Huang, J. (2020). Improving lung cancer risk stratification leveraging whole transcriptome RNA sequencing and machine learning across multiple cohorts. *BMC Med Genomics*, *13*(Suppl 10), 151. https://doi.org/10.1186/s12920-020-00782-1

D'Andrea, E., Choudhry, N. K., Raby, B., Weinhouse, G. L., & Najafzadeh, M. (2020). A bronchial-airway gene-expression classifier to improve the diagnosis of lung cancer: Clinical outcomes and cost-effectiveness analysis. *Int J Cancer*, *146*(3), 781-790. https://doi.org/10.1002/ijc.32333

Detterbeck, F. C., Lewis, S. Z., Diekemper, R., Addrizzo-Harris, D., & Alberts, W. M. (2013). Executive Summary: Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. *CHEST*, *143*(5 Suppl), 7s-37s. https://doi.org/10.1378/chest.12-2377

Doseeva, V., Colpitts, T., Gao, G., Woodcock, J., & Knezevic, V. (2015). Performance of a multiplexed dual analyte immunoassay for the early detection of non-small cell lung cancer. *J Transl Med*, *13*, 55. https://doi.org/10.1186/s12967-015-0419-y

EarlyCDT. (2024). *EarlyCDT Lung*. https://www.earlycdt.com/

ESMO. (2021). Early and locally advanced non-small-cell lung cancer: an update of the ESMO Clinical Practice Guidelines focusing on diagnosis, staging and systemic and local therapy https://www.esmo.org/guidelines/guidelines-by-topic/esmo-clinical-practice-guidelines-lung-and-chest-tumours/early-stage-and-locally-advanced-non-metastatic-non-small-cell-lung-cancer-esmo-clinical-practice-guidelines/eupdate-early-and-locally-advanced-non-small-cell-lung-cancer-nsclc-treatment-recommendations2

Fahrmann, J. F., Marsh, T., Irajizad, E., Patel, N., Murage, E., Vykoukal, J., Dennison, J. B., Do, K. A., Ostrin, E., Spitz, M. R., Lam, S., Shete, S., Meza, R., Tammemagi, M. C., Feng, Z., & Hanash, S. M. (2022). Blood-Based Biomarker Panel for Personalized Lung Cancer Risk Assessment. *J Clin Oncol*, *40*(8), 876-883. https://doi.org/10.1200/JCO.21.01460

Feller-Kopman, D., Liu, S., Geisler, B. P., DeCamp, M. M., & Pietzsch, J. B. (2017). Cost-Effectiveness of a Bronchial Genomic Classifier for the Diagnostic Evaluation of Lung Cancer. *J Thorac Oncol*, *12*(8), 1223-1232. https://doi.org/10.1016/j.jtho.2017.04.030

Ferguson, J. S., Van Wert, R., Choi, Y., Rosenbluth, M. J., Smith, K. P., Huang, J., & Spira, A. (2016). Impact of a bronchial genomic classifier on clinical decision making in patients undergoing diagnostic evaluation for lung cancer. *BMC Pulm Med*, *16*(1), 66. https://doi.org/10.1186/s12890-016-0217-1

Giraudo, C., Schoot, R., Cardoen, L., Stramare, R., Coppadoro, B., Bisogno, G., Bouhamama, A., Brennan, B., Brisse, H. J., Orbach, D., Coma, A., Di Paolo, P. L., Fayard, C., McDonald, L., Moalla, S., Morosi, C., Pace, E., Tang, V., van Noesel, M. M., . . . van Rijn, R. (2024). Indeterminate pulmonary nodules in non-rhabdomyosarcoma soft tissue sarcoma: A study of the European paediatric Soft Tissue Sarcoma Study Group. *Cancer*, *130*(4), 597-608. https://doi.org/https://doi.org/10.1002/cncr.35061

Goebel, C., Louden, C. L., McKenna, R., Jr., Onugha, O., Wachtel, A., & Long, T. (2020). Blood test shows high accuracy in detecting stage I non-small cell lung cancer. *BMC Cancer*, *20*(1), 137. https://doi.org/10.1186/s12885-020-6625-x

Gould, M. K., Donington, J., Lynch, W. R., Mazzone, P. J., Midthun, D. E., Naidich, D. P., & Wiener, R. S. (2013). Evaluation of individuals with pulmonary nodules: when is it lung cancer? Diagnosis and management of lung cancer, 3rd ed: American College of Chest Physicians evidence-based clinical practice guidelines. *CHEST*, *143*(5 Suppl), e93S-e120S. https://doi.org/10.1378/chest.12-2351

Hendriks, L. E., Kerr, K. M., Menis, J., Mok, T. S., Nestle, U., Passaro, A., Peters, S., Planchard, D., Smit, E. F., Solomon, B. J., Veronesi, G., & Reck, M. (2023). Oncogene-addicted metastatic non-small-cell lung cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up[☆]. *Annals of Oncology*, *34*(4), 339-357. https://doi.org/10.1016/j.annonc.2022.12.009

Hu, Z., Whitney, D., Anderson, J. R., Cao, M., Ho, C., Choi, Y., Huang, J., Frink, R., Smith, K. P., Monroe, R., Kennedy, G. C., & Walsh, P. S. (2016). Analytical performance of a bronchial genomic classifier. *BMC Cancer*, *16*, 161. https://doi.org/10.1186/s12885-016-2153-0

Islam, S. (2024, May 24). *Flexible bronchoscopy in adults: Associated diagnostic and therapeutic procedures*. https://www.uptodate.com/contents/flexible-bronchoscopy-in-adults-associated-diagnostic-and-therapeutic-procedures

Johnson, M. K., Wu, S., Pankratz, D. G., Fedorowicz, G., Anderson, J., Ding, J., Wong, M., Cao, M., Babiarz, J., Lofaro, L., Walsh, P. S., Kennedy, G. C., & Huang, J. (2021). Analytical validation of the Percepta genomic sequencing classifier; an RNA next generation sequencing assay for the assessment of Lung Cancer risk of suspicious pulmonary nodules. *BMC Cancer*, *21*(1), 400. https://doi.org/10.1186/s12885-021-08130-x

Kearney, P., Hunsucker, S. W., Li, X. J., Porter, A., Springmeyer, S., & Mazzone, P. (2017). An integrated risk predictor for pulmonary nodules. *PLoS One*, *12*(5), e0177635. https://doi.org/10.1371/journal.pone.0177635

King, T. (2024, March 7 2024). *Clinical manifestations and diagnosis of idiopathic pulmonary fibrosis*. https://www.uptodate.com/contents/clinical-manifestations-and-diagnosis-of-idiopathic-pulmonary-fibrosis

Lee, H., Whitten, P., Bhadra, K., Dotson, T., Hogarth, D., Benzaquen, S., Pritchett, M., Gordon, J., Bedekar, A., Esterle, M., Smith, K., Bianchi, P., Lenburg, M., & Spira, A. (2017). Prospective Utility of a Bronchial Genomic Classifier for Lung Cancer Detection: Interim Results From a Multicenter Prospective Registry. *CHEST*, *152*(4). https://doi.org/10.1016/j.chest.2017.08.658

Lee, H. J., Mazzone, P., Feller-Kopman, D., Yarmus, L., Hogarth, K., Lofaro, L. R., Griscom, B., Johnson, M., Choi, Y., Huang, J., Bhorade, S., Spira, A., Kennedy, G. C., & Wahidi, M. M. (2021). Impact of the Percepta Genomic Classifier on Clinical Management Decisions in a Multicenter Prospective Study. *CHEST*, *159*(1), 401-412. https://doi.org/10.1016/j.chest.2020.07.067

Li, X. J., Hayward, C., Fong, P. Y., Dominguez, M., Hunsucker, S. W., Lee, L. W., McLean, M., Law, S., Butler, H., Schirm, M., Gingras, O., Lamontagne, J., Allard, R., Chelsky, D., Price, N. D., Lam, S., Massion, P. P., Pass, H., Rom, W. N., . . . Kearney, P. (2013). A blood-based proteomic classifier for the molecular characterization of pulmonary nodules. *Sci Transl Med*, *5*(207), 207ra142. https://doi.org/10.1126/scitranslmed.3007013

Lynch, D. A., Sverzellati, N., Travis, W. D., Brown, K. K., Colby, T. V., Galvin, J. R., Goldin, J. G., Hansell, D. M., Inoue, Y., Johkoh, T., Nicholson, A. G., Knight, S. L., Raoof, S., Richeldi, L., Ryerson, C. J., Ryu, J. H., & Wells, A. U. (2018). Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner Society White Paper. *Lancet Respir Med*, *6*(2), 138-153. https://doi.org/10.1016/s2213-2600(17)30433-2

Mazzone, P. J., Silvestri, G. A., Patel, S., Kanne, J. P., Kinsinger, L. S., Wiener, R. S., Soo Hoo, G., & Detterbeck, F. C. (2018). Screening for Lung Cancer: CHEST Guideline and Expert Panel Report. *CHEST*, *153*(4), 954-985. https://doi.org/10.1016/j.chest.2018.01.016

Mazzone, P. J., Silvestri, G. A., Souter, L. H., Caverly, T. J., Kanne, J. P., Katki, H. A., Wiener, R. S., & Detterbeck, F. C. (2021). Screening for Lung Cancer: CHEST Guideline and Expert Panel Report. *CHEST*. https://doi.org/10.1016/j.chest.2021.06.063

Mazzone, P. J., Wang, X. F., Han, X., Choi, H., Seeley, M., Scherer, R., & Doseeva, V. (2018). Evaluation of a Serum Lung Cancer Biomarker Panel. *Biomark Insights*, *13*, 1177271917751608. https://doi.org/10.1177/1177271917751608

NCCN. (2024a, October 18, 2023). *Lung Cancer Screening Version 2.2024*. https://www.nccn.org/professionals/physician_gls/pdf/lung_screening.pdf

NCCN. (2024b, April 10, 2024). *Non-Small Cell Lung Cancer Version 4.2024*. https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf

NCCN. (2024c). *Small Cell Lung Cancer Version 2.2024*. https://www.nccn.org/professionals/physician_gls/pdf/sclc.pdf

Ostrin, E. J., Sidransky, D., Spira, A., & Hanash, S. M. (2020). Biomarkers for Lung Cancer Screening and Detection. *Cancer Epidemiol Biomarkers Prev*, *29*(12), 2411-2415. https://doi.org/10.1158/1055-9965.Epi-20-0865

Pankratz, D. G., Choi, Y., Imtiaz, U., Fedorowicz, G. M., Anderson, J. D., Colby, T. V., Myers, J. L., Lynch, D. A., Brown, K. K., Flaherty, K. R., Steele, M. P., Groshong, S. D., Raghu, G., Barth, N. M., Walsh, P. S., Huang, J., Kennedy, G. C., & Martinez, F. J. (2017). Usual Interstitial Pneumonia Can Be Detected in Transbronchial Biopsies Using Machine Learning. *Ann Am Thorac Soc*, *14*(11), 1646-1654. https://doi.org/10.1513/AnnalsATS.201612-947OC

Planchard, D., Popat, S., Kerr, K., Novello, S., Smit, E., Faivre-Finn, C., Mok, T., Reck, M., Van Schil, P., & Hellmann, M. (2018). Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of Oncology*, *29*, iv192-iv237. https://doi.org/10.1093/annonc/mdy275

Postmus, P. E., Kerr, K. M., Oudkerk, M., Senan, S., Waller, D. A., Vansteenkiste, J., Escriu, C., Peters, S., & on behalf of the, E. G. C. (2017). Early and locally advanced non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. *Annals of Oncology*, *28*(suppl_4), iv1-iv21. https://doi.org/10.1093/annonc/mdx222

Raghu, G., Flaherty, K. R., Lederer, D. J., Lynch, D. A., Colby, T. V., Myers, J. L., Groshong, S. D., Larsen, B. T., Chung, J. H., Steele, M. P., Benzaquen, S., Calero, K., Case, A. H., Criner, G. J., Nathan, S. D., Rai, N. S., Ramaswamy, M., Hagmeyer, L., Davis, J. R., . . . Martinez, F. J. (2019). Use of a molecular classifier to identify usual interstitial pneumonia in conventional transbronchial lung biopsy samples: a prospective validation study. *Lancet Respir Med*, *7*(6), 487-496. https://doi.org/10.1016/s2213-2600(19)30059-1

Raghu, G., Mikacenic, Carmen. (2023, November 2023). *Pathogenesis of idiopathic pulmonary fibrosis*. https://www.uptodate.com/contents/pathogenesis-of-idiopathic-pulmonary-fibrosis

Raghu, G., Remy-Jardin, M., Myers, J. L., Richeldi, L., Ryerson, C. J., Lederer, D. J., Behr, J., Cottin, V., Danoff, S. K., Morell, F., Flaherty, K. R., Wells, A., Martinez, F. J., Azuma, A., Bice, T. J., Bouros, D., Brown, K. K., Collard, H. R., Duggal, A., . . . Wilson, K. C. (2018). Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. *American Journal of Respiratory and Critical Care Medicine*, *198*(5), e44-e68. https://doi.org/10.1164/rccm.201807-1255ST

Raghu, G., Remy-Jardin, M., Richeldi, L., Thomson, C. C., Inoue, Y., Johkoh, T., Kreuter, M., Lynch, D. A., Maher, T. M., Martinez, F. J., Molina-Molina, M., Myers, J. L., Nicholson, A. G., Ryerson, C. J., Strek, M. E., Troy, L. K., Wijsenbeek, M., Mammen, M. J., Hossain, T., . . . Wilson, K. C. (2022). Idiopathic Pulmonary Fibrosis (an Update) and Progressive Pulmonary Fibrosis in Adults: An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. *Am J Respir Crit Care Med*, *205*(9), e18-e47. https://doi.org/10.1164/rccm.202202-0399ST

Roncarati, R., Lupini, L., Miotto, E., Saccenti, E., Mascetti, S., Morandi, L., Bassi, C., Rasio, D., Callegari, E., Conti, V., Rinaldi, R., Lanza, G., Gafà, R., Papi, A., Frassoldati, A., Sabbioni, S., Ravenna, F., Casoni, G. L., & Negrini, M. (2020). Molecular testing on bronchial washings for the diagnosis and predictive assessment of lung cancer. *Mol Oncol*, *14*(9), 2163-2175. https://doi.org/10.1002/1878-0261.12713

Sethi, S., Oh, S., Chen, A., Bellinger, C., Lofaro, L., Johnson, M., Huang, J., Bhorade, S. M., Bulman, W., & Kennedy, G. C. (2022). Percepta Genomic Sequencing Classifier and decision-making in patients with high-risk lung nodules: a decision impact study. *BMC Pulm Med*, *22*(1), 26. https://doi.org/10.1186/s12890-021-01772-4

Silvestri, G. A., Tanner, N. T., Kearney, P., Vachani, A., Massion, P. P., Porter, A., Springmeyer, S. C., Fang, K. C., Midthun, D., Mazzone, P. J., & Team, P. T. (2018). Assessment of Plasma Proteomics Biomarker's Ability to Distinguish Benign From

Malignant Lung Nodules: Results of the PANOPTIC (Pulmonary Nodule Plasma Proteomic Classifier) Trial. *CHEST*, *154*(3), 491-500. https://doi.org/10.1016/j.chest.2018.02.012

Silvestri, G. A., Vachani, A., Whitney, D., Elashoff, M., Porta Smith, K., Ferguson, J. S., Parsons, E., Mitra, N., Brody, J., Lenburg, M. E., & Spira, A. (2015). A Bronchial Genomic Classifier for the Diagnostic Evaluation of Lung Cancer. *N Engl J Med*, *373*(3), 243-251. https://doi.org/10.1056/NEJMoa1504601

Tanner, N. T., Springmeyer, S. C., Porter, A., Jett, J. R., Mazzone, P., Vachani, A., & Silvestri, G. A. (2021). Assessment of Integrated Classifier's Ability to Distinguish Benign From Malignant Lung Nodules: Extended Analyses and 2-Year Follow-Up Results of the PANOPTIC (Pulmonary Nodule Plasma Proteomic Classifier) Trial. *CHEST*, *159*(3), 1283-1287. https://doi.org/10.1016/j.chest.2020.10.069

Vaarwerk, B., Bisogno, G., McHugh, K., Brisse, H. J., Morosi, C., Corradini, N., Jenney, M., Orbach, D., Chisholm, J. C., Ferrari, A., Zanetti, I., De Salvo, G. L., van Rijn, R. R., & Merks, J. H. M. (2019). Indeterminate Pulmonary Nodules at Diagnosis in Rhabdomyosarcoma: Are They Clinically Significant? A Report From the European Paediatric Soft Tissue Sarcoma Study Group. *Journal of Clinical Oncology*, *37*(9), 723-730. https://doi.org/10.1200/JCO.18.01535

Veracyte. (2023). *Percepta® Nasal Swab*. https://lung.veracyte.com/pipeline/

Veracyte. (2024a). *Envisia*. Retrieved 2024 from https://lung.veracyte.com/envisia/

Veracyte. (2024b). *INTERSTITIAL LUNG DISEASES*. https://www.veracyte.com/interstitial-lung-disease/

Veracyte. (2024c). *Percepta® GSC stratifies the risk of lung cancer to guide patient management when bronchoscopy is inconclusive*. https://lung.veracyte.com/percepta-gsc/

Weinberger, S., McDermott, Shaunagh. (2024, March 24). *Diagnostic evaluation of the incidental pulmonary nodule*. https://www.uptodate.com/contents/diagnostic-evaluation-of-the-incidental-pulmonary-nodule

Whitney, D. H., Elashoff, M. R., Porta-Smith, K., Gower, A. C., Vachani, A., Ferguson, J. S., Silvestri, G. A., Brody, J. S., Lenburg, M. E., & Spira, A. (2015). Derivation of a bronchial genomic classifier for lung cancer in a prospective study of patients undergoing diagnostic bronchoscopy. *BMC Med Genomics*, *8*, 18. https://doi.org/10.1186/s12920-015-0091-3

Mutation Analysis in Myeloproliferative Neoplasms

Policy Number: AHS – M2101 – Mutation Analysis in Myeloproliferative Neoplasms	Prior Policy Name and Number, as applicable: • AHS-M2101-20180316-*JAK2*, CALR or MPL Mutation Analysis in Myeloproliferative Neoplasms
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Myeloproliferative neoplasms (MPN) are a heterogeneous group of clonal disorders characterized by overproduction of one or more differentiated myeloid lineages (Grinfeld et al., 2017). These include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). The majority of MPN result from somatic mutations in the three driver genes, *JAK2*, *CALR*, and *MPL*, which represent major diagnostic criteria in combination with hematologic and morphological abnormalities (Rumi & Cazzola, 2017).

Terms such as male and female are used when necessary to refer to sex assigned at birth.

II. Related Policies

Policy Number	Policy Title
AHS-M2182	Genomic Testing for Hematopoietic Neoplasms

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For the diagnosis of individuals presenting with clinical, laboratory, or pathological findings suggesting classic forms of myeloproliferative neoplasms (MPN)(e.g., polycythemia vera [PV], essential thrombocythemia [ET], or primary myelofibrosis [PMF]), *JAK2, CALR* or *MPL* mutation testing **MEETS COVERAGE CRITERIA** in any of the following situations:

   a) For individuals suspected to have PV who meet at least **one** of the following testing criteria:

      i) Hemoglobin greater than 16.5 g/dL in men or greater than 16.0 g/dL in women; **or** hematocrit greater than 49% in men or greater than 48% in women; **or** increased red cell mass (more than 25% above mean normal predicted value), **and** no other known cause of erythrocytosis, when measured on two separate occasions.

      ii) A bone marrow (BM) biopsy showing hypercellularity for age with trilineage hyperplasia including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size).

   b) For individuals suspected to have ET who meet at least **one** of the following testing criteria:

      i) Platelet count greater than or equal to 450 × 10^9/L that has persisted for more than three months.

      ii) A BM biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers.

   c) For individuals suspected to have PMF who meet at least **one** of the following testing criteria:

      i) The individual has demonstrated leukocytosis of greater than or equal to 11 x 10^9/L on two separate occasions in the absence of other conditions that can cause leukocytosis.

      ii) The individual has an enlarged spleen.

      iii) A BM biopsy shows megakaryocytic proliferation and atypia, without reticulin fibrosis >grade 1, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation, and often decreased erythropoiesis.

      iv) A BM biopsy shows presence of megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3.

2) To exclude a diagnosis of chronic myeloid leukemia (CML) for individuals with a suspected MPN, fluorescence in situ hybridization (FISH) or reverse transcriptase polymerase chain reaction (RT-PCR) testing on a peripheral blood sample to detect *BCR::ABL1* transcripts **MEETS COVERAGE CRITERIA**.

3) For individuals with a clinical suspicion of prePMF or overt PMF who have already tested negative for mutations in *JAK2, CALR,* or *MPL* and who do not meet the WHO criteria for BCR-AB1+ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms, screening for mutations in *ASXL1, CBL, DNMT3A, EZH2, IDH1/IDH2, RAS, SRSF2, SFS3B1*, *TET2, TP53,* and *U2AF1* (see Note 1) **MEETS COVERAGE CRITERIA**.

4) For individuals diagnosed with Budd-Chiari Syndrome, *JAK2, CALR,* or *MPL* mutation testing **MEETS COVERAGE CRITERIA**.

5) For individuals with normal blood counts and unexplained splanchnic vein thrombosis, screening for *JAK2* V617F **MEETS COVERAGE CRITERIA**.

6) For individuals suspected to have chronic neutrophilic leukemia, testing for *CSF3R* mutations **MEETS COVERAGE CRITERIA**.

7) For individuals with a clinical suspicion of mastocytosis, screening for *KIT* D816V **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

8) For all other situations not described above, *JAK2* tyrosine kinase, *CALR*, and *MPL* mutation testing **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: For 5 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
ABL	*Abelson murine leukemia viral oncogene*
aCML	Atypical chronic myeloid leukemia
ARMS	Amplification refractory mutation system

ASXL1	*additional sex combs like 1, transcriptional regulator*
BCR	Breakpoint cluster region
BPGM	*Bisphosphoglycerate mutase*
BSH	British Society for Haematology
CALR	*Calreticulin*
CBL	*Casitas B-lineage lymphoma* proto-oncogene
CELNOS	Chronic eosinophilic leukemia, not otherwise specified
CML	Chronic myeloid leukemia
CMML	Chronic myelomonocytic leukemia
CNL	Chronic neutrophilic leukemia
CSF3R	*Colony stimulating factor 3 receptor*
DNM3TA	*DNA methyltransferase 3 alpha*
EASL	European Association for the Study of the Liver
ELN	European Leukemia Net
ESMO	European Society of Medical Oncology
ET	Essential thrombocythemia
EZH2	*Enhancer of zeste 2 polycomb repressive complex 2 subunit*
FLT3	*Fms related receptor tyrosine kinase 3*
FLT3-ITD	*FLT3*- internal duplications
HBA1	*Hemoglobin subunit alpha 1*
HBA2	*Hemoglobin subunit alpha 2*
HBB	*Hemoglobin subunit beta*
HSC	Hematopoietic stem cell
HTLV	Human T-lymphotropic virus type 1
IDH1	*Isocitrate dehydrogenase (NADP(+)) 1*
IDH2	*Isocitrate dehydrogenase (NADP(+)) 2*
IR	Ionizing radiation
JAK2	*Janus Kinase 2*
JMML	Juvenile myelomonocytic leukemia
LNK	*Lymphocyte adapter protein*
MONU	MPN unclassifiable
MPL	*MPL proto-oncogene, thrombopoietin receptor*
MPN	Myeloproliferative neoplasms
NCCN	National Comprehensive Cancer Network
PCR	Polymerase chain reaction
PMF	Primary myelofibrosis

PV	Polycythemia vera
RAS	*Rat sarcoma virus* gene
SETBP	*SET binding protein*
SF3B1	*Splicing factor 3b subunit 1*
SH2B3	*SH2B adaptor protein 3*
SRSF2	*Serine and arginine rich splicing factor 2*
STAT	Signal transducer and activator of transcription
TET2	*Tet methylcytosine dioxygenase 2*
TP53	*Tumor protein p53*
U2AF1	*U2 small nuclear RNA auxiliary factor 1*
WHO	World Health Organization

V. Scientific Background

Myeloproliferative neoplasms, including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF), arise from somatic mutation in hematopoietic stem cell (HSC) that clonally expand resulting in single or multilineage hyperplasia (Vainchenker & Kralovics, 2017). They are relatively rare, affecting 0.84 (PV), 1.03 (ET), and 0.47 (PMF) per 100,000 people worldwide; however, these may not be reflective of its true incidence due to the high heterogeneity of MPN (Titmarsh et al., 2014).

Myeloproliferative neoplasms share features of bone marrow hypercellularity, increased incidence of thrombosis or hemorrhage, and an increased rate of progression to acute myeloid leukemia. Abnormalities in cytokine signaling pathways are common and usually lead to increased JAK-STAT signaling (Grinfeld et al., 2017). PV is characterized by erythrocytosis with suppressed endogenous erythropoietin production, bone marrow panmyelosis, and *JAK2* mutation leading to constitutive activation. ET is defined by thrombocytosis; bone marrow megakaryocytic proliferation; and presence of *JAK2*, *CALR*, or *MPL* mutation. PMF is characterized by bone marrow megakaryocytic proliferation; reticulin and/or collagen fibrosis; and presence of *JAK2*, *CALR*, or *MPL* mutation (Rumi & Cazzola, 2017). Mutations in other genes involved in signal transduction (*CBL, LNK/SH2B3*), chromatin modification (*TET2*, *EZH2*, *IDH1/2, ASXL1*, *DNM3TA*), RNA splicing (*SF3B1*, *SRSF2*, *U2AF1*), and tumor suppressor function (*TP53*) have also been reported and are considered "high-risk" (NCCN, 2019, 2023)

The gene *JAK2*, which stands for "Janus Kinase 2," is a gene whose mutation is responsible for a significant amount of MPNs. It is a mutation that causes hypersensitivity of hematopoietic progenitor cells to other cytokines, and this mutation typically appears on red blood cells or bone marrow cells. This mutation is often found

on exon 12 or 14, and the exon 14 mutation results in a cytokine-independent activation of several regulatory pathways. *JAK2* mutations contribute to at least 95% of PV cases, about 50-65% of ET cases, and 60-65% of PMF cases (Tefferi, 2022a, 2023a, 2023b).

The gene *MPL*, which encodes a thrombopoietin receptor, also contributes to MPNs. *MPL* mutations result in a similar phenotype to *JAK2* mutations; both result in cytokine-independent growth of their targets. However, *MPL* mutations are not nearly as common as *JAK2* and *CALR* mutations, casting doubt on the clinical utility for testing. *MPL* mutations comprise up to 4% of ET cases and 5% of PMF cases (Tefferi, 2022a, 2023a, 2023b).

The gene *CALR* encodes calreticulin (or calregulin), which is a Ca^{2+} binding protein. The mutation typically involves the creation of the incorrect Ca^{2+} binding region, thereby not allowing the protein to perform its regular duties such as maintaining calcium homeostasis. This results in a similar phenotype to the *JAK2* mutation, which is the cytokine-independent activation of regulatory pathways. *CALR* mutations contribute to approximately 15-25% of ET cases and 20-25% of PMF cases, and about 70% of ET or PMF patients without a *JAK2* or *MPL* mutation have this mutation (Tefferi, 2022a, 2023a, 2023b).

The significance of *JAK2*, *MPL*, *CALR* and other mutations in the genesis of the MPNs as well as their roles in determining phenotype are unclear (Tefferi, 2022b). However, integrated genomic analyses suggest that regardless of diagnosis or *JAK2* mutational status, MPNs are characterized by upregulation of JAK-STAT target genes, demonstrating the central importance of this pathway in the pathogenesis (Rampal et al., 2014). This may lead to development of novel *JAK2* therapeutics (Silvennoinen & Hubbard, 2015). Thus, mutation analysis at the time of diagnosis has value for determining prognosis as well as individual risk assessment and guide treatment-making decisions (Hussein et al., 2013; Tefferi, 2022b).

Neutrophilia, an increase in peripheral blood neutrophils at least two standard deviations above the mean, can be associated with any the MPNs. In chronic neutrophilic leukemia (CNL), *CSF3R* mutations have been discovered in most patients with CNL (Coates, 2022; Tefferi et al., 2014). A study released in 2013 reported 16 of 27 patients with CNL or atypical chronic myeloid leukemia (aCML) had activating mutations in *CSF3R* (Maxson et al., 2013). *SETBP1* has also been used as a part of comprehensive mutation profiling in distinguishing aCML and chronic myelomonocytic leukemia (CMML). A 2019 NGS study reports significant differences in the profiles of patients with aCML or CMML when comparing *TET2*, *SETBP1*, and *CSF3R*. The researchers conclude, "differential mRNA expression could be detected between both

cohorts in a subset of genes (*FLT3*, *CSF3R*, and *SETBP1* showed the strongest correlation). However, due to high variances in the mRNA expression, the potential utility for the clinic is limited" (Faisal et al., 2019).

Proprietary Testing

In 2017 the FDA approved ipsogen® *JAK2* RGQ PCR Kit (FDA, 2017b) to detect Janus Tyrosine Kinase 2 (*JAK2*) gene mutation G1849T (V617F) with an allele-specific, quantitative, polymerase chain reaction (PCR) using an amplification refractory mutation system (ARMS). The device marketing authorization was based on data from a clinical study of 473 suspected patients with MPNs, 276 with suspected PV, 98 with suspected ET, and 99 with suspected PMF. The study compared results from the ipsogen *JAK2* RGQ PCR Kit to results obtained with independently validated bi-directional sequencing. The study found that the ipsogen *JAK2* RGQ PCR Kit test was in 96.8% agreement with the reference method, 100% in positive agreement, and 95.1% in negative agreement, with 458 samples in agreement out of 473. The concordance with each condition was also high; agreement of 90.8% within the ET samples (89/98), 94.9% agreement within the PMF samples (94/99), and 99.6% within the PV samples (275/276). All three conditions had positive agreements of 100%. The authors went on to note that the 15 samples with disagreeing results had mutation levels under the detection capability of bi-directional sequencing. To validate these 15 samples, an independently validated NGS panel was used to compare results with the kit, and all 15 samples were found to test positive, thereby agreeing with the kit. The authors concluded that the kit was accurate for any mutation levels at or above 1% (FDA, 2017a).

Other proprietary tests are available for mutational analysis in MPN. IntelliGEN® Myeloid is a NGS assay that analyzes fifty genes for somatic mutations that could be useful in providing diagnostic or prognostic information for patients with MDS, AML, or MPN (labcorp, 2023). The LeukoVantage® Myeloid Neoplasm Mutation Panel detects myeloid neoplasm-associated mutations in 48 genes associated with AML, MDS, and MPN. The LeukoVantage AML panel can be used to assess AML subclass and prognosis based on genetic abnormalities in *NPM1, CEBPA*, and *RUNX1* (Quest_Diagnostics, 2020). NeoGenomics offers two tests which include the MPN Reflex Test and NeoTYPE® Myeloid Disorders Profile. The MPN Reflex Test is a sequential testing panel for qualitative detection of *JAK2* V617F*, JAK2 Exon 12-14, CALR exon 9*, and *MPL exon 10 (NeoGenomics, 2022a)*. NeoTYPE® Myeloid Disorders Profile is a 63 gene panel that targets known mutations associated with AML, MPN, MDS, CML, chronic myelomonocytic leukemia (CMML) and juvenile myelomonocytic leukemia (JMML) (NeoGenomics, 2022b). Centogene has released a Myeloid Tumor

Panel which targets 35 genes that are associated with myeloid malignancies which also include AML, MPN, MDS, CML, CMML, and JMML (Centogene, 2022).

Analytical Validity

Poluben et al. (2019) analyzed the characteristics of myeloproliferative neoplasms (MPN) in patients exposed to ionizing radiation (IR) from the 1986 Chernobyl accident. 281 patients (90 exposed to radiation, 181 unexposed) were included. *JAK2*, *MPL*, and *CALR* mutations were identified. IR-exposed patients had several different genetic features compared to the unexposed cohort: lower rate of *JAK2* V617F mutations (58.4% vs 75.4%), higher rate of type 1-like *CALR* mutations (12.2% vs 3.1%), higher rate of triple-negative cases (27.8% vs 16.2%), and higher rate of "potentially pathogenic" sequence variants (4.8 vs 3.1). The authors suggested IR-exposed patients as a cohort with "distinct" genomic characteristics (Poluben et al., 2019).

Rosenthal et al. (2021) studied the analytical validity of a 48-gene NGS panel for detecting mutations in myeloid neoplasms. The panel detects detect single nucleotide variations (SNVs), insertions/deletions, and *FLT3* internal tandem duplications (*FLT3*-ITD). 184 samples were analyzed using the 48-gene panel and compared to those identified by a 35-gene hematologic neoplasms panel using an additional 137 samples. Analytical validation yielded 99.6% sensitivity and 100% specificity. Concordance of variants detected by the 2 tested panels was 100%. "Among patients with suspected myeloid neoplasms, 54.5% patients had at least one clinically significant mutation: 77% in AML patients, 48% in MDS, and 45% in MPN." The authors conclude that "the assay can identify mutations associated with diagnosis, prognosis, and treatment options of myeloid neoplasms even in technically challenging genes" (Rosenthal et al., 2021).

Clinical Utility and Validity

An Argentinean study focusing on establishing the frequency of *JAK2*, *MPL*, and *CALR* mutations and comparing their clinical and hematological features corroborates this importance. Mutations of *JAK2*V617F, *JAK2* exon 12, *MPL* W515L/K and *CALR* were analyzed in 439 patients with *BCR-ABL1*-negative MPN, and it was demonstrated that these mutations were present in 94.9% of the cases of polycythemia vera (PV), 85.5% in patients with essential thrombocythemia (ET), and 85.2% with primary myelofibrosis, leading the researchers to conclude that "the combined genetic tests of these driver mutations are essential for accurate diagnoses of *BCR-ABL1*-negative MPN" (Ojeda et al., 2018).

VI. Guidelines and Recommendations

World Health Organization (WHO)

The 2017 edition of the World Health Organization's classification of myeloid neoplasm and acute leukemia proposed the following criteria for the diagnosis of PV, ET and PMF.

WHO Criteria for PV

Diagnosis of PV requires meeting either all three major criteria, or the first two major criteria and the minor criterion:

Major Criteria

1. Hemoglobin >16.5 g/dL in men; Hemoglobin >16.0 g/dL in women, or Hematocrit >49% in men; Hematocrit >48% in women, or Increased red cell mass (More than 25% above mean normal predicted value)
2. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (differences in size)
3. Presence of *JAK2V617F* or *JAK2* exon 12 mutation

Minor Criteria

Subnormal serum erythropoietin level

WHO Criteria for ET

Diagnosis of ET requires meeting all four major criteria or the first three major criteria and the minor criterion:

Major Criteria

1. Platelet count ≥450 × 10^9/L
2. Bone marrow biopsy showing proliferation mainly of the megakaryocyte lineage with increased numbers of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase or left shift in neutrophil granulopoiesis or erythropoiesis and very rarely minor (grade 1) increase in reticulin fibers
3. Not meeting WHO criteria for BCR-ABL1+ CML, PV, PMF, myelodysplastic syndromes, or other myeloid neoplasms
4. Presence of *JAK2*, *CALR*, or *MPL* mutation

Minor Criteria

Presence of a clonal marker or absence of evidence for reactive thrombocytosis

WHO Criteria for PrePMF

Diagnosis of prePMF requires meeting all three major criteria, and at least one minor criterion:

Major Criteria

1. Megakaryocytic proliferation and atypia, without reticulin fibrosis >grade 1, accompanied by increased age-adjusted BM cellularity, granulocytic proliferation, and often decreased erythropoiesis
2. Not meeting the WHO criteria for BCR-ABL1$^+$ CML, PV, ET, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker (e.g., *ASXL1*, *EZH2*, *TET2*, *IDH1/IDH2*, *SRSF2*, *SF3B1*), or absence of minor reactive BM reticulin fibrosis

Minor Criteria (presence of one of the following):

1. Anemia not attributed to a comorbid condition
2. Leukocytosis ≥11 × 10^9/L
3. Palpable splenomegaly
4. LDH increased to above upper normal limit of institutional reference range

The minor criteria must be confirmed in two consecutive determinations.

WHO Criteria for Overt PMF

Diagnosis of overt PMF requires meeting all three major criteria, and at least 1 minor criterion

Major Criteria

1. Presence of megakaryocytic proliferation and atypia, accompanied by either reticulin and/or collagen fibrosis grades 2 or 3
2. Not meeting WHO criteria for ET, PV, BCR-ABL1$^+$ CML, myelodysplastic syndromes, or other myeloid neoplasms
3. Presence of *JAK2*, *CALR*, or *MPL* mutation or in the absence of these mutations, presence of another clonal marker (e.g., *ASXL1*, *EZH2*, *TET2*, *IDH1/IDH2*, *SRSF2*, *SF3B1*), or absence of reactive myelofibrosis

Minor Criteria

1. Anemia not attributed to a comorbid condition
2. Leukocytosis ≥11 × 10^9/L
3. Palpable splenomegaly
4. LDH increased to above upper normal limit of institutional reference range

5. Leukoerythroblastosis (Barbui, Thiele, et al., 2018)

These guidelines also list four additional "clinicopathologic entities" for MPNs: "chronic myeloid leukemia (CML), chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia, not otherwise specified (CELNOS) and MPN, unclassifiable (MPN-U)". The guidelines note that although *CSF3R* mutations are "specific" to WHO-defined CNL, they also remark that "the presence of a membrane proximal *CSF3R* mutation in a patient with neutrophilic granulocytosis should be sufficient for the diagnosis of CNL, regardless of the degree of leukocytosis" (Barbui, Thiele, et al., 2018).

European LeukemiaNet (ELN)

ELN guidelines also recommend "strict adherence" to these guidelines for the three categories of Philadelphia-negative MPNs, (i.e. ET, PV, and MF) (Barbui, Tefferi, et al., 2018).

However, they also recommend "searching" for complementary clonal markers such as *ASXL1*, *EZH2*, *IDH1/2*, and *SRSF2* for patients that tested negative for the three driver mutations and have bone marrow features as well as a clinical phenotype consistent with myelofibrosis (Barbui, Tefferi, et al., 2018).

National Comprehensive Cancer Network (NCCN)

The NCCN Guidelines Version 1.2024 Myeloproliferative Neoplasms recommends molecular testing for *JAK2* V617F mutations as part of an initial workup for all patients. If *JAK2* mutation testing is negative, molecular testing for *CALR* and *MPL* mutations should be performed for patients with suspected ET and MF, and molecular testing for *JAK2* exon 12 should be done for patients who test negative for *JAK2* but are suspected of PV. An NGS panel including *JAK2*, *CALR*, and *MPL* may also be used for the workup of all patients. The NCCN follows the 2017 edition of the WHO diagnostic criteria for all three conditions. The NCCN does state that NGS "may be useful to establish clonality in selected circumstances (e.g., triple negative non-mutated *JAK2*, *MPL*, and *CALR*)". The NCCN includes a list of somatic mutations with prognostic significance in individuals with MPN that includes the *ASXL1*, *EZH2*, *RAS, IDH1/2*, *SRSF2*, *TP53*, *U2AF1* Q157, *DNMT3A,* and *CBL*.

For individuals suspected of MPN, the NCCN recommends excluding a diagnosis of chronic myeloid leukemia: "Fluorescence in situ hybridization (FISH) or a multiplex reverse transcriptase polymerase chain reaction (RT-PCR), if available, on peripheral blood to detect *BCR::ABL1* transcripts and exclude the diagnosis of CML is especially recommended for patients with left-shifted leukocytosis and/or thrombocytosis with basophilia" (NCCN, 2023).

British Society for Haematology (BSH)

The BSH recommends testing for *CALR* for patients suspected of ET and PMR, as *CALR* mutations account for most patients without either a *JAK2* or *MPL* mutation. The authors found that as many as one third of ET and PMF patients had a mutation in exon 9 of the *CALR* gene (Harrison et al., 2014).

The BSH also published guidelines on the diagnosis of polycythaemia vera (PV). In it, they divide PV into *JAK2*-positive and *JAK2*-negative PV. For *JAK2*-positive PV, the only two diagnostic criteria are as follows:

- "High haematocrit (>0·52 in men, >0·48 in women) OR raised red cell mass (>25% above predicted)"
- "Mutation in *JAK2*"

For *JAK2*-negative PV, the diagnostic criteria are as follows (requiring A1-A4, as well as another "A" criteria or two "B" criteria).

- "A1 Raised red cell mass (>25% above predicted) OR haematocrit ≥0·60 in men, ≥0·56 in women"
- "A2 Absence of mutation in *JAK2*"
- "A3 No cause of secondary erythrocytosis"
- "A4 Bone marrow histology consistent with polycythaemia vera"
- "A5 Palpable splenomegaly"
- "A6 Presence of an acquired genetic abnormality (excluding BCR-ABL1) in the haematopoietic cells"
- "B1 Thrombocytosis (platelet count >450 × 10^9 /l)"
- "B2 Neutrophil leucocytosis (neutrophil count >10 × 10^9 /l in non-smokers, ≥12.5 × 10^9 /l in smokers)"
- "B3 Radiological evidence of splenomegaly"
- "B4 Low serum erythropoietin"

The guidelines also note that investigation of erythrocytosis should be undertaken to properly identify the diagnosis. The BSH remarks that EPO receptor mutations may be a primary cause for erythrocytosis and that *EGNL1*, *VHL*, and *EPAS1* mutations may be a secondary cause. Other hemoglobinopathies caused by mutations in genes such as *HBA1*, *HBA2*, *HBB*, or *BPGM* may also be a factor (McMullin et al., 2019).

In 2021, the BSH published guidelines on the use of genetic tests to diagnose and manage patients with myeloproliferative neoplasms. The following recommendations were made:

1. "Molecular screening for *JAK2*, *CALR* and *MPL* variants as appropriate is recommended in patients with persistent erythrocytosis or thrombocytosis (GRADE 1B).
2. Screening for *JAK2* V617F is recommended in cases with normal blood counts and unexplained splanchnic vein thrombosis (GRADE 1B) and may be considered in selected patients with unexplained cerebral vein thrombosis (GRADE 2C).
3. Screening for *CALR* variants may be considered in patients with splanchnic vein thrombosis or cerebral vein thrombosis (GRADE 2C).
4. Screening for *JAK2*, *CALR* and *MPL* variants should be considered for patients with arterial or unprovoked venous thrombosis who have a mildly or variably elevated haematocrit or platelet count that persists for 2–3 months (GRADE 2C).
5. *BCR–ABL1* should be excluded in cases with persistent thrombocytosis negative for *JAK2, CALR* and *MPL* variants or with atypical features (GRADE 1B).
6. Younger patients (e.g., under 60 years) with bone marrow histology typical of ET [or myeloproliferative neoplasm, unclassifiable (MPN-U) or suspected prefibrotic MF] where confirmation of a clonal disorder would be useful in view of the patient's likely long-term disease course and ideally where a broad panel that covers non-canonical variants in *JAK2* and *MPL* and a range of other driver genes is available.
7. Patients with significant thrombocytosis (e.g., platelet count > 600 × 10^9/l), no reactive cause and borderline bone marrow histology, where cytoreduction would be indicated if there was convincing evidence of a clonal disorder. Examples would include those with an unexplained thrombotic event, particularly younger patients. For older patients without thrombosis, testing may be considered but results must be interpreted with caution in view of the possibility of incidental CH.
8. A myeloid gene panel and cytogenetic analysis (or equivalent) is recommended for patients with bone marrow histology and clinical features consistent with PMF (+/− suggestive features of MDS or MDS/MPN) who test negative for *JAK2*/*CALR*/*MPL* (GRADE 1B).
9. A myeloid gene panel and cytogenetic analysis (or equivalent) is not recommended for most patients with *JAK2*/*CALR*/*MPL*-negative erythrocytosis or thrombocytosis but may be considered in individual cases (GRADE 2C).
10. Myeloid gene panel testing is recommended for MPN cases who test positive for *JAK2*/*CALR*/*MPL* mutations and have additional cytopenias(s) at diagnosis, unexplained ring sideroblasts or other dysplasia, increased blasts (including blastic transformation), peripheral-blood monocytosis or atypical clinical features (GRADE 1B).

11. Myeloid gene panel testing and conventional karyotyping are recommended for all patients with PMF, post-PV or post-ET MF who are candidates for allogeneic stem cell transplant (GRADE 1B).
12. Myeloid gene panel testing should be considered for other patients if the additional genomic data will guide clinical management (GRADE 2C).
13. High-sensitivity assays of mutant allele burden are recommended following post-allogeneic stem cell transplant to monitor for residual disease (GRADE 1C).
14. Quantitative assays of mutant allele burden are not recommended for most MPN patients but may be considered where demonstration of molecular response would influence clinical management (GRADE 2C).
15. Patients with persistent eosinophilia should be investigated initially for *FIP1L1–PDGFRA* by FISH and/or nested RT-PCR (GRADE 1B).
16. BM cytogenetics or FISH is recommended to screen for other fusion genes, which must then be confirmed by molecular methods (GRADE 1B).
17. Myeloid gene panel and *KIT* D816V testing should be considered for patients with persistent unexplained eosinophilia who test negative for fusion genes (GRADE 2B).
18. Testing for *CSF3R* variants, preferably as part of wider myeloid panel, is recommended for all patients with suspected CNL (Grade 2B).
19. Sensitive testing for *KIT* D816V is recommended for all patients with a clinical suspicion of mastocytosis (GRADE 1B).
20. If negative for *KIT* D816V, screening for other *KIT* mutations should be considered for adults (but is recommended for children) (GRADE 1B).
21. Myeloid panel analysis is recommended for patients with advanced SM who are candidates for allogeneic stem cell transplantation (GRADE 1B).
22. Myeloid panel analysis may be considered for other SM patients if the apparent aggressiveness of the disease might influence options for therapy (GRADE 2B).
23. Myeloid panel and/or BM cytogenetics should be considered to characterise the AHN component of SM-AHN (GRADE 2B).
24. *BCR–ABL1* should be excluded in all cases of suspected MDS/MPN, and rearrangements associated with MLN-eo should be excluded in cases with eosinophilia (GRADE 1B).
25. Myeloid gene panel analysis and BM cytogenetics or SNP array is recommended for patients diagnosed with MDS/MPN and for cases with suspected MDS/MPN but with indeterminate morphology (GRADE 1B)" (Cross et al., 2021).

European Association for the Study of the Liver (EASL)

For myeloproliferative neoplasms, the EASL recommends testing for *JAK2* V617F mutations in splanchnic vein thrombosis patients, as well as patients with normal peripheral blood cell counts. If the *JAK2* mutation test is negative, a calreticulin

mutation test should be performed, and if both are negative, a bone marrow histology analysis should be performed (EASL, 2016).

European Society of Medical Oncology (ESMO)

The ESMO recommends that anyone with a suspected MPN be tested for the three driver mutations (*JAK2*, *CALR*, *MPL*) and that genotyping should be obtained at diagnosis. However, the ESMO states that it is not recommended to repeat testing in follow-up or assessing response to treatment, except for "allogeneic stem-cell transplantation and possibly interferon treatment". For these two assessments a detection limit of ≤1% is recommended. The ESMO also notes that conventional sequencing methods (PCR, melting analysis) may be used for detecting mutations (Vannucchi et al., 2015).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website http://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, please visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

On July 28, 2017 the FDA approved ipsogen® JAK2 RGQ PCR Kit (FDA, 2017b) to detect Janus Tyrosine Kinase 2 (JAK2) gene mutation G1849T (V617F) with an allele-specific, quantitative, polymerase chain reaction (PCR) using an amplification refractory mutation system (ARMS). This is the first FDA-authorized test intended to help physicians in evaluating patients for suspected Polycythemia Vera (PV). However, the FDA specifically states that this test is not intended for a stand-alone diagnosis of an MPN, nor can it detect less common mutations for MPN such as an exon 12 mutation (FDA, 2017a).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	CPT Description
81120	IDH1 (isocitrate dehydrogenase 1 [NADP+], soluble) (eg, glioma), common variants (eg, R132H, R132C)
81121	IDH2 (isocitrate dehydrogenase 2 [NADP+], mitochondrial) (eg, glioma), common variants (eg, R140W, R172M)
81175	ASXL1 (additional sex combs like 1, transcriptional regulator) (eg, myelodysplastic syndrome, myeloproliferative neoplasms, chronic myelomonocytic leukemia), gene analysis; full gene sequence
81176	ASXL1 (additional sex combs like 1, transcriptional regulator) (eg, myelodysplastic syndrome, myeloproliferative neoplasms, chronic myelomonocytic leukemia), gene analysis; targeted sequence analysis (eg, exon 12)
81206	BCR/ABL1 (t(9;22)) (eg, chronic myelogenous leukemia) translocation analysis; major breakpoint, qualitative or quantitative
81207	BCR/ABL1 (t(9;22)) (eg, chronic myelogenous leukemia) translocation analysis; minor breakpoint, qualitative or quantitative
81208	BCR/ABL1 (t(9;22)) (eg, chronic myelogenous leukemia) translocation analysis; other breakpoint, qualitative or quantitative
81219	CALR (calreticulin) (eg, myeloproliferative disorders), gene analysis, common variants in exon 9
81236	EZH2 (enhancer of zeste 2 polycomb repressive complex 2 subunit) (eg, myelodysplastic syndrome, myeloproliferative neoplasms) gene analysis, full gene sequence
81237	EZH2 (enhancer of zeste 2 polycomb repressive complex 2 subunit) (eg, diffuse large B-cell lymphoma) gene analysis, common variant(s) (eg, codon 646)
81270	JAK2 (Janus kinase 2) (eg, myeloproliferative disorder) gene analysis, p.Val617Phe (V617F) variant
81273	KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) (eg, mastocytosis), gene analysis, D816 variant(s)
81275	KRAS (Kirsten rat sarcoma viral oncogene homolog) (eg, carcinoma) gene analysis; variants in exon 2 (eg, codons 12 and 13)
81276	KRAS (Kirsten rat sarcoma viral oncogene homolog) (eg, carcinoma) gene analysis; additional variant(s) (eg, codon 61, codon 146)
81279	JAK2 (Janus kinase 2) (eg, myeloproliferative disorder) targeted sequence analysis (eg, exons 12 and 13)
81311	NRAS (neuroblastoma RAS viral [v-ras] oncogene homolog) (eg, colorectal carcinoma), gene analysis, variants in exon 2 (eg, codons 12 and 13) and exon 3 (eg, codon 61)

CPT	CPT Description
81338	MPL (MPL proto-oncogene, thrombopoietin receptor) (eg, myeloproliferative disorder) gene analysis; common variants (eg, W515A, W515K, W515L, W515R)
81339	MPL (MPL proto-oncogene, thrombopoietin receptor) (eg, myeloproliferative disorder) gene analysis; sequence analysis, exon 10
81347	SF3B1 (splicing factor [3b] subunit B1) (eg, myelodysplastic syndrome/acute myeloid leukemia) gene analysis, common variants (eg, A672T, E622D, L833F, R625C, R625L)
81348	SRSF2 (serine and arginine-rich splicing factor 2) (eg, myelodysplastic syndrome, acute myeloid leukemia) gene analysis, common variants (eg, P95H, P95L)
81351	TP53 (tumor protein 53) (eg, Li-Fraumeni syndrome) gene analysis; full gene sequence
81352	TP53 (tumor protein 53) (eg, Li-Fraumeni syndrome) gene analysis; targeted sequence analysis (eg, 4 oncology)
81353	TP53 (tumor protein 53) (eg, Li-Fraumeni syndrome) gene analysis; known familial variant
81357	U2AF1 (U2 small nuclear RNA auxiliary factor 1) (eg, myelodysplastic syndrome, acute myeloid leukemia) gene analysis, common variants (eg, S34F, S34Y, Q157R, Q157P)
81403	Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of >**10** amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81432	Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer); genomic sequence analysis panel, must include sequencing of at least 10 genes, always including BRCA1, BRCA2, CDH1, MLH1, MSH2, MSH6, PALB2, PTEN, STK11, and TP53
81442	Noonan spectrum disorders (eg, Noonan syndrome, cardio-facio-cutaneous syndrome, Costello syndrome, LEOPARD syndrome, Noonan-like syndrome), genomic sequence analysis panel, must include sequencing of at least 12 genes, including BRAF, CBL, HRAS, KRAS, MAP2K1, MAP2K2, NRAS, PTPN11, RAF1, RIT1, SHOC2, and SOS1
81450	Hematolymphoid neoplasm or disorder, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants, and copy number variants or

CPT	CPT Description
	rearrangements, or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis
81455	Solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes, genomic sequence analysis panel, interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis
81479	Unlisted molecular pathology procedure
0017U	Oncology (hematolymphoid neoplasia), JAK2 mutation, DNA, PCR amplification of exons 12-14 and sequence analysis, blood or bone marrow, report of JAK2 mutation not detected or detected. Proprietary test: JAK2 Mutation Lab/Manufacturer: University of Iowa, Department of Pathology
0027U	JAK2 (Janus kinase 2) (e.g., myeloproliferative disorder) gene analysis, targeted sequence analysis exons 12-15 Proprietary test: JAK2 Exons 12 to 15 Sequencing Lab/Manufacturer: Mayo Clinic

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Barbui, T., Tefferi, A., Vannucchi, A. M., Passamonti, F., Silver, R. T., Hoffman, R., Verstovsek, S., Mesa, R., Kiladjian, J. J., Hehlmann, R., Reiter, A., Cervantes, F., Harrison, C., McMullin, M. F., Hasselbalch, H. C., Koschmieder, S., Marchetti, M., Bacigalupo, A., Finazzi, G., . . . Barosi, G. (2018). Philadelphia chromosome-negative classical myeloproliferative neoplasms: revised management recommendations from European LeukemiaNet. *Leukemia*, *32*(5), 1057-1069. https://doi.org/10.1038/s41375-018-0077-1

Barbui, T., Thiele, J., Gisslinger, H., Kvasnicka, H. M., Vannucchi, A. M., Guglielmelli, P., Orazi, A., & Tefferi, A. (2018). The 2016 WHO classification and diagnostic criteria for myeloproliferative neoplasms: document summary and in-depth discussion. *Blood Cancer J*, *8*(2), 15. https://doi.org/10.1038/s41408-018-0054-y

Centogene. (2022). Myeloid Tumor Panel. https://www.centogene.com/diagnostics/our-tests/somatic-mutation-testing/somatic-mutation-testing-for-myeloid-tumors

Coates, T. D. (2022, May 5). *Approach to the patient with neutrophilia*. https://www.uptodate.com/contents/approach-to-the-patient-with-neutrophilia

Cross, N. C. P., Godfrey, A. L., Cargo, C., Garg, M., & Mead, A. J. (2021). The use of genetic tests to diagnose and manage patients with myeloproliferative and myeloproliferative/myelodysplastic neoplasms, and related disorders. *British Journal of Haematology*, *195*(3), 338-351. https://doi.org/https://doi.org/10.1111/bjh.17766

EASL. (2016). EASL Clinical Practice Guidelines: Vascular diseases of the liver. *J Hepatol*, *64*(1), 179-202. https://doi.org/10.1016/j.jhep.2015.07.040

Faisal, M., Stark, H., Busche, G., Schlue, J., Teiken, K., Kreipe, H. H., Lehmann, U., & Bartels, S. (2019). Comprehensive mutation profiling and mRNA expression analysis in atypical chronic myeloid leukemia in comparison with chronic myelomonocytic leukemia. *Cancer Med*. https://doi.org/10.1002/cam4.1946

FDA. (2017a). https://www.accessdata.fda.gov/cdrh_docs/pdf17/K172287.pdf

FDA. (2017b). *Approved Drugs - Ipsogen JAK2 RGQ PCR Kit* [WebContent]. https://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm551474.htm

Grinfeld, J., Nangalia, J., & Green, A. R. (2017). Molecular determinants of pathogenesis and clinical phenotype in myeloproliferative neoplasms. *Haematologica*, *102*(1), 7-17. https://doi.org/10.3324/haematol.2014.113845

Harrison, C. N., Butt, N., Campbell, P., Conneally, E., Drummond, M., Green, A. R., Murrin, R., Radia, D. H., Mead, A., Reilly, J. T., Cross, N. C. P., & McMullin, M. F. (2014). Modification of British Committee for Standards in Haematology diagnostic criteria for essential thrombocythaemia. *British Journal of Haematology*, *167*(3), 421-423. https://doi.org/10.1111/bjh.12986

Hussein, K., Granot, G., Shpilberg, O., & Kreipe, H. (2013). Clinical utility gene card for: familial polycythaemia vera. *Eur J Hum Genet*, *21*(6). https://doi.org/10.1038/ejhg.2012.216

labcorp. (2023). IntelliGEN® Myeloid. https://oncology.labcorp.com/cancer-care-team/test-menu/intelligen-myeloid

Maxson, J. E., Gotlib, J., Pollyea, D. A., Fleischman, A. G., Agarwal, A., Eide, C. A., Bottomly, D., Wilmot, B., McWeeney, S. K., Tognon, C. E., Pond, J. B., Collins, R. H., Goueli, B., Oh, S. T., Deininger, M. W., Chang, B. H., Loriaux, M. M., Druker, B. J., & Tyner, J. W. (2013). Oncogenic CSF3R mutations in chronic neutrophilic leukemia and atypical CML. *N Engl J Med*, *368*(19), 1781-1790. https://doi.org/10.1056/NEJMoa1214514

McMullin, M. F., Harrison, C. N., Ali, S., Cargo, C., Chen, F., Ewing, J., Garg, M., Godfrey, A., S, S. K., McLornan, D. P., Nangalia, J., Sekhar, M., Wadelin, F., Mead, A. J., & the, B. S. H. C. (2019). A guideline for the diagnosis and management of polycythaemia vera. A British Society for Haematology Guideline. *British Journal of Haematology*, *184*(2), 176-191. https://doi.org/10.1111/bjh.15648

NCCN. (2019). *NCCN Clinical Practice Guidelines in Oncology; Myeloproliferative Neoplasms v3.2019* https://www.nccn.org/professionals/physician_gls/pdf/mpn.pdf

NCCN. (2023). *NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines: Myeloproliferative Neoplasms v1.2024*. NCCN. Retrieved December 21 from https://www.nccn.org/professionals/physician_gls/pdf/mpn.pdf

NeoGenomics. (2022a). MPN JAK2 V617F with Sequential Reflex to JAK2 Exon 12-13, CALR, and MPL. https://neogenomics.com/test-menu/mpn-jak2-v617f-sequential-reflex-jak2-exon-12-13-calr-and-mpl

NeoGenomics. (2022b). NeoTYPE® Myeloid Disorders Profile. https://neogenomics.com/test-menu/neotyper-myeloid-disorders-profile

Ojeda, M. J., Bragós, I. M., Calvo, K. L., Williams, G. M., Carbonell, M. M., & Pratti, A. F. (2018). CALR, JAK2 and MPL mutation status in Argentinean patients with BCR-ABL1-negative myeloproliferative neoplasms. *Hematology*, *23*(4), 208-211. https://doi.org/https://doi.org/10.1080/10245332.2017.1385891

Poluben, L., Puligandla, M., Neuberg, D., Bryke, C. R., Hsu, Y., Shumeiko, O., Yuan, X., Voznesensky, O., Pihan, G., Adam, M., Fraenkel, E., Rasnic, R., Linial, M., Klymenko, S., Balk, S. P., & Fraenkel, P. G. (2019). Characteristics of myeloproliferative neoplasms in patients exposed to ionizing radiation following the Chernobyl nuclear accident. *Am J Hematol*, *94*(1), 62-73. https://doi.org/10.1002/ajh.25307

Quest_Diagnostics. (2020). LeukoVantage® Myeloid Neoplasm Mutation Panels. https://www.questdiagnostics.com/healthcare-professionals/clinical-education-center/faq/faq208

Rampal, R., Al-Shahrour, F., Abdel-Wahab, O., Patel, J. P., Brunel, J. P., Mermel, C. H., Bass, A. J., Pretz, J., Ahn, J., Hricik, T., Kilpivaara, O., Wadleigh, M., Busque, L., Gilliland, D. G., Golub, T. R., Ebert, B. L., & Levine, R. L. (2014). Integrated genomic analysis illustrates the central role of JAK-STAT pathway activation in myeloproliferative neoplasm pathogenesis. *Blood*, *123*(22), e123-133. https://doi.org/10.1182/blood-2014-02-554634

Rosenthal, S. H., Gerasimova, A., Ma, C., Li, H. R., Grupe, A., Chong, H., Acab, A., Smolgovsky, A., Owen, R., Elzinga, C., Chen, R., Sugganth, D., Freitas, T., Graham, J., Champion, K., Bhattacharya, A., Racke, F., & Lacbawan, F. (2021). Analytical validation and performance characteristics of a 48-gene next-generation sequencing panel for detecting potentially actionable genomic alterations in myeloid neoplasms. *PLoS One*, *16*(4), e0243683. https://doi.org/10.1371/journal.pone.0243683

Rumi, E., & Cazzola, M. (2017). Diagnosis, risk stratification, and response evaluation in classical myeloproliferative neoplasms. *Blood*, *129*(6), 680-692. https://doi.org/10.1182/blood-2016-10-695957

Silvennoinen, O., & Hubbard, S. R. (2015). Molecular insights into regulation of JAK2 in myeloproliferative neoplasms. *Blood*, *125*(22), 3388-3392. https://doi.org/10.1182/blood-2015-01-621110

Tefferi, A. (2022a, September 13). *Clinical manifestations and diagnosis of primary myelofibrosis*. https://www.uptodate.com/contents/clinical-manifestations-and-diagnosis-of-primary-myelofibrosis

Tefferi, A. (2022b, September 13). *Overview of the myeloproliferative neoplasms*. https://www.uptodate.com/contents/overview-of-the-myeloproliferative-neoplasms

Tefferi, A. (2023a, January 3). *Clinical manifestations and diagnosis of polycythemia vera*. https://www.uptodate.com/contents/clinical-manifestations-and-diagnosis-of-polycythemia-vera

Tefferi, A. (2023b, January 9). *Clinical manifestations, pathogenesis, and diagnosis of essential thrombocythemia*. https://www.uptodate.com/contents/diagnosis-and-clinical-manifestations-of-essential-thrombocythemia

Tefferi, A., Thiele, J., Vannucchi, A. M., & Barbui, T. (2014). An overview on CALR and CSF3R mutations and a proposal for revision of WHO diagnostic criteria for myeloproliferative neoplasms. *Leukemia*, *28*(7), 1407-1413. https://doi.org/10.1038/leu.2014.35

Titmarsh, G. J., Duncombe, A. S., McMullin, M. F., O'Rorke, M., Mesa, R., De Vocht, F., Horan, S., Fritschi, L., Clarke, M., & Anderson, L. A. (2014). How common are myeloproliferative neoplasms? A systematic review and meta-analysis. *Am J Hematol*, *89*(6), 581-587. https://www.ncbi.nlm.nih.gov/pubmed/24971434

Vainchenker, W., & Kralovics, R. (2017). Genetic basis and molecular pathophysiology of classical myeloproliferative neoplasms. *Blood*, *129*(6), 667-679. https://doi.org/10.1182/blood-2016-10-695940

Vannucchi, A. M., Barbui, T., Cervantes, F., Harrison, C., Kiladjian, J. J., Kröger, N., Thiele, J., & Buske, C. (2015). Philadelphia chromosome-negative chronic myeloproliferative neoplasms: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, *26 Suppl 5*, v85-99. https://doi.org/10.1093/annonc/mdv203

Pancreatic Cancer Risk Testing Using Pancreatic Cyst Fluid

Policy Number: AHS – M2114 – Pancreatic Cancer Risk Testing Using Pancreatic Cyst Fluid	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Pancreatic cancer is the fourth leading cause of death among cancers in the United States, and neoplasms frequently arise from pancreatic cysts that require investigation to differentiate benign neoplasms from malignant ones (Suriawinata, 2024). Up to 10% of pancreatic adenocarcinoma instances are "familial" in nature (Stoffel et al., 2018). Pathogenic germline variants in specific genes play a role in a 4% to 40% risk of developing pancreatic cancer over a lifetime (Canto et al., 2013). In such patients who are determined to be at risk, screening and meaningful intervention can make the difference in detecting pancreatic neoplasms early, but the risk of unnecessary and invasive intervention is also high.

First-line tests for pathologic diagnosis of a pancreatic cyst include cytology, imaging, and fluid chemistry. Integrated molecular pathology (IMP) testing combines molecular analysis with first-line test results (cytology, imaging, and fluid chemistry) to assess malignant potential (Al-Haddad et al., 2015). It is currently most commonly used adjunctively as a second-line testing strategy when a definitive pathologic diagnosis cannot be made because of inadequate specimen or equivocal histologic or cytologic findings.

II. Related Policies

Policy Number	Policy Title
AHS-G2124	Serum Tumor Markers for Malignancies
AHS-G2153	Pancreatic Enzyme Testing for Acute Pancreatitis
AHS-M2066	Genetic Cancer Susceptibility Using Next Generation Sequencing
AHS-M2079	Genetic Testing for Hereditary Pancreatitis

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) As a first step analysis of pancreatic cyst fluid, cytology and/or testing of carcinoembryonic antigen (CEA) and/or amylase **MEETS COVERAGE CRITERIA.**

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

2) For all situations, pancreatic cancer risk testing using molecular classifiers (e.g., PancraGEN) to evaluate pancreatic cyst fluid **DOES NOT MEET COVERAGE CRITERIA**.

IV. Table of Terminology

Term	Definition
ACG	American College of Gastroenterology
ACR	American College of Radiology
AGA	American Gastroenterological Association
ASGE	American Society for Gastrointestinal Endoscopy
CA 19-9	Carbohydrate antigen 19-9
CAPS	International Cancer of The Pancreas Screening
CEA	Carcinoembryonic antigen
CF	Cystic fluid
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare and Medicaid Services
CTNNB1	*Catenin beta 1* gene
DNA	Deoxyribonucleic acid
ESG	European Study Group on Cystic Tumours of the Pancreas
EUS	Endoscopic ultrasound
EUS-FNA	Endoscopic ultrasound fine-needle aspiration
FDA	Food And Drug Administration
FNA	Fine-needle aspirates
GNAS	*Guanine nucleotide binding protein, alpha stimulating* gene
IAP	International Association of Pancreatology
ICG	International Consensus Guideline

IMP	Integrated molecular pathology
indels	Insertions/deletions
IPMN	Intraductal papillary mucinous neoplasm
KRAS	*Kirsten rat sarcoma viral oncogene homolog* gene
LDT	Laboratory-developed test
MCN	Mucinous cystic neoplasm
MRI	Magnetic resonance imaging
MvP	Metastasis versus primary tumors
NCCN	National Comprehensive Cancer Network
NGS	Next-generation sequencing
NT	Neoplastic tissue
PCF	Pancreatic cyst fluid
PCN	Pancreatic cystic neoplasm
POLD1	*DNA polymerase delta 1, catalytic subunit* gene
PTPRD	*Protein tyrosine phosphatase receptor type D* gene
QALY	Quality-adjusted life-years
RAF	*Rapidly accelerated fibrosarcoma* gene
RNF43	*Ring finger protein 4* gene
SCA	Serous cystadenoma
SNV	Single nucleotide variant
SPN	Solid pseudopapillary neoplasm
TG	Topographic genotyping
TP53	Tumor protein p53
WGO	World Gastroenterology Organization

V. Scientific Background

Discovery of pancreatic cysts is becoming increasingly common as imaging technologies such as computed tomography and magnetic resonance imaging scans have become widespread in use. Though data suggests that malignant transformation of cysts is rare, due to the overall poor prognosis of pancreatic cancer, an incidental finding of a cyst can lead to an aggressive clinical workup. Cysts may be neoplastic with malignant potential, whereas non-neoplastic cysts only require treatment if they are symptomatic. Pancreatic cysts are divided pathologically into three different categories: inflammatory fluid collections, non-neoplastic pancreatic cysts, and pancreatic cystic neoplasms (PCNs) (Khalid & McGrath, 2023). Non-neoplastic cysts are often only identified after a surgical resection (Khalid & McGrath, 2023).

Pancreatic cystic neoplasms are further categorized into four subtypes with varying degrees of potential towards malignancy:

- Serous neoplasms
- Mucinous cystic neoplasms (MCNs)
- Intraductal papillary mucinous neoplasms (IPMNs)
- Solid pseudopapillary neoplasms (SPNs)

Evaluating tissues samples pathologically is a critical component of diagnosing patients with malignancy. Many cysts are first surveilled by imaging technology. The management of PCNs focuses on preventing progression of malignancy, while also avoiding unnecessary and invasive surgical intervention. If imaging is inconclusive, an evaluation is usually performed by endoscopic, ultra-sound guided fine needle aspiration sampling of fluid and the cyst wall using cytologic examination and analysis. Generally, before a diagnosis is made, the patient may have undergone any or all of the following diagnostic procedures: computed tomography, magnetic resonance imaging/magnetic resonance cholangiopancreatography, and/or endoscopic ultrasound (with or without fine needle aspiration) (Scholten et al., 2018). Additional tests may include amylase, lipase, and carcinoembryonic antigen (CEA) levels on cyst fluid, but these may still leave uncertainty as to a diagnosis.

Combining pathological study with molecular analysis and/or serum biomarkers is proposed to enhance the ability for greater diagnostic confirmation. One method used to ascertain subtypes of PCN and to identify malignancy is the use of biomarkers in peripheral blood such as serum carbohydrate antigen (CA) 19-9. Serum CA 19-9 levels that exceed 37 U/ml, may provide information on potential malignancy or invasive IPMN.

Cystic fluid analysis is also purported as useful to further analyze PCN by subtype. Analyses of pancreatic cystic fluid for this purpose may include CEA, CA 19-9, amylase and lipase, viscosity, mucin stain, and cytology (Khalid & McGrath, 2023).

Molecular analysis of DNA has been suggested as another way to gather important diagnostic information on pancreatic cysts. DNA markers such as *GNAS* and *KRAS* have evidenced specificity and sensitivity for diagnosis of whether a cyst is an IPMN (Scholten et al., 2018).

Proprietary tests are available that propose that they can estimate the chances of pancreatic cancer with pancreatic cyst fluid and integrated molecular pathology or molecular anatomic pathology.

Proprietary Testing

RedPath Integrated Pathology developed and patented a proprietary platform, PathFinderTG®, based on topographic genotyping (TG), which integrates microscopic

analysis (anatomic pathology) with molecular tissue analysis (RedPath Integrated Pathology, 2001). RedPath developed five different Pathfinder GT tests (Pancreas, Biliary, Barrett, Glioma, and Metastasis versus Primary Tumors (MvP) before the company was purchased by Interpace Diagnostics. Interpace Diagnostics has continued development of these molecular pathology panels and markets them separately as PancraGEN (Interpace, 2023b).

PancraGEN is a DNA-based, integrated molecular pathology test that helps to assess the cancer risk in aspirated pancreatic cyst fluid. This test uses extracted DNA from aspirated pancreatic cyst fluid to test tumor suppressor genes (such as *PTEN* and *TP53*) and oncogenes (such as *KRAS* and *NRAS*). PancraGEN is intended as a supplement to other diagnostic tools such as cytology and imaging, and is proposed to enhance assessments of malignancy risk (Interpace, 2023a). The DNA abnormalities identified by this technology include tumor suppressor gene panel (Loss of Heterozygosity) analysis of *VHL, OGG1; PTEN, MXI1; TP53; SMAD4, DCC; CDKN2A; RNF43, NME1; PSEN2, TFF1; CMM1v; MCC, APC; NF2*. Oncogene point mutations provided by this test are those in KRAS and GNAS. The report provides summary of specific molecular results and details of each result with the possible clinical meanings of those results. Interpace also offers PanDNA, which provides molecular-only results to enhance risk stratification of pancreatic cysts. DNA abnormalities are identified by PanDNA technology from aspirated pancreatic cyst fluid without the integration with first-line testing results (Interpace, 2023b).

PancraSeq, from UPMC, is a test that detects "74 pancreatic cyst- and pancreatic cancer-related genes for main classes of molecular alterations, including mutations, gene fusions, copy number alterations, and gene expression alterations." The test also detects *CEA* gene expression. The test identifies the following mutations (SNVs/indels): *KRAS, GNAS, BRAF, VHL, TP53, PIK3CA, PTEN, CTNNB1, MEN1, AKT1, APC, HRAS, NRAS, IDH1, IDH2, MET, NF2, STK11, TERT, TSC2*; the following copy number alterations: *RNF43* (17q), *SMAD4* (18q), *TP53* (17p), *VHL* (3p*), NF2* (22q), and *PTEN* (10q) tumor suppressor genes and 13 other chromosomal regions; the following gene expression alterations: *KRT7, KRT20, CHGA, CEACAM5, PGK*; and over 170 types of gene fusions, including *PRKACA/B* fusions for detection of IOPN, and *ALK, NTRK1/3, BRAF, RET* gene fusions as potential therapeutic targets for PDAC. The test reports alterations in any of its genes, its allele frequency, and whether the variant is of clinical or "potential" clinical significance. PancraSeq reports the test to have a "95% sensitivity and 100% specificity for detection of cystic precursor neoplasms (IPMN, MCN, IOPN), and the 82% sensitivity and 100% specificity for detection of advanced neoplasia" (Pancreaseq, 2024).

PanreaSeq has a sensitivity of 82% - 98%, specificity of 94% - 100%, positive predictive value of 84% - 100%, and negative predictive value of 62% - 98% (Nikiforova et al., 2023; Paniccia et al., 2023). PancraGen/PanDNA has a sensitivity: 70% - 100%, and a specificity of 77% - 99%. The positive predictive value and negative predictive value of PancraGen/PanDNA is not explicitly identified (Bell et al., 2022; Rahal et al., 2022; Simpson et al., 2018, 2019).

Clinical Utility and Validity

Malhotra et al. (2014) evaluated the supporting role that mutational profiling of DNA may play in the diagnosis of malignancy in fine-needle aspirates (FNA) and biliary brushing specimens from patients with pancreaticobiliary masses. The study included 30 patients who presented with pancreaticobiliary masses were evaluated and had minimum follow-up of three months. PathFinderTG® mutational profiling was done and analyzed in 26 patients with atypical, negative, or indeterminate cytology. Cytology correctly diagnosed four of 21 malignant cases (sensitivity, 19%), and identified seven of nine patients with non-aggressive disease (specificity, 78%). PathFinderTG® correctly diagnosed eight of 17 malignant cases (sensitivity, 47%) and identified all nine patients with non-aggressive disease (specificity, 100%). When first-line malignant cytology results were combined with positive second-line mutational profiling results, sensitivity improved to 57% (12/21 cases of aggressive disease were identified). The investigators concluded that mutational profiling provided additional information regarding the presence of aggressive disease. When used in conjunction with first-line cytology, mutational profiling increased detection of aggressive disease without compromising specificity in patients that were difficult to diagnose by cytology alone (Malhotra et al., 2014)

Al-Haddad et al. (2015) published a study that examined the diagnostic accuracy of IMP for pancreatic adenocarcinoma. A total of 492 samples were assessed, and out of the benign or indolent IMP diagnoses, 97% had a benign follow-up for up to seven years, eight months after IMP testing. Statistically higher risk and aggressive diagnoses had hazard ratios for malignancy of 30.8 and 76.3, respectively. The Sendai surveillance criteria had identical chances of benign follow-up over the same timeframe, but the Sendai surgical criteria only had a hazard ratio of 9.0. The authors concluded, “IMP more accurately determined the malignant potential of pancreatic cysts than a Sendai 2012 guideline management criteria model. IMP may improve patient management by justifying more relaxed observation in patients meeting Sendai surveillance criteria. IMP can more accurately differentiate between the need for surveillance or surgery in patients meeting Sendai surgical criteria” (Al-Haddad et al., 2015).

Loren et al. (2016) performed a study evaluating the impact of IMP testing on clinical management decisions. A total of 491 patients were examined, and 66 had a malignant outcome (425 benign). The IMP testing was compared to the 2012 International Consensus Guideline (ICG) recommendations. When the two methods agreed, surveillance and surgery was undertaken in 83% and 88% of the cases, respectively. However, when the methods disagreed, the clinicians tended to agree with the IMP method. Eighty eight percent of patients had an intervention when ICG recommended surveillance, but IMP indicated "high-risk," and 55% of patients underwent surveillance when the ICG recommended surgery, but IMP indicated low risk. The authors concluded that "DNA-based IMP diagnoses were predictive of real-world management decisions. Importantly, when International Consensus Guidelines and IMP were discordant, IMP influence benefitted patients by increasing confidence in surveillance and surgery decisions and reducing the number of unnecessary surgeries in patients with benign disease" (Loren et al., 2016).

Springer et al. (2015) evaluated "whether a combination of molecular markers and clinical information could improve the classification of pancreatic cysts and management of patients." A total of 130 patients with resected pancreatic cystic neoplasms were enrolled. The cyst fluid was evaluated for the following genetic alterations: "*BRAF, CDKN2A, CTNNB1, GNAS, KRAS, NRAS, PIK3CA, RNF43, SMAD4, TP53* and *VHL*); loss of heterozygosity at *CDKN2A, RNF43, SMAD4, TP53*, and *VHL* tumor suppressor loci; and aneuploidy." The authors found this panel to identify 67 of the 74 patients who did not require surgery and estimated the sensitivity to be 90-100% and the specificity to be 92-98% (Springer et al., 2015).

Singhi et al. (2016) assessed the accuracy of the AGA guidelines in detecting advanced neoplasia and presented an alternative approach to pancreatic cysts. The clinical findings and molecular testing of pancreatic cyst fluid of 225 patients who underwent EUS-guided FNA for pancreatic cysts were reviewed. The authors found that "Diagnostic pathology results were available for 41 patients with 13 harboring advanced neoplasia. Among these cases, the AGA guidelines identified advanced neoplasia with 62% sensitivity, 79% specificity, 57% positive predictive value, and 82% negative predictive value. Moreover, the AGA guidelines missed 45% of intraductal papillary mucinous neoplasms with adenocarcinoma or high-grade dysplasia. For cases without confirmatory pathology, 27 of 184 patients (15%) with serous cystadenomas (SCAs) based on EUS findings and/or VHL alterations would continue magnetic resonance imaging (MRI) surveillance. In comparison, a novel algorithmic pathway using molecular testing of pancreatic cyst fluid detected advanced neoplasias with 100% sensitivity, 90% specificity, 79% positive predictive value, and 100% negative predictive value" (Singhi et al., 2016).

Singhi et al. (2018) also evaluated the accuracy of pancreatic cyst fluid (PCF) DNA testing. A total of 626 PCF samples were taken from 595 patients. *KRAS/GNAS* mutations were identified in 308 samples (49%), and *PIK3CA/PTEN/TP53* mutations were identified in 35 samples (6%). A total of 102 patients had a surgical follow-up, and *KRAS/GNAS* mutations were detected in 56 intraductal papillary mucinous neoplasms (IPMNs) and three mucinous cystic neoplasms (MCNs), which corresponded to an 89% sensitivity and 100% specificity for a mucinous pancreatic cyst. Next generation sequencing identified the combination of *KRAS/GNAS* mutations and *TP53/PTEN/PIK3CA* alterations at an 89% sensitivity and 100% specificity. The authors concluded, "In contrast to Sanger sequencing, preoperative NGS of PCF for *KRAS/GNA*S mutations is highly sensitive for IPMNs and specific for mucinous PCs. In addition, the combination of *TP53/PIK3CA/PTEN* alterations is a useful preoperative marker for advanced neoplasia" (Singhi et al., 2018).

Das et al. (2015) investigated the cost efficiency of IMP in a "third-party-payer perspective Markov decision model" of a hypothetical cohort of 1000 asymptomatic patients with a three cm solitary pancreatic cyst. They used four different strategies to evaluate the cost efficiency in terms of quality-adjusted life-years (QALY): "Strategy I used cross-sectional imaging, recommended surgery only if symptoms or risk factors emerged. Strategy II considered patients for resection without initial EUS. Strategy III (EUS + CEA + Cytology) referred only those with mucinous cysts (CEA > 192 ng/mL) for resection. Strategy IV implemented IMP; a commercially available panel provided a 'Benign,' 'Mucinous,' or 'Aggressive' classification based on the level of mutational change in cyst fluid. 'Benign' and 'Mucinous' patients were followed with surveillance; 'Aggressive' patients were referred for resection." The authors report that the IMP-based Strategy IV provided the greatest increase in QALY at approximately the same cost as the "cheapest approach," concluding that "use of IMP was the most cost-effective strategy, supporting its routine clinical use" (Das et al., 2015). It should be noted, however, that two of the authors listed on the study were employed by RedPath Integrated Pathology, the developer of the IMP test.

Laquiere et al. (2019) investigated the concordance of mutation analysis between pancreatic cyst fluid and neoplastic tissue. The authors used next-generation sequencing to compare DNA collected from both cystic fluid (CF) and neoplastic tissue (NT). A total of 17 patients were included, and concordant CF-NT genotypes were found in 15 of 17 patients. A higher proportion of mutated alleles were found in CF compared to NT. The authors also noted that "the sensitivity and specificity of *KRAS/GNAS* mutations in CF to predict an appropriate indication for surgical resection were 0.78 and 0.62, respectively. The sensitivity and specificity of *RAF/PTPRD/CTNNB1 /RNF43/POLD1/TP53* mutations in CF were 0.55 and 1.0, respectively." Although the authors remarked that mutational analysis between both media were highly

concordant, they also stated that the results "need to be confirmed on a larger scale" (Laquiere et al., 2019).

Volckmar et al. (2019) published preliminary results from the "prospective ZYSTEUS biomarker study." This study is intended to investigate "(i) whether detection of driver mutations in IPMN [intraductal papillary mucinous neoplasm] by liquid biopsy is technically feasible, (ii) which compartment of IPMN is most suitable for analysis, and (iii) implications for clinical diagnostics." A total of 15 patients with pancreatic cysts larger than 10 mm were included, 12 of which had an IPMN and three acute pancreatitis controls. All 12 IPMN cases were found to harbor at least one mutation in either KRAS (n = 11) or GNAS (n = 4), with three cases harboring both mutations. In three cases with "pseudocysts," no alterations were identified. The authors also found that DNA yields were higher and showed higher mutation diversity in the cellular fraction and concluded that "mutation detection in pancreatic cyst fluid is technically feasible with more robust results in the cellular than in the liquid fraction." The authors also suggested that their results, "targeted sequencing supports discrimination of IPMN from pseudocysts" when combined with imaging (Volckmar et al., 2019).

Pérez et al. (2021) studied the impact of molecular analysis on the detection of mucinous cysts and malignancy. Currently, recommendations suggest endoscopic ultrasound fine-needle aspiration (EUS-FNA) with molecular analysis to improve the diagnosis of pancreatic cysts. EUS-FNA and next-generation sequencing was performed in 36 pancreatic cysts, which were classified as mucinous, non-mucinous, and malignant. Of the 36 lesions, 28 (82.4%) were classified as mucinous, six (17.6%) were classified as non-mucinous, and five (13.9%) were classified as malignant lesions. *KRAS* and *GNAS* genes were analyzed for mutations. Analysis of *KRAS* and *GNAS* showed 83.33% sensitivity, 60% specificity, 88.24% positive predictive value, and 50% negative predictive value for the diagnosis of mucinous cystic lesions. Mutations in *KRAS* and *GNAS* were found in two of five (40%) of the lesions classified as non-mucinous, so they were recategorized as mucinous neoplasms. These led to a modification of the follow up plan in 8% of the cysts. Additionally, one indeterminate cyst showed a mutation in both *KRAS* and *GNAS*, so it could also be classified as mucinous. Therefore, performing molecular analysis in cases of uncertain diagnosis improved categorization of the cyst. One hundred percent of the malignant cysts had mutations in *KRAS* and/or *GNAS*. However, the presence of a mutation was not related to malignancy. Overall, the authors conclude that "molecular analysis can improve the classification of pancreatic cysts as mucinous or non-mucinous. This is important as mucinous cysts are premalignant lesions and have a higher risk of concomitant pancreatic adenocarcinoma, thus implying long-term follow-up" (Pérez et al., 2021).

Buerlein and Shami (2021) published an overview of current guidelines for gastroenterologists as part of recommendations for pancreatic cysts. The prevalence of pancreatic cysts has increased as technology has improved. However, incidental identification of asymptomatic pancreatic cysts causes patient concern as malignancy varies greatly and surgical resection is an invasive technique. Pancreatic cystic neoplasms (PCNs) fall into one of two categories: mucinous PCNs, which create mucus and have a greater potential for malignancy, as compared to non-mucinous PCNs. Regarding biomarker identification, EUS-guided fluid samples taken from PCNs combined with ways of acquiring tissue to analyze PCN malignancy "could improve our ability to accurately diagnose PCNs and understand their risk of malignant transformation." However, "these are not currently recommended for usage by any of the guidelines." The authors briefly discussed several methods of risk-stratifying pancreatic cysts through identifying mucinous versus non-mucinous cysts: (1) next-generation sequencing of PCN fluid (2) cyst fluid glucose level (3) microbiopsy, and (4) confocal laser endomicroscopy. All four methods were described as requiring further clinical validation (Buerlein & Shami, 2021).

Nagula et al. (2010) evaluated the use of cyst fluid CEA analysis in the diagnosis of mucinous cysts of the pancreas. A group of 267 patients was identified by pathological diagnosis. Mucinous cysts were identified in 66 of 97 patient cases (68%) by CEA value. A CEA greater than 192 ng/mL had a sensitivity of 73% and specificity of 65% when it came to identifying mucinous cysts. However, cyst fluid CEA was not found to be associated with malignancy. A non-surgical strategy was used to manage the 178 patients identified to have mucinous cysts. Eight of these patients later had radiographic developments that required surgery. Results from pathology indicated seven benign mucinous cysts and one retention cyst. The conclusion of the study was that "cyst fluid CEA is a useful test for identifying mucinous cysts, including MCN and IPMN. In mucinous cysts, cyst fluid CEA is not associated with malignancy or radiographic progression" (Nagula et al., 2010).

Oh et al. (2014) used a pancreatic cyst database containing the profiles of 78 patients with histologically proven cysts to study the differential diagnosis of pancreatic cysts using cyst fluid amylase and CEA. The median age of the 78 patients was 60.4 years. Cyst fluid amylase levels showed a significant difference between pseudocysts (PP) and mucinous cystic neoplasms (MCNs) but did not aid in distinguishing between MCNs and IPMN. The cyst fluid CEA showed a significant difference between pseudocysts and mucinous cystic neoplasms (median: 26.00 versus 627.50 ng/mL respectively, $p < 0.001$) and between pseudocysts (26.00 ng/mL) and IPMN (356.50 ng/mL). Overall, the established optimal cut-off values from the study were 6,800 U/mL for amylase and 50 ng/mL for CEA. These correlated with the "crossover of the sensitivity and specificity

curves for differentiating PP and mucinous neoplasms. The overall accuracies of cyst fluid amylase and CEA were 69% and 85%, respectively" (Oh et al., 2014).

Smith et al. (2016) performed a retrospective study on cytology, amylase, and CEA in the preoperative diagnosis of pancreatic cysts. The goal of the study was to reclassify and analyze malignancy risk in cysts that were already histologically proven to be pancreatic neoplastic mucinous cysts using Pap Center guidelines, ancillary testing through amylase and CEA values, and cytology. First, a database search was conducted. Pancreatic neoplastic mucinous cyst resections using EUS-FNA technique in the prior year were identified. One hundred and thirty-eight cases of pancreatic neoplastic mucinous cysts were retrieved. Eleven cases were excluded for missing slides. Of the remaining 127 cases, there were 81 IPMNs and 86 MCNs. Cysts that were atypical, suspicious, or positive were re-reviewed, blinded to the previous diagnosis and categorization. The authors concluded that the sensitivity of cytology for diagnosis of neoplastic mucinous cysts (with ancillary information from amylase and CEA values) was 76.4%. Diagnosis of malignancy using cytology had a sensitivity of 48.3%, specificity of 94.9% and accuracy of 84.3%. The authors concluded that a "purely cytologic approach is inferior to an integrated approach of cytology with ancillary testing in diagnosing a neoplastic mucinous cyst of the pancreas" (Smith et al., 2016).

Gorris et al. (2023) studied the value of combined carcinoembryonic antigen (CEA) and glucose testing in pancreatic cyst fluid when differentiating mucinous from non-mucinous pancreatic cystic neoplasms. Pancreatic cyst fluid was collected from 63 patients. Histopathology, cytopathology, clinical, and/or radiological diagnosis was used as a reference standard; 33 (52%) participants had mucinous pancreatic cystic neoplasms and 30 (48%) had non-mucinous pancreatic cystic neoplasms. The authors performed laboratory measurements of CEA and used a hand glucometer to measure glucose. Combined CEA and glucose results had 92% specificity and 48% sensitivity. Either a positive CEA or glucose result alone had 97% sensitivity and 50% specificity. The authors conclude that "combined CEA and glucose testing in PCF reached high specificity and sensitivity for differentiating mucinous from non-mucinous PCN" and note that changing the CEA cut-off levels could increase sensitivity (Gorris et al., 2023).

Pflüger et al. (2023) conducted a systematic review of 42 studies to analyze the predictive ability of pancreatic cyst fluid biomarkers. The authors included studies that evaluated the diagnostic performance of cyst fluid, particularly those that emphasized DNA-based biomarkers. "Mutations in *KRAS* and/or *GNAS* allowed identification of mucinous cysts with a sensitivity of 79% and specificity of 98%," which "exceeded the performance of the traditional biomarker carcinoembryonic antigen (CEA; sensitivity 58%, specificity 87%)." Additionally, mutations in *VHL* were specific to serous cystadenomas, and mutations of *CDKN2A, PIK3CA, SMAD4,* and *TP53* all had high

specificities to high-grade dysplasia or pancreatic ductal adenocarcinoma. The authors concluded that the results "support the use of DNA-based cyst fluid biomarkers in the multidisciplinary diagnostic work-up of pancreatic cysts" (Pflüger et al., 2023).

Cui et al. (2023) studied potential cyst fluid biomarkers to be used for the diagnosis of PCN. The authors analyzed the glycoproteomic and proteomic characteristics of pancreatic cyst fluid samples from PCN patients using mass spectrometry-based glycosite- and glycoform-specific glycoproteomics and proteomics. N-glycosylated PHKB (Asn-935, H5N2F0S0; Asn-935, H4N4F0S0; Asn-935, H5N4F0S0), CEACAM5 (Asn-197, H5N4F0S0) and ATP6V0A4 (Asn-367, H6N4F0S0) were confirmed as "promising diagnostic biomarkers for distinguishing malignant PCNs" with an area under the curve ranging from 0.771 to 0.943. The authors concluded that the results "hold significant clinical implications, providing valuable insights for PCN decision-making, and potentially offering therapeutic targets for PCN treatment" (Cui et al., 2023).

VI. Guidelines and Recommendations

American Gastroenterological Association (AGA)

In 2015, the AGA published guidelines on the diagnosis and management of asymptomatic neoplastic pancreatic cysts. These guidelines only state, "Molecular techniques to evaluate pancreatic cysts remain an emerging area of research, and the diagnostic utility of these tests is uncertain" (Vege et al., 2015).

American College of Gastroenterology (ACG)

In 2018, the ACG updated its recommendations (Elta et al., 2018) on the diagnosis and management of pancreatic cysts, stating that "Molecular markers may help identify IPMNs and MCNs. Their use may be considered in cases in which the diagnosis is unclear and the results are likely to change management (Conditional recommendation, very low quality of evidence)."

The ACG also acknowledges the cost of cyst analysis, noting that "The cost of cyst analysis and cyst surveillance is high, and the benefit in terms of cancer prevention is unproven. There have been no dedicated cost effectiveness analyses about surveillance of incidental pancreatic cysts" (Elta et al., 2018).

American Society for Gastrointestinal Endoscopy (ASGE)

The ASGE states that "additional research is needed to determine the precise role molecular analysis of cyst fluid will play in evaluating pancreatic cystic lesions." However, the ASGE suggests that "molecular testing of the cyst be considered when

initial ancillary testing of cytology and CEA is inconclusive and when test results may alter management" (Muthusamy et al., 2016).

National Comprehensive Cancer Network (NCCN)

The current NCCN clinical practice guidelines for pancreatic adenocarcinoma do not include recommendations for assessment of pancreatic cyst fluid (NCCN, 2024).

International Consensus Fukuoka Guidelines

The International Association of Pancreatology (IAP) (Tanaka et al., 2017) held a consensus symposium to examine the guidelines regarding prediction of invasive carcinoma and high-grade dysplasia, surveillance, and postoperative follow-up of IPMN. They found that "At present, EUS-FNA with cytological and molecular analyses is still considered investigational and should be performed only in centers with expertise in performing EUS-FNA and interpreting the results. More data are needed to accurately determine the sensitivity, specificity, and safety of this procedure and if results can be generalized." Overall, the guideline remarked that molecular analysis of cyst fluid is "still evolving" (Tanaka et al., 2017).

European Study Group on Cystic Tumours of the Pancreas

This guideline is considered "a joint initiative of the European Study Group on Cystic Tumors of the Pancreas, United European Gastroenterology, European Pancreatic Club, European-African Hepato-Pancreato-Biliary Association, European Digestive Surgery, and the European Society of Gastrointestinal Endoscopy."

The guidelines state that "DNA markers, in particular, mutations in *GNAS* and *KRAS*, have shown promise in identifying mucin-producing cysts. In cases in which the diagnosis is unclear, and a change in diagnosis will alter management, analysis of these mutations using highly sensitive techniques, such as next-generation sequencing (NGS), may be considered." This recommendation was given a grade of "2C." The guidelines also remarked that there is "insufficient evidence" to support the use of RNA or non-carcinoembryonic antigen protein markers in pancreatic cysts. This recommendation was given a grade of "1B" (The European Study Group on Cystic Tumours of the Pancreas, 2018).

International Cancer of the Pancreas Screening (CAPS) Consortium

The CAPS Consortium was convened to update the consensus recommendations for "management of individuals with increased risk of pancreatic cancer based on family history or germline mutation status (high-risk individuals)." In this Consortium, the authors state that "In some cases, evidence of pancreatic neoplasia can be inferred by

the presence of mutations detected in secretin-stimulated pancreatic fluid samples…but further investigation is needed to determine the value of these tests for patients under pancreatic surveillance" (Goggins et al., 2020).

World Gastroenterology Organization (WGO)

The WGO Global Guidelines provided key guidelines in diagnosis and management of pancreatic cystic lesions. The following recommendations were made.

1. "At the initial cyst fluid aspiration: carry out carcinoembryonic antigen (CEA), amylase, and cytology testing.
2. Molecular testing is not routinely done because of limited data and the expense, but it does hold promise for the future.
3. When fluid is aspirated, the following tests are recommended in the sequence described, depending on the volume of the aspirate:
    - Cytology: glycogen-rich cells (SCNs) or mucin-containing cells (MCNs and IPMNs), but the sensitivity is low.
    - Tumor markers: CEA level, an accurate tumor marker for diagnosing a mucinous PCN (the accuracy and cut-off level vary among laboratories).
    - Diagnostic molecular markers: KRAS, GNAS, VHL, CTNNB1.
    - Prognostic molecular markers: TP53, PIK3CA, PTEN.
    - Mucins: assessment of cyst mucin is complementary to cyst CEA levels and cytology
    - Viscosity: the "string sign" concept is an indirect, inexpensive, but subjective measurement of viscosity, assessed by placing a sample of aspirated fluid between the thumb and index finger and measuring the length of stretch prior to disruption.
    - Amylase (or lipase)" (Malagelada et al., 2019).

The guidelines also state that "molecular testing is not routinely done because of limited data and the expense, but it does hold promise for the future" (Malagelada et al., 2019).

American College of Radiology (ACR)

The Expert Panel on Gastrointestinal Imaging of the American College of Radiology created guidelines to determine the appropriate initial imaging study to further evaluate a pancreatic cyst that was incidentally detected on a nondedicated imaging study. ACR mentions that molecular assays for markers such as *K-ras, GNAS, PTEN, VHL, TP53*, and *PIK3CA* "may also assist in differentiating neoplastic cystic lesions and predicting cyst behavior. When performed in centers with expertise in EUS-FNA,

cytological evaluation can identify atypia, dysplasia, or neoplasia" (Fábrega-Foster et al., 2020).

American Society of Clinical Oncology (ASCO)

In 2019, ASCO published a guideline on the susceptibility to pancreatic cancer. The authors note the importance of emerging data despite very few clinical trials. In a disclaimer, they note: "This PCO should be read with the understanding that randomized clinical trial data are not available for these guidance statements, but it is the opinion of the Expert Panel that the statements made represent the state of the data available." Generally, they note that there are "currently no approved biomarkers for screening or surveillance" (Stoffel et al., 2018).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81479	Unlisted molecular pathology procedure
82150	Amylase
82378	Carcinoembryonic antigen (CEA)
84999	Unlisted chemistry procedure
88108	Cytopathology, concentration technique, smears and interpretation (eg, Saccomanno technique)

88112	Cytopathology, selective cellular enhancement technique with interpretation (eg, liquid based slide preparation method), except cervical or vaginal
88173	Cytopathology, evaluation of fine needle aspirate; interpretation and report
0313U	Oncology (pancreas), DNA and mRNA next-generation sequencing analysis of 74 genes and analysis of CEA (CEACAM5) gene expression, pancreatic cyst fluid, algorithm reported as a categorical result (ie, negative, low probability of neoplasia or positive, high probability of neoplasia) Proprietary test: PancreaSeq® Genomic Classifier Lab/Manufacturer: Molecular and Genomic Pathology Laboratory/University of Pittsburgh Medical Center

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Al-Haddad, M. A., Kowalski, T., Siddiqui, A., Mertz, H. R., Mallat, D., Haddad, N., Malhotra, N., Sadowski, B., Lybik, M. J., Patel, S. N., Okoh, E., Rosenkranz, L., Karasik, M., Golioto, M., Linder, J., & Catalano, M. F. (2015). Integrated molecular pathology accurately determines the malignant potential of pancreatic cysts. *Endoscopy*, *47*(2), 136-142. https://doi.org/10.1055/s-0034-1390742

Bell, M., Feng, J., Chow, K. W., Reicher, S., & Eysselein, V. (2022). INTEGRATED MOLECULAR PATHOLOGY AS A PREDICTOR OF MALIGNANT TRANSFORMATION OF PANCREATIC CYSTS WITH A MAXIMUM 11-YEAR FOLLOW-UP. *Gastrointestinal Endoscopy*, *95*(6), AB542. https://www.sciencedirect.com/science/article/abs/pii/S0016510722016145

Buerlein, R. C. D., & Shami, V. M. (2021). Management of pancreatic cysts and guidelines: what the gastroenterologist needs to know. *Therapeutic advances in gastrointestinal endoscopy*, *14*, 26317745211045769-26317745211045769. https://doi.org/10.1177/26317745211045769

Canto, M. I., Harinck, F., Hruban, R. H., Offerhaus, G. J., Poley, J. W., Kamel, I., Nio, Y., Schulick, R. S., Bassi, C., Kluijt, I., Levy, M. J., Chak, A., Fockens, P., Goggins, M., & Bruno, M. (2013). International Cancer of the Pancreas Screening (CAPS) Consortium summit on the management of patients with increased risk for familial pancreatic cancer. *Gut*, *62*(3), 339-347. https://doi.org/10.1136/gutjnl-2012-303108

Cui, M., Hu, Y., Zhang, Z., Chen, T., Dai, M., Xu, Q., Guo, J., Zhang, T., Liao, Q., Yu, J., & Zhao, Y. (2023). Cyst fluid glycoproteins accurately distinguishing malignancies of pancreatic cystic neoplasm. *Signal Transduct Target Ther*, *8*(1), 406. https://doi.org/10.1038/s41392-023-01645-8

Das, A., Brugge, W., Mishra, G., Smith, D. M., Sachdev, M., & Ellsworth, E. (2015). Managing incidental pancreatic cystic neoplasms with integrated molecular pathology is a cost-effective strategy. *Endosc Int Open*, *3*(5), E479-486. https://doi.org/10.1055/s-0034-1392016

Elta, G. H., Enestvedt, B. K., Sauer, B. G., & Lennon, A. M. (2018). ACG Clinical Guideline: Diagnosis and Management of Pancreatic Cysts. *Am J Gastroenterol*, *113*(4), 464-479. https://doi.org/10.1038/ajg.2018.14

Fábrega-Foster, K., Kamel, I. R., Horowitz, J. M., Arif-Tiwari, H., Bashir, M. R., Chernyak, V., Goldstein, A., Grajo, J. R., Hindman, N. M., Kamaya, A., McNamara, M. M., Porter, K. K., Scheiman, J. M., Solnes, L. B., Srivastava, P. K., Zaheer, A., & Carucci, L. R. (2020). ACR Appropriateness Criteria® Pancreatic Cyst. *J Am Coll Radiol*, *17*(5s), S198-s206. https://doi.org/10.1016/j.jacr.2020.01.021

Goggins, M., Overbeek, K. A., Brand, R., Syngal, S., Del Chiaro, M., Bartsch, D. K., Bassi, C., Carrato, A., Farrell, J., Fishman, E. K., Fockens, P., Gress, T. M., van Hooft, J. E., Hruban, R. H., Kastrinos, F., Klein, A., Lennon, A. M., Lucas, A., Park, W., . . . Bruno, M. (2020). Management of patients with increased risk for familial pancreatic cancer: updated recommendations from the International Cancer of the Pancreas Screening (CAPS) Consortium. *Gut*, *69*(1), 7. https://doi.org/10.1136/gutjnl-2019-319352

Gorris, M., Dijk, F., Farina, A., Halfwerk, J. B., Hooijer, G. K., Lekkerkerker, S. J., Voermans, R. P., Wielenga, M. C., Besselink, M. G., & van Hooft, J. E. (2023). Validation of combined carcinoembryonic antigen and glucose testing in pancreatic cyst fluid to differentiate mucinous from non-mucinous cysts. *Surg Endosc*. https://doi.org/10.1007/s00464-022-09822-6

Interpace. (2023a). How PancraGEN Works. https://pancragen.com/how-it-works/

Interpace. (2023b). *Molecular Testing with PancraGEN*. https://pancragen.com/power-of-pancragen/

Khalid, A., & McGrath, K. (2023, September 29). *Classification of pancreatic cysts*. https://www.uptodate.com/contents/classification-of-pancreatic-cysts

Laquiere, A. E., Lagarde, A., Napoleon, B., Bourdariat, R., Atkinson, A., Donatelli, G., Pol, B., Lecomte, L., Curel, L., Urena-Campos, R., Helbert, T., Valantin, V., Mithieux, F., Buono, J. P., Grandval, P., & Olschwang, S. (2019). Genomic profile concordance between pancreatic cyst fluid and neoplastic tissue. *World J Gastroenterol*, *25*(36), 5530-5542. https://doi.org/10.3748/wjg.v25.i36.5530

Loren, D., Kowalski, T., Siddiqui, A., Jackson, S., Toney, N., Malhotra, N., & Haddad, N. (2016). Influence of integrated molecular pathology test results on real-world management decisions for patients with pancreatic cysts: analysis of data from a national registry cohort. *Diagn Pathol*, *11*. https://doi.org/10.1186/s13000-016-0462-x

Malagelada, J., Guda, N., Goh, K.-L., Hackert, T., Layer, P., Molero, X., Pandol, S., Tanaka, M., Umar, M., & LeMair, A. (2019). Pancreatic cystic lesions.

https://www.worldgastroenterology.org/UserFiles/file/guidelines/pancreatic-cystic-lesions-english-2019.pdf

Malhotra, N., Jackson, S. A., Freed, L. L., Styn, M. A., Sidawy, M. K., Haddad, N. G., & Finkelstein, S. D. (2014). The added value of using mutational profiling in addition to cytology in diagnosing aggressive pancreaticobiliary disease: review of clinical cases at a single center. *BMC Gastroenterol*, *14*, 135. https://doi.org/10.1186/1471-230x-14-135

Muthusamy, V. R., Chandrasekhara, V., Acosta, R. D., Bruining, D. H., Chathadi, K. V., Eloubeidi, M. A., Faulx, A. L., Fonkalsrud, L., Gurudu, S. R., Khashab, M. A., Kothari, S., Lightdale, J. R., Pasha, S. F., Saltzman, J. R., Shaukat, A., Wang, A., Yang, J., Cash, B. D., & DeWitt, J. M. (2016). The role of endoscopy in the diagnosis and treatment of cystic pancreatic neoplasms. *Gastrointest Endosc*, *84*(1), 1-9. https://doi.org/10.1016/j.gie.2016.04.014

Nagula, S., Kennedy, T., Schattner, M. A., Brennan, M. F., Gerdes, H., Markowitz, A. J., Tang, L., & Allen, P. J. (2010). Evaluation of cyst fluid CEA analysis in the diagnosis of mucinous cysts of the pancreas. *J Gastrointest Surg*, *14*(12), 1997-2003. https://doi.org/10.1007/s11605-010-1281-0

NCCN. (2024). *Pancreatic Adenocarcinoma Version 3.2024 — April 30, 2024*. https://www.nccn.org/professionals/physician_gls/pdf/pancreatic.pdf

Nikiforova, M. N., Wald, A. I., Spagnolo, D. M., Melan, M. A., Grupillo, M., Lai, Y. T., Brand, R. E., O'Broin-Lennon, A. M., McGrath, K., Park, W. G., Pfau, P. R., Polanco, P. M., Kubiliun, N., DeWitt, J., Easler, J. J., Dam, A., Mok, S. R., Wallace, M. B., Kumbhari, V., . . . Singhi, A. D. (2023). A Combined DNA/RNA-based Next-Generation Sequencing Platform to Improve the Classification of Pancreatic Cysts and Early Detection of Pancreatic Cancer Arising From Pancreatic Cysts. *Ann Surg*, *278*(4), e789-e797. https://doi.org/10.1097/SLA.0000000000005904

Oh, H. C., Kang, H., & Brugge, W. R. (2014). Cyst fluid amylase and CEA levels in the differential diagnosis of pancreatic cysts: a single-center experience with histologically proven cysts. *Dig Dis Sci*, *59*(12), 3111-3116. https://doi.org/10.1007/s10620-014-3254-8

Pancreaseq. (2024). *Pancreaseq*. https://pancreaseq.com/

Paniccia, A., Polanco, P. M., Boone, B. A., Wald, A. I., McGrath, K., Brand, R. E., Khalid, A., Kubiliun, N., O'Broin-Lennon, A. M., Park, W. G., Klapman, J., Tharian, B., Inamdar, S., Fasanella, K., Nasr, J., Chennat, J., Das, R., DeWitt, J., Easler, J. J., . . . Singhi, A. D. (2023). Prospective, Multi-Institutional, Real-Time Next-Generation Sequencing of Pancreatic Cyst Fluid Reveals Diverse Genomic Alterations That Improve the Clinical Management of Pancreatic Cysts. *Gastroenterology*, *164*(1), 117-133 e117. https://doi.org/10.1053/j.gastro.2022.09.028

Pérez, R. H., de la Morena López, F., Rodríguez, P. L. M., Jiménez, F. M., Piris, L. V., & Vaquero, C. S. (2021). Molecular analysis of pancreatic cystic neoplasm in routine

clinical practice. *World journal of gastrointestinal endoscopy*, *13*(2), 56-71. https://doi.org/10.4253/wjge.v13.i2.56

Pflüger, M. J., Jamouss, K. T., Afghani, E., Lim, S. J., Franco, S. R., Mayo, H., Spann, M., Wang, H., Singhi, A., & Lennon, A. M. (2023). Predictive ability of pancreatic cyst fluid biomarkers: a systematic review and meta-analysis. *Pancreatology*.

Rahal, M. A., DeWitt, J. M., Patel, H., Schmidt, C. M., Ceppa, E. P., Simpson, R. E., Sherman, S., & Al-Haddad, M. (2022). Serial EUS-Guided FNA for the Surveillance of Pancreatic Cysts: A Study of Long-Term Performance of Tumor Markers. *Dig Dis Sci*, *67*(11), 5248-5255. https://doi.org/10.1007/s10620-022-07427-6

RedPath Integrated Pathology, I. (2001, 11-05-2001). *Topographic genotyping*. https://patents.justia.com/patent/7014999

Scholten, L., van Huijgevoort, N. C. M., van Hooft, J. E., Besselink, M. G., & Del Chiaro, M. (2018). Pancreatic Cystic Neoplasms: Different Types, Different Management, New Guidelines. *Visceral Medicine*, *34*(3), 173-177. https://doi.org/10.1159/000489641

Simpson, R. E., Cockerill, N. J., Yip-Schneider, M. T., Ceppa, E. P., House, M. G., Zyromski, N. J., Nakeeb, A., Al-Haddad, M. A., & Schmidt, C. M. (2018). DNA profile components predict malignant outcomes in select cases of intraductal papillary mucinous neoplasm with negative cytology. *Surgery*, *164*(4), 712-718. https://doi.org/10.1016/j.surg.2018.05.033

Simpson, R. E., Cockerill, N. J., Yip-Schneider, M. T., Ceppa, E. P., House, M. G., Zyromski, N. J., Nakeeb, A., Al-Haddad, M. A., & Schmidt, C. M. (2019). Clinical criteria for integrated molecular pathology in intraductal papillary mucinous neoplasm: less is more. *HPB (Oxford)*, *21*(5), 574-581. https://doi.org/10.1016/j.hpb.2018.09.004

Singhi, A. D., McGrath, K., Brand, R. E., Khalid, A., Zeh, H. J., Chennat, J. S., Fasanella, K. E., Papachristou, G. I., Slivka, A., Bartlett, D. L., Dasyam, A. K., Hogg, M., Lee, K. K., Marsh, J. W., Monaco, S. E., Ohori, N. P., Pingpank, J. F., Tsung, A., Zureikat, A. H., . . . Nikiforova, M. N. (2018). Preoperative next-generation sequencing of pancreatic cyst fluid is highly accurate in cyst classification and detection of advanced neoplasia. *Gut*, *67*(12), 2131-2141. https://doi.org/10.1136/gutjnl-2016-313586

Singhi, A. D., Zeh, H. J., Brand, R. E., Nikiforova, M. N., Chennat, J. S., Fasanella, K. E., Khalid, A., Papachristou, G. I., Slivka, A., Hogg, M., Lee, K. K., Tsung, A., Zureikat, A. H., & McGrath, K. (2016). American Gastroenterological Association guidelines are inaccurate in detecting pancreatic cysts with advanced neoplasia: a clinicopathologic study of 225 patients with supporting molecular data. *Gastrointest Endosc*, *83*(6), 1107-1117.e1102. https://doi.org/10.1016/j.gie.2015.12.009

Smith, A. L., Abdul-Karim, F. W., & Goyal, A. (2016). Cytologic categorization of pancreatic neoplastic mucinous cysts with an assessment of the risk of malignancy: A retrospective study based on the Papanicolaou Society of Cytopathology guidelines. *Cancer Cytopathology*, *124*(4), 285-293. https://acsjournals.onlinelibrary.wiley.com/doi/abs/10.1002/cncy.21657

Springer, S., Wang, Y., Dal Molin, M., Masica, D. L., Jiao, Y., Kinde, I., Blackford, A., Raman, S. P., Wolfgang, C. L., Tomita, T., Niknafs, N., Douville, C., Ptak, J., Dobbyn, L., Allen, P. J., Klimstra, D. S., Schattner, M. A., Schmidt, C. M., Yip-Schneider, M., . . . Lennon, A. M. (2015). A combination of molecular markers and clinical features improve the classification of pancreatic cysts. *Gastroenterology*, *149*(6), 1501-1510. https://doi.org/10.1053/j.gastro.2015.07.041

Stoffel, E. M., McKernin, S. E., & Khorana, A. A. (2018). Evaluating Susceptibility to Pancreatic Cancer: ASCO Clinical Practice Provisional Clinical Opinion Summary. *Journal of Oncology Practice*, *15*(2), 108-111. https://doi.org/10.1200/JOP.18.00629

Suriawinata, A. (2024, February 14, 2024). *Pathology of exocrine pancreatic neoplasms*. https://www.uptodate.com/contents/pathology-of-exocrine-pancreatic-neoplasms

Tanaka, M., Fernandez-Del Castillo, C., Kamisawa, T., Jang, J. Y., Levy, P., Ohtsuka, T., Salvia, R., Shimizu, Y., Tada, M., & Wolfgang, C. L. (2017). Revisions of international consensus Fukuoka guidelines for the management of IPMN of the pancreas. *Pancreatology*, *17*(5), 738-753. https://doi.org/10.1016/j.pan.2017.07.007

The European Study Group on Cystic Tumours of the Pancreas. (2018). European evidence-based guidelines on pancreatic cystic neoplasms. *Gut*, *67*(5), 789-804. https://doi.org/10.1136/gutjnl-2018-316027

Vege, S. S., Ziring, B., Jain, R., & Moayyedi, P. (2015). American gastroenterological association institute guideline on the diagnosis and management of asymptomatic neoplastic pancreatic cysts. *Gastroenterology*, *148*(4), 819-822; quize812-813. https://doi.org/10.1053/j.gastro.2015.01.015

Volckmar, A. L., Endris, V., Gaida, M. M., Leichsenring, J., Stogbauer, F., Allgauer, M., von Winterfeld, M., Penzel, R., Kirchner, M., Brandt, R., Neumann, O., Sultmann, H., Schirmacher, P., Rudi, J., Schmitz, D., & Stenzinger, A. (2019). Next generation sequencing of the cellular and liquid fraction of pancreatic cyst fluid supports discrimination of IPMN from pseudocysts and reveals cases with multiple mutated driver clones: First findings from the prospective ZYSTEUS biomarker study. *Genes Chromosomes Cancer*, *58*(1), 3-11. https://doi.org/10.1002/gcc.22682

Pharmacogenetic Testing

Policy Number: AHS – M2021 – Pharmacogenetic Testing	Prior Policy Name and Number, as applicable: • M2021 – Cytochrome P450
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Pharmacogenetics aims to study the influence of genetic variation on drug response and drug toxicity, which allows physicians to select a more targeted therapeutic strategy to suit each patient's genetic profile (Aka et al., 2017). Genetic variations in human proteins, such as, cytochrome P450 enzymes, Thiopurine methyltransferase (*TPMT*), dihydropyrimidine dehydrogenase (*DPD*), and cell surface proteins, highlights the clinical importance of pharmacogenetic testing.

Cytochrome (CYP) P450 enzymes are a class of enzymes essential in the synthesis and breakdown metabolism of various molecules and chemicals. Found primarily in the liver, these enzymes are also essential for the metabolism of many medications. CYP P450 enzymes, approximately 58 CYP human genes, are essential to produce many biochemical building blocks, such as cholesterol, fatty acids, and bile acids. Additional cytochrome P450 are involved in the metabolism of drugs, carcinogens, and internal substances, such as toxins formed within cells. Mutations in CYP P450 genes can result in the inability to properly metabolize medications and other substances, leading to increased levels of toxic substances in the body (Bains, 2013; Tantisira & Weiss, 2023).

Thiopurine methyltransferase (*TPMT*) is an enzyme that methylates azathioprine, mercaptopurine and thioguanine into active thioguanine nucleotide metabolites. Azathioprine and mercaptopurine are used for treatment of nonmalignant immunologic disorders; mercaptopurine is used for treatment of lymphoid malignancies; and thioguanine is used for treatment of myeloid leukemias (Relling et al., 2013).

Dihydropyrimidine dehydrogenase (DPD), encoded by the gene *DPYD*, is a rate-limiting enzyme responsible for fluoropyrimidine catabolism. The fluoropyrimidines (5-fluorouracil and capecitabine) are drugs used in the treatment of solid tumors, such as colorectal, breast, and aerodigestive tract tumors (Amstutz et al., 2018).

A variety of cell surface proteins, such as antigen-presenting molecules and other proteins, are encoded by the human leukocyte antigen genes (*HLA*s). HLAs are also known as major histocompatibility complex (MHC) (Viatte, 2023).

II. Related Policies

Policy Number	Policy Title
AHS-G2100	In Vitro Chemoresistance and Chemosensitivity Assays
AHS-G2115	Metabolite Markers of Thiopurines Testing
AHS-M2038	Genetic Testing for Familial Alzheimer Disease
AHS-M2067	Therapeutic Drug Monitoring for 5-Fluorouracil
AHS-M2082	Genetic Testing for Lipoprotein A Variant(s) as a Decision Aid for Aspirin Treatment and/or CVD Risk Assessment

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) To aid in therapy selection and/or dosing for individuals being considered for therapy or who are in their course of therapy with any of the medications listed below, testing for the *CYP2D6* genotype once per lifetime (see Note 1) **MEETS COVERAGE CRITERIA**:
    a) Amphetamine
    b) Aripiprazole
    c) Aripiprazole Lauroxil
    d) Atomoxetine
    e) Brexpiprazole
    f) Carvedilol
    g) Cevimeline
    h) Clozapine

i) Codeine
j) Desipramine
k) Deutetrabenazine
l) Eliglustat
m) Fluvoxamine
n) Gefitinib
o) Iloperidone
p) Lofexidine
q) Meclizine
r) Metoclopramide
s) Nortriptyline
t) Oliceridine
u) Ondansetron
v) Paroxetine
w) Perphenazine
x) Pimozide
y) Pitolisant
z) Propafenone
aa) Tamoxifen
bb) Tetrabenazine
cc) Thioridazine
dd) Tolterodine
ee) Tramadol
ff) Tropisetron
gg) Valbenazine
hh) Venlafaxine
ii) Vortioxetine

2) To aid in therapy selection and/or dosing for individuals being considered for therapy or who are in their course of therapy with any of the medications listed

below, testing for the *CYP2D6* and *CYP2C19* genotype once per lifetime (see Note 1) **MEETS COVERAGE CRITERIA:**

a) Amitriptyline
b) Clomipramine
c) Doxepin
d) Imipramine
e) Trimipramine

3) To aid in therapy selection and/or dosing for individuals being considered for therapy or who are in their course of therapy, testing for the *CYP2C19* genotype once per lifetime (see Note 1) **MEETS COVERAGE CRITERIA**:

a) Abrocitinib
b) Brivaracetam
c) Citalopram
d) Clobazam
e) Clopidogrel
f) Dexlansoprazole (see Note 2)
g) Escitalopram
h) Flibanserin
i) Lansoprazole (see Note 2)
j) Mavacamten
k) Omeprazole (see Note 2)
l) Pantoprazole (in pediatric individuals) (see Note 2)
m) Sertraline
n) Voriconazole (see Note 2)

4) To aid in therapy selection and/or dosing for individuals being considered for therapy or who are in their course of therapy with any of the medications listed below, testing for the *CYP2C9* genotype once per lifetime (see Note 1) **MEETS COVERAGE CRITERIA**:

a) Celecoxib
b) Dronabinol

c) Erdafitinib
d) Flurbiprofen
e) Lornoxicam
f) Meloxicam
g) Nateglinide
h) Piroxicam
i) Siponimod
j) Tenoxicam

5) For individuals being considered for warfarin therapy, testing for the *CYP2C9*, *CYP4F2*, *VKORC1*, and rs12777823 genotype once per lifetime (see Note 1) **MEETS COVERAGE CRITERIA**.

6) To aid in therapy selection and/or dosing for individuals being considered for therapy or who are in their course of therapy with the below medications, testing for the *TPMT* and *NUDT15* genotype once per lifetime (see Note 1) **MEETS COVERAGE CRITERIA**:
   a) Azathioprine
   b) Mercaptopurine
   c) Thioguanine

7) To aid in therapy selection and/or dosing for individuals being considered for therapy or who are in their course of therapy with the below medications, testing for the *DPYD* genotype once per lifetime (see Note 1) **MEETS COVERAGE CRITERIA**:
   a) Capecitabine
   b) Flucytosine
   c) Fluorouracil
   d) Tegafur

8) To aid in therapy selection and/or dosing for individuals being considered for therapy or who are in their course of therapy with the below medications, testing for the following human leukocyte antigens (HLAs) genotypes once per lifetime (see Note 1) **MEETS COVERAGE CRITERIA**:
   a) *HLA-B*57:01* before treatment with Abacavir
   b) *HLA-B*58:01* before treatment with Allopurinol

c) *HLA-B*15:02* for treatment with Oxcarbazepine

d) *HLA-B*15:02* and *HLA-A*31:01* for treatment with Carbamazepine

9) To aid in therapy selection and/or dosing for individuals being considered for therapy or who are in their course of therapy with phenytoin/fosphenytoin, testing for the *CYP2C9* and *HLA-B*15:02* genotype once per lifetime (see Note 1) **MEETS COVERAGE CRITERIA**.

10) To aid in therapy selection and/or dosing for individuals being considered for therapy or who are in their course of therapy with the medications listed below, testing for the *G6PD* genotype once per lifetime (see Note 1) **MEETS COVERAGE CRITERIA**:

a) Pegloticase

b) Primaquine

c) Rasburicase

d) Tafenoquine

11) To aid in therapy selection and/or dosing for individuals being considered for therapy or who are in their course of therapy with the below medications, testing for the following genotypes once per lifetime (see Note 1) **MEETS COVERAGE CRITERIA**:

a) *BCHE* for treatment with mivacurium or succinylcholine.

b) *CFTR* for treatment with ivacaftor, elexacaftor and tezacaftor, ivacaftor and lumacaftor, or ivacaftor and tezacaftor.

c) *CYP2B6* for treatment with efavirenz.

d) *CYP3A5* for treatment with tacrolimus.

e) *IFNL3* treatment with peginterferon alfa-2a, peginterferon alfa-2b or ribavirin.

f) *NAT2* for treatment with amifampridine or amifampridine phosphate.

g) *UGT1A1* for treatment with atazanavir, belinostat, irinotecan, nilotinib, pazopanib, or sacituzumab govitecan-hziy.

12) To aid in therapy selection and/or dosing for individuals being considered for therapy or who are in their course of therapy with belzutifan, testing for the *CYP2C19* and *UGT2B17* genotype once per lifetime (see Note 1) **MEETS COVERAGE CRITERIA**.

13) For individuals being considered for the use of halogenated volatile anesthetics or depolarizing muscle relaxants, testing for the *RYR1* and *CACNA1S* genotype once per lifetime (see Note 1) **MEETS COVERAGE CRITERIA**.

14) When formulary coverage allows a pharmacotherapy that is dependent on a known genetic status (e.g., *APOE* testing prior to lecanemab-irmb treatment), gene specific testing **MEETS COVERAGE CRITERIA.**

15) To identify patients at risk of statin-induced myopathy, genetic testing for the presence of variants in the *SLCO1B1* gene **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

16) The following pharmacogenetic testing **DOES NOT MEET COVERAGE CRITERIA**:

a) Genotyping more than once per lifetime (see Note 1) for any medication therapy.

b) Genotyping of the general population.

c) Pharmacogenetic testing (e.g., single nucleotide polymorphism [SNP] testing or SNP panel testing; single gene or multi-gene panel testing [see Note 3]) for all other situations not addressed above.

NOTES:

Note 1: Any gene may only be tested **once** per lifetime, regardless of the indication (an exception would be for *HLA* where a specific variant is tested for the medication). For example, if *CYP2C19* was tested for therapy with citalopram, additional testing for *CYP2C19* for treatment with clopidogrel is not needed and **DOES NOT MEET COVERAGE CRITERIA**. Testing in a patient post-liver transplant is not indicated.

Note 2: Pharmacogenetic testing for proton pump inhibitor therapies (PPIs) ***ONLY MEETS COVERAGE CRITERIA*** if the patient has an active *H. pylori* infection.

Note 3: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162-Reimbursement Policy.

IV. Table of Terminology

Term	Definition
AACAP	American Academy of Child and Adolescent Psychiatry

AACC	American Association for Clinical Chemistry
AACF	American College of Cardiology Foundation
AAFP	American Family Physician
AAN	American Academy of Neurology
ACMG	American College of Medical Genetics and Genomics
AHA	American Heart Association
AMP	Association For Molecular Pathology
APOE	*Apolipoprotein E*
ARIA	Amyloid related imaging abnormalities
AS	Activity score
ASCPT	American Society for Clinical Pharmacology and Therapeutics
ASHP	American Society of Health System Pharmacists
BCHE	*Butyrylcholinesterase*
BDI	Beck's Depression Inventory
CACNA1S	*Calcium voltage-gated channel subunit alpha1 S*
CFTR	*Cystic fibrosis transmembrane conductance regulator*
COMT	*Catechol-O-methyltransferase*
CPIC	Clinical Pharmacogenetics Implementation Consortium
CYP	Cytochrome
CYP1A2	*Cytochrome P450 family 1 subfamily A member 2*
CYP2B6	*Cytochrome P450 family 2 subfamily B member 6*
CYP2C9	*Cytochrome P450 family 2 subfamily C member 9*
CYP2C19	*Cytochrome P450 family 2 subfamily C member 19*
CYP2D6	*Cytochrome P450 family 2 subfamily D member 6*
CYP3A4	*Cytochrome P450 family 3 subfamily A member 4*
CYP3A5	*Cytochrome P450 family 3 subfamily A member 5*
CYP4F2	*Cytochrome P450 family 4 subfamily F member 2*
DAT1	Former gene name of *solute carrier family 6 member 3*
DBH	*Dopamine beta-hydroxylase*
DPD	Dihydropyrimidine dehydrogenase
DPWG	Dutch Pharmacogenetics Working Group
DPYD	*Dihydropyrimidine dehydrogenase gene*
DRD1/2/4	*Dopamine receptor gene 1/2/4*
EMA	European Medicines Agency
FDA	Food and Drug Administration

G6PD	*Glucose-6-phosphate dehydrogenase gene*
HLA	Human leukocyte antigen
HLA-A	*Major histocompatibility complex, class I, A*
HLA-B	*Major histocompatibility complex, class I, B*
IFNL3	*Interferon lambda 3*
IM	Intermediate metabolizer
ISPG	The International Society of Psychiatric Genetics
MDD	Major depressive disorder
MHC	Major histocompatibility complex
MP	Mercaptopurine
MTHFR	*Methylenetetrahydrofolate reductase*
NAT2	*N-acetyltransferase 2*
NM	Normal metabolizer
NUDT15	*Nudix hydrolase 15*
OPRK1	*Opioid receptor kappa 1*
OPRM1	*Opioid receptor mu 1*
PM	Poor metabolizer
PPIs	Proton pump inhibitor therapies
RM	Rapid metabolizer
rs12777823	SNP that can affect warfarin sensitivity
RYR1	*Ryanodine receptor 1 gene*
SJS	Stevens Johnson Syndrome
SLC6A3	*Solute carrier family 6 member 3*
SLC6A4	*Solute carrier family 6 member 4*
SLCO1B1	*Solute carrier organic anion transporter family member 1B1*
SNP	Single nucleotide polymorphism
TAU	Treatment as usual
TCAs	Tricyclic antidepressants
TEN	Toxic epidermal necrolysis
TG	Thioguanine
TPMT	*Thiopurine methyltransferase*
TYMS	*Thymidylate synthetase*
UGT1A1	*UDP glucuronosyltransferase family 1 member A1*
UGT2B15	*Uridine diphosphate glycosyltransferase 2 family, member 15*
UGT2B17	*UDP glucuronosyltransferase family 2 member B17*
URM	Ultra-rapid metabolizer
VKORC1	*Vitamin K epoxide reductase complex subunit 1*

V. Scientific Background

Genetic variations play a potentially large role in an individual's response to medications. However, drug metabolism and responses are affected by many other factors, including age, sex, interactions with other drugs, and disease states (Tantisira & Weiss, 2023). Nonetheless, inherited differences in the metabolism and disposition of drugs and genetic polymorphisms in the targets of drug therapy can have a significant influence on the efficacy and toxicity of medications potentially even more so than clinical variables such as age and organ function (Kapur et al., 2014; Ting & Schug, 2016). Genetic variation can influence pharmacodynamic factors through variations affecting drug target receptors and downstream signal transduction, or pharmacokinetic factors, affecting drug metabolism and/or elimination (Tantisira & Weiss, 2023).

The Cytochrome P450 (CYP 450) system is a group of enzymes responsible for the metabolism of many endogenous and exogenous substances, including many pharmaceutical agents. This system may serve to "activate" an inactive form of a drug, as well as inactivate and/or clear a drug from circulation. The CYP 450 enzymes are responsible for the clearance of over half of all drugs, and their activity can be affected by diet, age, and other medications. The genes encoding for the CYP 450 enzymes are highly variable with multiple alleles that confer various levels of metabolic activity for specific substrates. In some cases, alleles can be highly correlated with ethnic background. Generally, there are three categories of metabolizer; ultra-rapid metabolizers, normal metabolizers, and poor metabolizers (Tantisira & Weiss, 2023).

Due to the variations in enzyme activity conferred by allelic differences, some CYP 450 alleles are associated with an increased risk for certain conditions or adverse outcomes with certain drugs. Knowledge of the allele type may assist in the selection of a drug, or in drug dosing. Three CYP 450 enzymes are most often considered regarding clinical use for drug selection and/or dosing. Phenotypes, such as CYP2D6, CYP2C9 and CYP2C19, have been associated with the metabolism of several therapeutic drugs, and various alleles of the *CYP450* gene confer differences in metabolic function. For these CYP 450 enzymes, it is thought that "poor metabolizers" could have less efficient elimination of a drug, and therefore may be at risk for side effects due to drug accumulation. For drugs that require activation by a specific CYP 450 enzyme, lower activity may yield less of the biologically active drug, which could result in lower drug efficacy. Individuals considered as "ultra-rapid metabolizers" may clear the drug more quickly than normal, and therefore may require higher doses to yield the desired therapeutic effect. Likewise, for drugs that require activation, these individuals may produce higher levels of the active drug, potentially causing unwanted side effects.

Due to these differences in enzyme activity, some alleles are associated with a higher risk of adverse outcomes depending on the drug prescribed (Tantisira & Weiss, 2023).

ApoE

Apolipoprotein E (*APOE*) is the gene most strongly associated as a genetic risk factor for late-onset Alzheimer disease. *APOE* can have three alleles: ε1, ε2, and ε4 (Sherva & Kowall, 2022). *APOE ε4* is a susceptibility gene, meaning it is associated with increased risk but does not cause Alzheimer disease, and not all patients with Alzheimer disease will carry *APOE ε4*. In one study of 1303 patients, 55% of those homozygous for *ε4* developed Alzheimer disease, while 27% of those heterozygous and 9% with no *ε4* allele also developed Alzheimer disease (Myers et al., 1996). *APOE*, as well as *CYP2D6,* carrier status may have an effect on a patient's response to drugs, "with *CYP2D6*-PMs [poor metabolizers], *CYP2D6*-Ums [ultrarapid metabalizers], and *APOE-4/4* carriers acting as the worst responders" (Cacabelos et al., 2012).

In 2023, the FDA approved Lecanemab (brand name Leqembi), an amyloid beta-directed antibody, for the treatment of Alzheimer disease in adult patients (FDA, 2023). One potential side effect of Leqembi is amyloid related imaging abnormalities (ARIA), which may be more likely to occur in people who are homozygous *APOE ε4* carries (Leqembi, 2024). The FDA includes that "the prescribing information states that testing for ApoE ε4 status should be performed before starting treatment with Leqembi to inform the risk of developing ARIA" (FDA, 2023).

CYP2C9

Warfarin (brand name Coumadin) is widely used as an anticoagulant in the treatment and prevention of thrombotic disorders. *CYP2C9* participates in warfarin metabolism, and several *CYP2C9* alleles have reduced activity, resulting in a higher circulating drug concentration. *CYP2C9**2 and *CYP2C9**3 are the most common variants with reduced activity. Variations in a second gene, *VKORC1*, also can impact warfarin's effectiveness. This gene codes for the enzyme that is the target for warfarin. Genotypes resulting in reduced metabolism may need a higher dose to achieve the desired efficacy (Tantisira & Weiss, 2023).

CYP2C19

Clopidogrel (brand name Plavix) is used to inhibit platelet aggregation and is given as a pro-drug that is metabolized to its active form by *CYP2C19*. Alleles *CYP2C19**2 and *CYP2C19**3 are associated with reduced metabolism of clopidogrel. Individuals with

the "poor metabolizer" alleles may not benefit from clopidogrel treatment at standard doses (Tantry et al., 2021). Tuteja et al. (2020) studied *CYP2C19* genotyping to guide antiplatelet therapy. A total of 504 participants contributed to this study, with only 249 participants genotyped. The authors noted that genotyping results "significantly influenced antiplatelet drug prescribing; however, almost half of *CYP2C19* LOF [loss-of-function] carriers continued to receive clopidogrel. Interventional cardiologists consider both clinical and genetic factors when selecting antiplatelet therapy following PCI [percutaneous coronary intervention]" (Tuteja et al., 2020).

CYP2D6

Tetrabenazine (brand name Xenazine) is used in the treatment of chorea associated with Huntington disease. This drug is metabolized for clearance primarily by *CYP2D6*. Poor metabolizers are considered to be those individuals with impaired *CYP2D6* function, and dosing is often influenced by how well a patient metabolizes the drug. For example, a poor metabolizer will often have a maximum dose of 50 mg daily whereas an extensive metabolizer has a maximum dose of 100 mg daily (Suchowersky, 2023).

Tamoxifen, a drug commonly used for the treatment and prevention of recurrence of estrogen receptor positive breast cancer, is metabolized by *CYP2D6*. Polymorphisms of *CYP2D6* have been noted to affect the efficacy of tamoxifen by affecting the amount of active metabolite produced. Endoxifen, which is the primary active metabolite of tamoxifen, has a 100-fold affinity for the estrogen receptor compared to tamoxifen, but poor metabolizers have been demonstrated to show lower than expected levels of plasma endoxifen (Ahern et al., 2017).

Codeine, which is commonly used to treat mild to moderate pain, is metabolized to morphine, a much more powerful opioid, by *CYP2D6*. Individuals with varying CYP2D6 activity may see negative side effects or a shorter duration of pain relief. The effect is significant enough to have caused fatalities in unusual metabolizers; for instance, an ultra-rapid metabolizing toddler was reported to have passed away after being given codeine for a routine dental operation (Kelly et al., 2012; Tantisira & Weiss, 2023).

TPMT

Thiopurine methyltransferase (TPMT) is an enzyme that methylates thiopurines into active thioguanine nucleotides. The *TPMT* gene is inherited as a monogenic co-dominant trait with ethnic differences in the frequencies of low-activity variant alleles. Individuals who inherit two inactive *TPMT* alleles will develop severe myelosuppression. Individuals that inherit only one inactive *TPMT* allele will develop moderate to severe myelosuppression, and those individuals who inherit both active *TPMT* alleles will have

a lower risk of myelosuppression. Therefore, genotyping for *TPMT* is critical before starting therapy with thiopurine drugs (Relling et al., 2013).

DPYD

The dihydropyrimidine dehydrogenase (*DPYD*) gene encodes for the rate-limiting enzyme dihydropyrimidine dehydrogenase, which is involved in catabolism of fluoropyrimidine drugs used in the treatment of solid tumors. Decreased DPD activity increases the risk for severe or even fatal drug toxicity when patients are being treated with fluoropyrimidine drugs. Numerous genetic variants in the *DPYD* gene have been identified that alter the protein sequence or mRNA splicing; however, some of these variants have no effect on DPD enzyme activity. The most studied causal variant of *DPYD* haplotype (HapB3) spans intron 5 to exon 11 and affects protein function. The most common variant in Europeans is HapB3 with a c.1129–5923C>G *DPYD* variant which demonstrates decreased function with carrier frequency of 4.7%, followed by c.190511G>A (carrier frequency: 1.6%) and c.2846A>T (carrier frequency: 0.7%). Approximately 7% of Europeans carry at least one decreased function *DPYD* variant. In people with African ancestry, the most common variant is c.557A>G (rs115232898, p.Y186C) and is relatively common (3–5% carrier frequency). Other *DPYD* decreased function variants are rare. Therefore, most available genetic tests focus on identifying the most common variants with well-established risk: (c.190511G>A, c.1679T>G, c.2846A>T, c.1129– 5923C>G) (Amstutz et al., 2018).

TYMS

TYMS (thymidylate synthetase) encodes an enzyme necessary for thymidine production. As with *DPYD*, *TYMS* is thought to be involved with the toxicity of fluoropyrimidines. Fluorouracil (FU)'s primary metabolite inhibits thymidylate synthetase by forming a stable complex with thymidylate synthetase and folate, thereby blocking activity of the enzyme. Polymorphisms in the *TYMS* gene further affect the interaction between *TYMS* and FU, potentially increasing the toxicity of FU. Genotyping of *TYMS* prior to treatment with FU or capecitabine has been suggested for clinical practice, but data has been varied (Krishnamurthi & Kamath, 2024).

Castro-Rojas et al. (2017) evaluated *TYMS* genotypes as predictors of both clinical response and toxicity to fluoropyrimidine-based treatment for colorectal cancer. A total of 105 patients were genotyped. The authors noted that while the 2R/2R genotype was associated with clinical response (odds ratio = 3.45), the genotype was also associated with severe toxicity (odds ratio = 5.21). The genotype was thought to be associated with low *TYMS* expression. The authors further identified the rs2853542

and rs151264360 alleles to be independent predictors of response failure to chemotherapy (Castro-Rojas et al., 2017).

HLAs

Human Leukocyte antigens (HLAs) are divided into three regions, such as class I, class II and class III. Each class has many gene loci, expressed genes and pseudogenes. The class I encodes HLA-A, HLA-B, HLA-C and other antigens. The class II encodes HLA-DP, DQ and DR. The class III region is located between class I and class II and does not encode any HLAs, but other immune response proteins (Viatte, 2023).

An article published by van der Wouden et al. (2019) reports on the development of the new PGx-Passport panel (pre-emptive pharmacogenetics-passport panel), which is able to test "58 germline variant alleles, located within 14 genes (*CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A5, DPYD, F5, HLA-A, HLA-B, NUDT15, SLCO1B1, TPMT, UGT1A1*, and *VKORC1*)"; this standardized panel is based on the Dutch Pharmacogenetics Working Group (DPWG) guidelines and will help physicians to optimize drug prescription in 49 common drugs. It is recommended by the authors that commercial and hospital laboratories utilize this panel for personalized medicinal purposes. Drug optimization in the 49 commonly prescribed drugs includes ten antidepressants, five immunosuppressants, five anti-cancer drugs, four anti-infectives, four anticoagulants, four antiepileptics, four antipsychotics, three proton pump inhibitors, two anti-arrhythmics, two analgesics, two antilipidemics, one antihypertensive, one psychostimulant, one anticontraceptive and one Gaucher disease drug (van der Wouden et al., 2019).

Proprietary Testing

Due to the increase in pharmacogenetic genotyping, proprietary gene panels have become commercially available. Panels encompassing the most common genes that influence drug metabolism have increased in usage. For example, Myriad's new proprietary panel "GeneSight" proposes it can "predict poorer antidepressant outcomes and to help guide healthcare providers to more genetically optimal medications," thereby leading to better patient outcomes. The test assesses every known metabolic pathway (CYP450 or otherwise) for a given drug and their metabolites, as well as the pharmacodynamic activity of the compound and its metabolites, any FDA information on that drug, and other validated research on the relevant alleles; this information is then integrated with the genetic test results. This allows the test to categorize the 64 medications into three categories: "green (use as directed), yellow (some moderate gene-drug interaction) and red (significant gene-

drug interaction)." Myriad states that this allows every metabolic pathway of a drug to be evaluated instead of the "one gene, one drug" view. Other GeneSight variations, such as GeneSight Psychotropic used for psychotropic medications, exist as well (Myriad, 2016, 2019, 2022). Still, other companies such as Mayo Clinic and Sema4 have developed their own pharmacogenetic panels, each with individually chosen analytes (Mayo, 2023; Sema4, 2022).

Benitez et al. (2018) assessed the cost-effectiveness of pharmacogenomics in treating psychiatric disorders. The authors compared 205 members that received guidance from GeneSight's Psychotropic to 478 members that received "treatment-as-usual" (TAU). Reimbursement costs were calculated over the 12 months pre- and post-index event periods. The authors found a total post-index cost savings of $5505, which was equivalent to a savings of $0.07 per-member-per-month (PMPM). The authors also evaluated the savings at different adoption rates of the GeneSight test. At 5% adoption, commercial payer savings was calculated at $0.02 PMPM and at 40% adoption, savings was $0.15 PMPM (Benitez et al., 2018).

The AmpliChip® (Roche Molecular Systems, Inc.) is the FDA-cleared test for CYP450 genotyping. This test genotypes *CYP2D6* and *CYP2C19*. From the FDA website: "The AmpliChip CYP4502C19 Test is designed to identify specific nucleic acid sequences and query for the presence of certain known sequence polymorphisms through analysis of the pattern of hybridization to a series of probes that are specifically complementary either to wild-type or mutant sequences" (FDA, 2005). The analytical accuracy was evaluated at 99.6%, or 806 of 809 samples identified correctly. This test assesses a total of 30 alleles, three for CYP219 and 27 for *CYP2D6* (FDA, 2005).

The OneOme RightMed Pharmacogenomic Test analyzes more than 100 variants in 27 genes to study how a patient may respond to certain medications. The test covers *CYP1A2, CYP2B6, CYP2C Cluster, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5, CYP4F2, COMT, DPYD, DRD2, F2, F5, GRIK4, HLA-A, HLA-B, HTR2A, HTR2C, IL28B (IFNL4), MTHFR*, NUDT15, OPRM1, SLC6A4, SLCO1B1, TPMT, UGT1A1*, and *VKORC1* (OneOme, 2021). Analytical validity of the test was assessed by comparing RightMed test results with bi-directional Sanger sequencing results, which resulted in 100% concordance. The RightMed test detects *CYP2D6* deletions, duplications, and hybrid alleles, but cannot differentiate duplications in the presence of a deletion (GTR, 2017).

Clinical Utility and Validity

A study evaluating GeneSight Psychotropic's clinical utility was performed by Greden et al. (2019); a total of 1167 patients with major depressive disorder were split into two randomized groups: treatment as usual (TAU) and pharmacogenetic-guided. Medications were classified as "congruent" (use as directed' or 'use with caution' test

categories) or "incongruent" ('use with increased caution and with more frequent monitoring' test category) with test results. After eight weeks, the authors found a statistically significant improvement in response and remission; 26% for the pharmacogenetic arm compared 19.9% for TAU and 15.3% for remission compared to 10.1% for TAU (Greden et al., 2019). The authors concluded that pharmacogenetic testing did not improve results, but significantly improved response and remission rates for "difficult-to-treat depression patients over standard of care" (Greden et al., 2019).

Kekic et al. (2020) studied genetic variants that commonly affect supportive care medications, which include, antidepressants, antiemetics, and analgesics, used in oncology practice. A total of 196 cancer patients were genotyped using a multi-gene panel, OneOme RightMed. The panel assessed 27 genes, including *CYP2C9, CYP2C19, CYP2D6, CYP3A4, COMT, OPRM1, GRIK4, HTR2A, SLC6A4,* associated with pain medications, antidepressants, and antiemetics. Of the 196 patients, 19.9% had prostate cancer, 17.9% had colorectal cancer, 14.8% had melanoma, and 47.4% had other cancer types. All 196 patients had at least one actionable polymorphism related to these supportive care medications, specifically, in *CYP2C19* and *CYP2D6*. Specifically, 67.3% of the patients had other than normal *CYP2D6* metabolizer phenotype and 57.1% had other than normal *CYP2C19* metabolizer phenotype. Based on the results, 37 patients were recommended an alternative analgesic, nine were recommended an alternative antiemetic, and 51 were recommended an alternative anti-depressant (Kekic et al., 2020).

Plumpton et al. (2019) evaluated the cost-effectiveness of panel tests with various pharmacogenes. The constructed multigene panel included *HLA-A*31:01, HLA-B*15:02, HLA-B*57:01, HLA-B*58:01, HLA-B (158T), and HLA-DQB1 (126Q)*, which are involved with various treatments (abacavir, carbamazepine, et al). The constructed multigene panel was found to provide a cost savings of $491 if all findings for all alleles were acted on, regardless of an allele's individual cost-effectiveness. Testing for patients eligible for abacavir (*HLA-B*57:01*) and clozapine (*HLA-B (158T*) and *HLA-DQB1 (126Q*)) was found to be cost-effective. However, testing for patients eligible for allopurinol (*HLA-B*58:01*) was not found to be cost-effective. Furthermore, testing for *HLA-A*31:01* for carbamazepine was found to be cost-effective, but not testing for *HLA-B*15:02* (Plumpton et al., 2019).

Braten et al. (2020) researched the impact of *CYP2C19* genotyping on the antidepressant drug sertraline, which is metabolized by the polymorphic *CYP2C19* enzyme. A total of 1202 patients participated and submitted 2190 sertraline serum samples. All patients were categorized based on *CYP2C19* genotype-predicted phenotype subgroups; these groups include normal (NM), ultra-rapid (UM),

intermediate (IM), and poor metabolizer (PM). Serum samples showed that *CYP2C19* IM and PM patients had significantly higher sertraline concentrations compared to NMs; "Based on the relative differences in serum concentrations compared to NMs, dose reductions of 60% and 25% should be considered in PMs and IMs, respectively, to reduce the risk of sertraline overexposure in these patients" (Braten et al., 2020).

Roscizewski et al. (2021) conducted a retrospective observational study to determine what effect pharmacogenomic testing had on "treatment decisions in patients with depressive symptoms in an interprofessional primary care setting." From April 2019 to March 2021, they identified 78 patients who underwent pharmacogenomic testing for psychotropic medications. They found that 53.8% of patients "experienced a change to their antidepressant regiment after [pharmacogenomic] testing," with the most cited change being addition of another antidepressant, followed by switching the antidepressant, then increased dose. This demonstrated how pharmacogenomic testing could be useful in informing clinical decision making at the beginning of treatment or "in those who experience an inadequate response to their prescribed regimen" and ensuring optimal patient recovery.

Stevenson et al. (2021) aimed to assess the potential impact of multigene pharmacogenomic testing among those hospitalized with COVID-19 in the United States. Through a cross-sectional analysis with electronic health records, researchers "characterized medication orders, focusing on medications with actionable guidance related to 14 commonly assayed genes (*CYP2C19, CYP2C9, CYP2D6, CYP3A5, DPYD, G6PD, HLA-A, HLA-B, IFNL3, NUDT15, SLCO1B1, TPMT, UGT1A1, and VKORC1*)." From their cohort, they found that 64 unique medications with pharmacogenomic guidance were ordered at least once, and about 89.7% of patients "had at least one order for a medication with PGx guidance and… (23.1%) had orders for 4 or more actionable medications." Through a simulation analysis, they estimated that "17 treatment modifications per 100 patients would be enabled if [pharmacogenomic] results were available," and that the genes *CYP2D6* and *CYP2C19* were responsible for most of the treatment modifications. Medications most affected included ondansetron, oxycodone, and clopidogrel. With additional investigations that support these findings, pharmacogenomic testing would better inform the curation of individualized treatment plans for patients suffering from severe COVID-19.

Galli et al. (2021) studied the use of guided selection of antiplatelet therapy for patients undergoing percutaneous coronary intervention. The authors conducted a meta-analysis that included 3656 relevant articles with 20743 patients. Overall, "guided selection of antiplatelet therapy was associated with a reduction in major adverse cardiovascular events and reduced bleeding, although not statistically significant." Additionally, cardiovascular death, myocardial infraction, stent thrombosis, and minor

bleeding were all reduced with guided therapy compared to standard therapy, but the risks of all-cause death and major bleeding did not differ. The authors concluded that "guided selection of antiplatelet therapy improved both composite and individual efficacy outcomes with a favourable safety profile, driven by a reduction in minor bleeding, supporting the use of platelet function or genetic testing to optimise the choice of agent in patients undergoing PCI" (Galli et al., 2021).

Oslin et al. (2022) conducted a randomized clinical trial that compared treatment guided by pharmacogenomic testing vs. usual care "to determine whether pharmacogenomic testing affects antidepressant medication selection and whether such testing leads to better clinical outcomes". Participants of this clinical trial included 676 clinicians and 1944 patients. Criteria for patient enrollment were those with major depressive disorder who were initiating or switching treatment with a single antidepressant and exclusion included those who have active substance use disorder, mania, psychosis, or concurrent treatment with a specified list of medications. Results of this study determined "remission rates over 24 weeks were higher among patients whose care was guided by pharmacogenomic testing than those in usual care (OR, 1.28 [95% CI, 1.05 to 1.57]; P = .02; risk difference, 2.8% [95% CI, 0.6% to 5.1%]) but were not significantly higher at week 24 when 130 patients in the pharmacogenomic-guided group and 126 patients in the usual care group were in remission (estimated risk difference, 1.5% [95%CI, -2.4% to 5.3%]; P = .45)". In conclusion, in provision of pharmacogenomic testing for drug-gene interaction amongst patients with major depressive disorder, pharmacogenomic testing "reduced prescription of medications with predicted drug-gene interactions compared to usual care. Provision test results had small nonpersistent effects on symptom remission" (Oslin et al., 2022).

Ghanbarian et al. (2023) studied the cost-effectiveness of pharmacogenetic testing used to guide prescription of antidepressants. The authors looked at data from patients with major depressive disorder in British Columbia, Canada. The data included unique patient characteristics, including metabolizer phenotypes, incremental costs, life-years, and quality-adjusted life-years. "Pharmacogenomic-guided care was associated with 37% fewer patients with refractory depression over 20 years." The costs of pharmacogenetic testing were estimated to be offset within about two years of use, with an overall saving of 956 million Canadian dollars (4926 Canadian dollars per patient) (Ghanbarian et al., 2023).

The 2023 PREPARE (preemptive pharmacogenomic testing for preventing adverse drug reactions) trial investigated the effects of pre-emptive genotyping using a pharmacogenetic panel on adverse drug reactions. Swen et al. (2023) conducted an "open-label, multicentre, controlled, cluster-randomised, crossover implementation

study of a 12-gene pharmacogenetic panel in 18 hospitals, nine community health centres, and 28 community pharmacies in seven European countries." A total of 6944 patients receiving their first prescription for a clinically recommended drug were included in the study. The participants were divided into a study group, which received genotyping and recommended treatment adjustments, and a control group, which received standard care. The primary outcome measured was the occurrence of clinically relevant adverse drug reactions within 12-weeks. A clinically relevant adverse drug reactions occurred in 21.5% of patients in the study group (N=2923), and 28.6% of patients in the control group (N=3270). The authors concluded that "genotype-guided treatment using a 12-gene pharmacogenetic panel significantly reduced the incidence of clinically relevant adverse drug reactions and was feasible across diverse European health-care system organisations and settings" (Swen et al., 2023)

VI. Guidelines and Recommendations

Clinical Pharmacogenetics Implementation Consortium (CPIC)

CPIC guidelines provide guidance to physicians on how to use genetic testing to help them to optimize drug therapy. The guidelines and projects were endorsed by several professional societies including The Association for Molecular Pathology (AMP), The American Society for Clinical Pharmacology and Therapeutics (ASCPT) and The American Society of Health-System Pharmacists (ASHP) (CPIC, 2023b).

In their list of guidelines, CPIC provides specific therapeutic recommendations for drugs metabolized by Cytochrome P450 enzymes and other important metabolic enzymes.

CYP2C9 Genotypes

Drug	***CYP2C9*/ Phenotype**	**Summary of CPIC Therapeutic Recommendations**	**Level of Recommendations**	**Reference**
Phenytoin/ fosphenytoin based on *HLA-B*15:02*	HLA-B*15:02 Positive- Normal Metabolizer (NM), Intermediate Metabolizer	If patient is phenytoin-naïve, do not use phenytoin/fosphenytoin. Avoid carbamazepine and oxcarbazepine. If the patient has previously used phenytoin continuously for longer than three months	Strong	(Caudle et al., 2014; Karnes et al., 2021)

	(EM), and Poor Metabolism (M)	without incidence of cutaneous adverse reactions, cautiously consider use of phenytoin in the future.		
	HLA-B*15:02 Negative-Normal Metabolizer (NM)	No adjustments needed from typical dosing strategies. Subsequent doses should be adjusted according to therapeutic drug monitoring, response, and side effects. An HLA-B*15:02 negative test does not eliminate the risk of phenytoin-induced SJS/TEN, and patients should be carefully monitored according to standard practice.	Strong	
	HLA-B*15:02 Negative-Intermediate Metabolizer (IM)	No adjustments needed from typical dosing strategies. Subsequent doses should be adjusted according to therapeutic drug monitoring, response and side effects. An HLA-B*15:02 negative test does not eliminate the risk of phenytoin-induced SJS/TEN, and patients should be carefully monitored according to standard practice. For first dose, use typical initial or loading dose. For subsequent doses, use approximately 25% less than typical maintenance dose. Subsequent doses should be adjusted according to therapeutic drug monitoring, response and side effects.	Moderate	

	HLA-B*15:02 Negative-Poor Metabolizer (PM)	For first dose, use typical initial or loading dose. For subsequent doses use approximately 50% less than typical maintenance dose. Subsequent doses should be adjusted according to therapeutic drug monitoring, response, and side effects. An HLA-B*15:02 negative test does not eliminate the risk of phenytoin-induced SJS/TEN, and patients should be carefully monitored according to standard practice.	Strong	
Warfarin	Various phenotypes	Genotype-guided warfarin dosing is very complex and involves a combination of *CYP2C9*, *VKORC1*, *CYP4F2* and rs12777823 as well as an algorithm including ancestry information.	Multiple	(Johnson et al., 2017)
Celecoxib, flurbiprofen, ibuprofen, lornoxicam	NM	"In accordance with the prescribing information, use the lowest effective dosage for shortest duration consistent with individual patient treatment goals"	Strong	(Theken et al., 2020)
	IM (Activity Score [AS] = 1.5)	"Initiate therapy with recommended starting dose. In accordance with the prescribing information, use the lowest effective dosage for shortest duration consistent with individual patient treatment goals."	Moderate	
	IM (AS = 1)	"Initiate therapy with lowest	Moderate	

		recommended starting dose. Titrate dose upward to clinical effect or maximum recommended dose with caution. In accordance with the prescribing information, use the lowest effective dosage for shortest duration consistent with individual patient treatment goals. Carefully monitor adverse events, such as blood pressure and kidney function during course of therapy."		
	PM	"Initiate therapy with 25–50% of the lowest recommended starting dose. Titrate dose upward to clinical effect or 25–50% of the maximum recommended dose with caution. In accordance with the prescribing information, use the lowest effective dosage for shortest duration consistent with individual patient treatment goals. Upward dose titration should not occur until after steady-state is reached (at least 8 days for celecoxib and 5 days for ibuprofen, flurbiprofen, and lornoxicam after first dose in PMs). Carefully monitor adverse events such as blood pressure and kidney function during	Moderate	

		course of therapy. Alternatively, consider an alternate therapy not metabolized by CYP2C9 or not significantly impacted by CYP2C9 genetic variants in vivo"		
Meloxicam	NM	"Initiate therapy with recommended starting dose. In accordance with the prescribing information, use the lowest effective dosage for shortest duration consistent with individual patient treatment goals"	Strong	(Theken et al., 2020)
	IM, AS 1.5	See NM	Moderate	
	IM, AS 1	"Initiate therapy with recommended starting dose. In accordance with the prescribing information, use the lowest effective dosage for shortest duration consistent with individual patient treatment goals. Upward dose titration should not occur until after steady-state is reached (at least 7 days). Carefully monitor adverse events, such as blood pressure and kidney function during course of therapy. Alternatively, consider alternative therapy. Choose an alternative therapy not metabolized by CYP2C9 or not significantly impacted by	Moderate	

		CYP2C9 genetic variants in vivo or choose an NSAID metabolized by CYP2C9 but with a shorter half-life"		
	PM	"Choose an alternative therapy not metabolized by CYP2C9 or not significantly impacted by CYP2C9 genetic variants in vivo or choose an NSAID metabolized by CYP2C9 but with a shorter half-life"	Moderate	
Piroxicam/ Tenoxicam	NM	"Initiate therapy with recommended starting dose. In accordance with the prescribing information, use the lowest effective dosage for shortest duration consistent with individual patient treatment goals."	Strong	(Theken et al., 2020)
	IM AS 1.5	"Initiate therapy with recommended starting dose. In accordance with the prescribing information, use the lowest effective dosage for shortest duration consistent with individual patient treatment goals."	Moderate	
	IM AS 1	"Choose an alternative therapy not metabolized by CYP2C9 or not significantly impacted by CYP2C9 genetic variants in vivo or choose an NSAID metabolized by CYP2C9 but with a shorter half-life"	Moderate (Optional for Tenoxicam)	

	PM	"Choose an alternative therapy not metabolized by CYP2C9 or not significantly impacted by CYP2C9 genetic variants in vivo or choose an NSAID metabolized by CYP2C9 but with a shorter half-life"	Moderate (Optional for Tenoxicam)	

***CYP2D6* Genotype**

Drug	***CYP2D6* Phenotype**	**Summary of CPIC Therapeutic Recommendations**	**Level of Recommendations**	**Reference**
Amitriptyline and Nortripylin e Other TCAs (tricyclic antidepressants): clomipramine, desipramine, doxepin, imipramine, and trimipramine	Ultra-rapid Metabolizer (URM)	Avoid tricyclic use due to potential lack of efficacy. Consider alternative drug not metabolized by *CYP2D6*. If a TCA is warranted, consider titrating to a higher target dose (compared to normal metabolizers). Utilize therapeutic drug monitoring to guide dose adjustments.	Strong (recommendation for other TCAs is Optional)	(Hicks et al., 2016)
	Normal Metabolizer (NM)	Initiate therapy with recommended starting dose.	Strong (recommendation for other TCAs is Strong)	
	IM	Consider a 25% reduction of recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments.	Moderate (recommendation for other TCAs is Optional)	
	PM	Avoid tricyclic use due to potential for side effects.	Strong	

Drug	*CYP2D6* Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
		Consider alternative drug not metabolized by *CYP2D6*. If a TCA is warranted, consider a 50% reduction of recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments.	(recommendation for other TCAs is Optional)	
Codeine	URM	Avoid codeine use due to potential for toxicity.	Strong	(Crews et al., 2021)
	EM	Use label-recommended age or weight-specific dosing.	Strong	
	IM	Use label-recommended age or weight-specific dosing. If no response, consider alternative analgesics such as morphine or a nonopioid.	Moderate	
	PM	Avoid codeine use because of possibility of diminished analgesia	Strong	
Paroxetine	URM	Select alternative drug not predominantly metabolized by *CYP2D6*	Moderate	(Bousman et al., 2023)
	M	Initiate therapy with recommended starting dose.	Strong	
	IM	Consider a lower starting dose and slower titration schedule as compared with normal metabolizers	Optional	
	PM	Consider a 50% reduction in recommended starting dose, slower titration schedule, and	Moderate	

Drug	*CYP2D6* Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
		a 50% lower maintenance dose as compared with normal metabolizers.		
Fluvoxamine	URM	No recommendation due to lack of evidence.	No recommendation	(Bousman et al., 2023)
	EM	Initiate therapy with recommended starting dose.	Strong	
	IM	Initiate therapy with recommended starting dose.	Moderate	
	PM	Consider a 25–50% lower starting dose and slower titration schedule as compared with normal metabolizers or consider a clinically appropriate alternative antidepressant not predominantly metabolized by *CYP2D6*	Optional	
Ondansetron and Tropisetron	URM	Select alternative drug not predominantly metabolized by *CYP2D6* (i.e., granisetron).	Moderate	(Bell et al., 2016)
	NM	Initiate therapy with recommended starting dose.	Strong	
	IM	Insufficient evidence demonstrating clinical impact based on *CYP2D6* genotype. Initiate therapy with recommended starting dose.	No recommendation	
	PM	Insufficient evidence demonstrating clinical impact based on *CYP2D6* genotype.	No recommendation	

Drug	*CYP2D6* Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
		Initiate therapy with recommended starting dose.		
Tamoxifen	URM	Avoid moderate and strong *CYP2D6* inhibitors. Initiate therapy with recommended standard of care dosing (tamoxifen 20 mg/day).	Strong	(Goetz et al., 2018)
	NM	Avoid moderate and strong *CYP2D6* inhibitors. Initiate therapy with recommended standard of care dosing (tamoxifen 20 mg/day).	Strong	
	NM/IM	Consider hormonal therapy such as an aromatase inhibitor for postmenopausal [individuals] or aromatase inhibitor along with ovarian function suppression in premenopausal [individuals], given that these approaches are superior to tamoxifen regardless of *CYP2D6* genotype. If aromatase inhibitor use is contraindicated, consideration should be given to use a higher but FDA approved tamoxifen dose (40 mg/day).45 Avoid *CYP2D6* strong to weak inhibitors.	Optional (Controversy remains)	
	IM	Consider hormonal therapy such as an aromatase inhibitor for postmenopausal	Moderate	

Drug	*CYP2D6* Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
		[individuals] or aromatase inhibitor along with ovarian function suppression in premenopausal [individuals], given that these approaches are superior to tamoxifen regardless of *CYP2D6* genotype. If aromatase inhibitor use is contraindicated, consideration should be given to use a higher but FDA approved tamoxifen dose (40 mg/day). Avoid *CYP2D6* strong to weak inhibitors.		
	PM	Recommend alternative hormonal therapy such as an aromatase inhibitor for postmenopausal [individuals] or aromatase inhibitor along with ovarian function suppression in premenopausal [individuals] given that these approaches are superior to tamoxifen regardless of *CYP2D6* genotype and based on knowledge that *CYP2D6* poor metabolizers switched from tamoxifen to anastrozole do not have an increased risk of recurrence. Note, higher dose tamoxifen (40 mg/day)	Strong	

Drug	*CYP2D6* Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
		increases but does not normalize endoxifen concentrations and can be considered if there are contraindications to aromatase inhibitor therapy.		
Atomoxetine (for children)	URM	Initiate with a dose of 0.5 mg/kg/day and increase to 1.2 mg/kg/day after 3 days. If no clinical response and in the absence of adverse events after 2 weeks, consider obtaining a peak plasma concentration (1–2 hours after dose administered). If < 200 ng/mL, consider a proportional increase in dose to approach 400 ng/mL	Moderate	(Brown et al., 2019)
	NM	Initiate with a dose of 0.5 mg/kg and increase to 1.2 mg/kg/day after 3 days. If no clinical response and in the absence of adverse events after 2 weeks, consider obtaining a peak plasma concentration (1–2 hours after dose administered). If < 200 ng/mL, consider a proportional increase in dose to approach 400 ng/mL.	Moderate	
	IM	Initiate with a dose of 0.5 mg/kg/day and if no clinical response and in the absence	Moderate	

Drug	*CYP2D6* Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
		of adverse events after 2 weeks, consider obtaining a plasma concentration 2–4 hours after dosing. If response is inadequate and concentration is < 200 ng/mL, consider a proportional dose increase to achieve a concentration to approach 400 ng/mL.b,c If unacceptable side effects are present at any time, consider a reduction in dose		
	PM	Initiate with a dose of 0.5 mg/kg/day and if no clinical response and in the absence of adverse events after 2 weeks, consider obtaining a plasma concentration 4 hours after dosing. If response is inadequate and concentration is < 200 ng/mL, consider a proportional dose increase to achieve a concentration to approach 400 ng/mL.b,c If unacceptable side effects are present at any time, consider a reduction in dose	Strong	
Atomoxetine (for adults)	URM	Initiate with a dose of 40 mg/day and increase to 80 mg/ day after 3 days. If no clinical response and in the absence of adverse events	Moderate	

Drug	*CYP2D6* Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
		after 2 weeks, consider increasing dose to 100 mg/day. If no clinical response observed after 2 weeks, consider obtaining a peak plasma concentration (1–2 hours after dose administered). If < 200 ng/mL, consider a proportional increase in dose to approach 400 ng/mL.b,c Dosages > 100 mg/day may be needed to achieve target concentrations.		
	NM	Initiate with a dose of 40 mg/day and increase to 80 mg/ day after 3 days. If no clinical response and in the absence of adverse events after 2 weeks, consider increasing dose to 100 mg/day. If no clinical response observed after 2 weeks, consider obtaining a peak plasma concentration (1–2 hours after dose administered). If < 200 ng/mL, consider a proportional increase in dose to approach 400 ng/mL.b,c Dosages > 100 mg/day may be needed to achieve target concentrations.	Moderate	
	IM	Initiate with a dose of 40 mg/day and if no clinical	Moderate	

Drug	*CYP2D6* Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
		response and in the absence of adverse events after 2 weeks increase dose to 80 mg/day. If response is inadequate after 2 weeks consider obtaining a plasma concentration 2–4 hours after dosing. If concentration is < 200 ng/mL, consider a proportional dose increase to achieve a concentration to approach 400 ng/mL.b,c If unacceptable side effects are present at any time, consider a reduction in dose.		
	PM	Initiate with a dose of 40 mg/day and if no clinical response and in the absence of adverse events after 2 weeks increase dose to 80 mg/day. If response is inadequate after 2 weeks, consider obtaining a plasma concentration 2–4 hours after dosing. If concentration is < 200 ng/mL, consider a proportional dose increase to achieve a concentration to approach 400 ng/mL.b,c If unacceptable side effects are present at any time, consider a reduction in dose	Moderate	

CYP2B6 Genotypes

Drug	CYP2B6 Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
Efavirenz (for children >40 kg and adults)	URM	Initiate efavirenz with standard dosing (600 mg/day)	Strong	(Desta et al., 2019)
	Rapid Metabolizer (RM)	Initiate efavirenz with standard dosing (600 mg/day)	Strong	
	NM	Initiate efavirenz with standard dosing (600 mg/day)	Strong	
	IM	Consider initiating efavirenz with decreased dose of 400 mg/day	Moderate	
	PM	Consider initiating efavirenz with decreased dose of 400 or 200 mg/ day.	Moderate	

CYP2C19 Genotype

Drug	Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations for amitriptyline	Reference
Amitriptyline and Nortripyline Other TCAs: clomipramine, doxepin,	URM, RM	Avoid tertiary amine use due to potential for sub-optimal response. Consider alternative drug not metabolized by *CYP2C19*. TCAs without major *CYP2C19* metabolism include the secondary amines nortriptyline and desipramine. If a tertiary amine is warranted,	Optional (Recommendation for other TCAs is Optional)	(Hicks et al., 2016)

Drug	Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations for amitriptyline	Reference
imipramine, and trimipramine		utilize therapeutic drug monitoring to guide dose adjustments.		
	NM	Initiate therapy with recommended starting dose.	Strong (Recommendation for other TCAs is Strong)	
	IM	Initiate therapy with recommended starting dose.	Strong (Recommendation for other TCAs is Optional)	
	PM	Avoid tertiary amine use due to potential for sub-optimal response. Consider alternative drug not metabolized by *CYP2C19*. TCAs without major *CYP2C19* metabolism include the secondary amines nortriptyline and desipramine. For tertiary amines, consider a 50% reduction of the recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments.	Moderate (Recommendation for other TCAs is Optional)	
Citalopram and Escitalopram	URM	Consider a clinically appropriate alternative antidepressant not predominantly metabolized by	Strong	(Bousman et al., 2023)

Drug	Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations for amitriptyline	Reference
		CYP2C19. If citalopram or escitalopram are clinically appropriate, and adequate efficacy is not achieved at standard maintenance dosing, consider titrating to a higher maintenance dose		
	EM	Initiate therapy with recommended starting dose.	Strong	
	IM	Initiate therapy with recommended starting dose. Consider a slower titration schedule and lower maintenance dose than normal metabolizers	Moderate	
	PM	Consider a clinically appropriate antidepressant not predominantly metabolized by CYP2C19. If citalopram or escitalopram are clinically appropriate, consider a lower starting dose, slower titration schedule, and 50% reduction of the standard maintenance dose as compared with normal metabolizers	Strong	
Sertraline	URM	Initiate therapy with recommended starting dose.	Strong	(Bousman et al., 2023)
	EM	Initiate therapy with recommended starting dose.	Strong	

Drug	Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations for amitriptyline	Reference
	IM	Initiate therapy with recommended starting dose. Consider a slower titration schedule and lower maintenance dose than CYP2C19 normal metabolizers	Moderate	
	PM	Consider a lower starting dose, slower titration schedule, and 50% reduction of standard maintenance dose as compared with CYP2C19 normal metabolizers or select a clinically appropriate alternative antidepressant not predominantly metabolized by CYP2C19	Moderate	
Clopidogrel	URM, RM, NM	If considering clopidogrel, use at standard dose (75 mg/day)	Strong	(Lee et al., 2022)
	IM, Likely IM	Avoid standard dose clopidogrel (75mg) if possible. Use prasugrel or ticagrelor at standard dose if no contraindication.	Strong	
	PM, Likely PM	Avoid clopidogrel if possible. Use prasugrel or ticagrelor at standard dose if no contraindication.	Strong	
Voriconazole	URM	Choose an alternative agent that is not dependent on *CYP2C19* metabolism as	Moderate	

Drug	Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations for amitriptyline	Reference
		primary therapy in lieu of voriconazole. Such agents include isavuconazole, liposomal amphotericin B, and posaconazole.		(Moriyama et al., 2017)
	RM	Choose an alternative agent that is not dependent on *CYP2C19* metabolism as primary therapy in lieu of voriconazole. Such agents include isavuconazole, liposomal amphotericin B, and posaconazole.	Moderate	
	NM	Initiate therapy with recommended starting dose.	Strong	
	IM	Initiate therapy with recommended starting dose.	Moderate	
	PM	Choose an alternative agent that is not dependent on *CYP2C19* metabolism as primary therapy in lieu of voriconazole. Such agents include isavuconazole, liposomal amphotericin B, and posaconazole. In the event that voriconazole is considered to be the most appropriate agent, based on clinical advice, for a patient with poor metabolizer genotype, voriconazole should be	Moderate	

Drug	Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations for amitriptyline	Reference
		administered at a preferably lower than standard dosage with careful therapeutic drug monitoring.		
Proton Pump Inhibitors (omeprazole, lansoprazole, and pantoprazole) All recommendations here are "Optional" for dexlansoprazole	URM	"Increase starting daily dose by 100%. Daily dose may be given in divided doses. Monitor for efficacy"	Optional	(Lima et al., 2020)
	RM	"Initiate standard starting daily dose. Consider increasing dose by 50–100% for the treatment of Helicobacter pylori infection and erosive esophagitis. Daily dose may be given in divided doses. Monitor for efficacy"	Moderate	
	Normal	"Initiate standard starting daily dose. Consider increasing dose by 50–100% for the treatment of H. pylori infection and erosive esophagitis. Daily dose may be given in divided doses. Monitor for efficacy"	Moderate	
	Likely IM/IM	"Initiate standard starting daily dose. For chronic therapy (> 12 weeks) and efficacy achieved, consider 50% reduction in daily dose and monitor for continued efficacy"	Optional	
	Likely PM/PM	"Initiate standard starting daily dose. For chronic therapy	Moderate	

Drug	Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations for amitriptyline	Reference
		(> 12 weeks) and efficacy achieved, consider 50% reduction in daily dose and monitor for continued efficacy"		

***CYP2D6* and *CYP2C19* Genotypes (Caudle et al., 2020; Hicks et al., 2016) for Amitriptyline, Clomipramine, Doxepin, Imipramine, and Trimipramine**

Phenotype	***CYP2D6***	***CYP2D6***	***CYP2D6***	***CYP2D6***
CYP2C19	**UM**	**NM**	**IM**	**PM**
URM	Avoid amitriptyline use Recommendation: Optional	Consider alternative drug not metabolized by *CYP2C19*. Recommendation: Optional	Consider alternative drug not metabolized by *CYP2C19*. Recommendation: Optional	Avoid amitriptyline use Recommendation: Optional
NM	Avoid amitriptyline use. If amitriptyline is warranted, consider titrating to a higher target dose (compared to normal metabolizers) Recommendation: Strong	Initiate therapy with recommended starting dose. Recommendation: Strong	Consider a 25% reduction of recommended starting dose. Recommendation: Moderate	Avoid amitriptyline use. If Amitriptyline is warranted, consider a 50% reduction of recommended starting dose. Recommendation: Strong

IM	Avoid amitriptyline use Recommendation: Optional	Initiate therapy with recommended starting dose. Recommendation: Strong	Consider a 25% reduction of recommended starting dose. Recommendation: Optional This recommendation may also be considered for diplotypes with an activity score of 1.	Avoid amitriptyline use. If Amitriptyline is warranted, consider a 50% reduction of recommended starting dose. Recommendation: Optional
PM	Avoid amitriptyline use Recommendation: Optional	Avoid amitriptyline use. If Amitriptyline is warranted, consider a 50% reduction of recommended starting dose. Recommendation: Moderate	Avoid amitriptyline use Recommendation: Optional	Avoid amitriptyline use Recommendation: Optional

***TPMT* Genotype**

Drug	***TPMT* Phenotype**	**Summary of CPIC Therapeutic Recommendations**	**Level of Recommendations**	**Reference**
Mercaptopurine (MP)	NM	Start with normal starting dose (e.g., 75 mg/m^2/d or 1.5 mg/kg/d) and adjust doses of MP (and of any other myelosuppressive therapy) without any special emphasis on MP compared to other agents.	Strong	(Relling et al., 2018)

		Allow 2 weeks to reach steady state after each dose adjustment. Consider evaluating TPMT erythrocyte activity to assess TPMT phenotype. If thiopurines are required and either TPMT or NUDT15 status is unknown, monitor closely for toxicity.		
	IM	Start with reduced doses (start at 30–70% of full dose: e.g., at 50 mg/m^2/d or 0.75 mg/kg/d) and adjust doses of MP based on degree of myelosuppression and disease-specific guidelines. Allow 2–4 weeks to reach steady state after each dose adjustment. In those who require a dosage reduction based on myelosuppression, the median dose may be ~40% lower (44 mg/m^2) than that tolerated in wild-type patients (75 mg/m^2). In setting of myelosuppression, and depending on other therapy, emphasis should be on reducing MP over other agents. Consider evaluating TPMT erythrocyte activity to assess TPMT phenotype. If thiopurines are required and either TPMT or NUDT15 status is unknown, monitor closely for toxicity.	Strong	
	PM	For malignancy, start with drastically reduced doses (reduce daily dose by 10-fold and reduce frequency to thrice weekly	Strong	

		instead of daily, e.g., 10 mg/m^2/d given just 3 days/week) and adjust doses of MP based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady state after each dose adjustment. In setting of myelosuppression, emphasis should be on reducing MP over other agents. For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy. Consider evaluating TPMT erythrocyte activity to assess TPMT phenotype. If thiopurines are required and either TPMT or NUDT15 status is unknown, monitor closely for toxicity.		
Azathioprine	NM	Start with normal starting dose (e.g., 2–3 mg/kg/d) and adjust doses of azathioprine based on disease-specific guidelines. Allow 2 weeks to reach steady state after each dose adjustment. Consider evaluating erythrocyte TPMT activity to assess TPMT phenotype. If thiopurines are required and TPMT status is unknown, monitor closely for toxicity.	Strong	(Relling et al., 2018)
	IM	Start with reduced starting doses (30%-80% of normal dose) if normal starting dose is 2-3 mg/kg/day, (e.g. 0.6 – 2.4 mg/kg/day), and adjust doses of azathioprine based on degree of	Strong	

		myelosuppression and disease-specific guidelines. Allow 2-4 weeks to reach steady-state after each dose adjustment. Consider evaluating erythrocyte TPMT activity to assess TPMT phenotype. If thiopurines are required and TPMT status is unknown, monitor closely for toxicity.		
	PM	For non-malignant conditions, consider alternative-nonthiopurine immunosuppressant therapy or malignancy, start with drastically reduced doses (reduce daily dose by 10-fold and dose thrice weekly instead of daily) and adjust doses of azathioprine based on degree of myelosuppression and disease specific guidelines. Allow 4-6 weeks to reach steady state after each dose adjustment. Consider evaluating erythrocyte TPMT activity to assess TPMT phenotype. If thiopurines are required and TPMT status is unknown, monitor closely for toxicity.	Strong	
Thioguanine	NM	Start with normal starting dose (e.g. 40-60 mg/m2 /day). Adjust doses of thioguanine (TG) and of other myelosuppressive therapy without any special emphasis on TG. Allow 2 weeks to reach steady state after each dose adjustment. Consider evaluating erythrocyte TPMT activity to	Strong	(Relling et al., 2013; Relling et al., 2018)

		assess TPMT phenotype. If thiopurines are required and TPMT status is unknown, monitor closely for toxicity.		
	IM	Start with reduced doses (50% to 80% of normal dose) if normal starting dose is ≥40-60 mg/m2 /day (e.g. 20-48 mg/m2 /day) and adjust doses of TG based on degree of myelosuppression and disease-specific guidelines. Allow 2–4 weeks to reach steady state after each dose adjustment. In setting of myelosuppression, and depending on other therapy, emphasis should be on reducing TG over other agents. Consider evaluating erythrocyte TPMT activity to assess TPMT phenotype. If thiopurines are required and TPMT status is unknown, monitor closely for toxicity.	Moderate	
	PM	Start with drastically reduced doses (reduce daily dose by 10-fold and dose thrice weekly instead of daily) and adjust doses of TG based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady state after each dose adjustment. In setting of myelosuppression, emphasis should be on reducing TG over other agents. For nonmalignant conditions, consider alternative	Strong	

		nonthiopurine immunosuppressant therapy. Consider evaluating erythrocyte TPMT activity to assess TPMT phenotype. If thiopurines are required and TPMT status is unknown, monitor closely for toxicity.		

***NUDT15* Genotype**

Drug	***NUDT15* Phenotype**	**Summary of CPIC Therapeutic Recommendations**	**Level of Recommendations**	**Reference**
Mercaptopurine	NM	Start with normal starting dose (e.g., 75 mg/m^2/d or 1.5 mg/kg/d) and adjust doses of MP (and of any other myelosuppressive therapy) without any special emphasis on MP compared to other agents. Allow 2 weeks to reach steady state after each dose adjustment.	Strong	(Relling et al., 2018)
	IM	Start with reduced doses (start at 30–80% of normal dose: if normal starting dose is ≥75 mg/m2 /day or ≥ 1.5 mg/kg/day (e.g. start at 25-60 mg/m2 /day or 0.45-1.2 mg/kg/day) and adjust doses of MP based on degree of myelosuppression and disease-specific guidelines. Allow 2–4 weeks to reach steady state after each dose adjustment. If myelosuppression occurs, and	Strong	

Drug	*NUDT15* Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
		depending on other therapy, emphasis should be on reducing mercaptopurine over other agents. If normal starting dose is already < 1.5mg/kg/day, dose reduction may not be recommended.		
	PM	For malignancy, initiate dose at 10 mg/m2 /day and adjust dose based on myelosuppression and disease specific guidelines. Allow 4-6 weeks to reach steady state after each dose adjustment. If myelosuppression occurs, emphasis should be on reducing mercaptopurine over other agents. For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy.	Strong	
Azathioprine	NM	Start with normal starting dose (e.g., 2–3 mg/kg/day) and adjust doses of azathioprine based on disease-specific guidelines. Allow 2 weeks to reach steady state after each dose adjustment.	Strong	(Relling et al., 2018)
	IM	Start with reduced doses (start at 30–80% of normal dose: if normal starting dose is 2-3 mg/kg/day, (e.g. 0.6 – 2.4 mg/kg/day) and adjust doses of MP based on degree of	Strong	

Drug	*NUDT15* Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
		myelosuppression and disease-specific guidelines. Allow 2–4 weeks to reach steady state after each dose adjustment.		
	PM	For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy. For malignant conditions, start with drastically reduced normal daily doses (reduce daily dose by 10-fold) and adjust doses of azathioprine based on degree of myelosuppression and disease specific guidelines. Allow 4-6 weeks to reach steady-state after each dose adjustment.	Strong	
Thioguanine	NM	Start with normal starting dose (40- 60 mg/day). Adjust doses of thioguanine and of other myelosuppressive therapy without any special emphasis on thioguanine. Allow 2 weeks to reach steady-state after each dose adjustment	Strong	(Relling et al., 2018)
	IM	Start with reduced doses (50% to 80% of normal dose) if normal starting dose is ≥40-60 mg/m2 /day (e.g. 20-48 mg/m2 /day) and adjust doses of thioguanine based on degree of myelosuppression and disease	Moderate	

Drug	*NUDT15* Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
		specific guidelines. Allow 2-4 weeks to reach steady-state after each dose adjustment. If myelosuppression occurs, and depending on other therapy, emphasis should be on reducing thioguanine over other agents.		
	PM	Reduce doses to 25% of normal dose and adjust doses of thioguanine based on degree of myelosuppression and disease specific guidelines. Allow 4-6 weeks to reach steady-state after each dose adjustment. In setting of myelosuppression, emphasis should be on reducing thioguanine over other agents. For non-malignant conditions, consider alternative nonthiopurine immunosuppressant therapy.	Strong	

***DPYD* Genotypes**

Drug	Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
5-Fluorouracil Capecitabine	NM	Based on genotype, there is no indication to change dose or therapy. Use label recommended dosage and administration.	Strong	(Amstutz et al., 2018)

	IM	Reduce starting dose based on activity score followed by titration of dose based on toxicity or therapeutic drug monitoring (if available). Activity score 1: Reduce dose by 50% Activity score 1.5: Reduce dose by 25% to 50%	Activity score 1: Strong Activity score 1.5: Moderate	
	PM	Activity score 0.5: Avoid use of 5-fluorouracil or 5-fluorouracil prodrug-based regimens. In the event, based on clinical advice, alternative agents are not considered a suitable therapeutic option, 5-fluorouracil should be administered at a strongly reduced dosed with early therapeutic drug monitoring. Activity score 0: Avoid use of 5-fluorouracil or 5-fluorouracil prodrug-based regimens.	Strong	

***HLA-B* Genotypes**

Drug	**Phenotype**	**Summary of CPIC Therapeutic Recommendations**	**Level of Recommendations**	**Reference**
Abacavir	Noncarrier of *HLA-B*57:01*	Low or reduced risk of abacavir hypersensitivity	Strong	(Martin et al., 2014)

Drug	Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
	Carrier of *HLA-B*57:01*	Abacavir is not recommended	Strong	
Allopurinol	Noncarrier of *HLA-B*5801* (*X/*X)	Use allopurinol per standard dosing guidelines	Strong	(Hershfield et al., 2013; Saito et al., 2016)
	Carrier of *HLA-B*5801* (*HLA-B*5801*/*X, b *HLA-B*5801/HLA-B*5801*)	Allopurinol is contraindicated	Strong	
Oxcarbazepine	*HLA-B*15:02* negative	Use oxcarbazepine per standard dosing guidelines	Strong	(Phillips et al., 2018)
	*HLA-B*15:02* positive	If patient is oxcarbazepine naive, do not use oxcarbazepine.	Strong	
Carbamazepine	*HLA-B*15:02* negative and *HLA-A*31:01* negative	Use carbamazepine per standard dosing guidelines.	Strong	(Phillips et al., 2018)
	*HLA-B*15:02* negative and *HLA-*	If patient is carbamazepine-naive and alternative agents are available, do not use carbamazepine.	Strong	

Drug	Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
	*A*31:01* positive			
	*HLA-B*15:02* positive and any *HLA-A*31:01* genotype (or *HLA-A*31:01* genotype unknown)	If patient is carbamazepine-naive, do not use carbamazepine.	Strong	

Additional Genotypes

Drug/Genotype	Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
UGT1A1 for Atazanavir	EM	There is no need to avoid prescribing of atazanavir based on *UGT1A1* genetic test result. Inform the patient that some patients stop atazanavir because of jaundice (yellow eyes and skin), but that this patient's genotype makes this unlikely (less than about a 1 in 20 chance of stopping atazanavir because of jaundice).	Strong	(Gammal et al., 2016)

Drug/Genotype	Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
	IM	There is no need to avoid prescribing of atazanavir based on *UGT1A1* genetic test result. Inform the patient that some patients stop atazanavir because of jaundice (yellow eyes and skin), but that this patient's genotype makes this unlikely (less than about a 1 in 20 chance of stopping atazanavir because of jaundice).	Strong	
	PM	Consider an alternative agent particularly where jaundice would be of concern to the patient. If atazanavir is to be prescribed, there is a high likelihood of developing jaundice that will result in atazanavir discontinuation (at least 20% and as high as 60%).	Strong	
UGT1A1 for Irinotecan	N/A	N/A	A, 1A level of evidence	(CPIC, 2023a)
CFTR for Ivacaftor	Homozygous or heterozygous G551D-*CFTR*—e.g. G551D/ F508del, G551D/G551	Use ivacaftor according to the product label (e.g., 150 mg every 12h for patients aged 6 years and older without other diseases; modify dose in patients with hepatic impairment)	Strong	(Clancy et al., 2014)

Drug/Genotype	Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
	D, rs75527207 genotype AA or AG			
	Noncarrier of G551D-*CFTR*— e.g. F508del/R553X, rs75527207 genotype GG	Ivacaftor is not recommended	Moderate	
	Homozygous for F508del-*CFTR* (F508del/F508del), rs113993960, or rs199826652 genotype del/ del	Ivacaftor is not recommended	Moderate	
G6PD for high-risk drugs (rasburicase and pegloticase)	Normal	No reason to avoid high-risk drugs based on G6PD status	Strong	(Gammal et al., 2023)
	Deficient or deficient with CNSHA	Avoid use of high-risk drugs	Strong	
	Variable	To ascertain G6PD status, enzyme activity must be measured. Drug use should be guided per the	Moderate	

Drug/Genotype	Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
		recommendations based on the activity-based phenotype		
SLCO1B1 for Simvastatin	SLCO1B1 decreased function or SLCO1B1 possible decreased function	Prescribe an alternative statin depending on the desired potency. If simvastatin therapy is warranted, limit dose to <20 mg/day	Strong	(Cooper-DeHoff et al., 2022)
	SLCO1B1 poor function	Prescribe an alternative statin depending on the desired potency	Strong	
CYP3A5 for treatment with Tacrolimus	EM	Increase starting dose 1.5–2 times recommended starting dose. Total starting dose should not exceed 0.3 mg/kg/day. Use therapeutic drug monitoring to guide dose adjustments.	Strong	(Birdwell et al., 2015)
	IM	Increase starting dose 1.5–2 times recommended starting dose. Total starting dose should not exceed 0.3 mg/kg/day. Use therapeutic drug monitoring to guide dose adjustments.	Strong	
	PM	Initiate therapy with standard recommended dose. Use therapeutic drug monitoring to guide dose adjustments.	Strong	

Drug/Genotype	Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
IFNL3 treatment with Peginterferon alfa-2a, Peginterferon alfa-2b or Ribavirin	Favorable response genotype	Approximately 90% chance for SVR after 24–48 weeks of treatment. Approximately 80–90% of patients are eligible for shortened therapy (24–28 weeks vs. 48 weeks). Weighs in favor of using PEG-IFN-α- and RBV-containing regimens.	Strong	(Muir et al., 2014)
	Unfavorable response genotype	Approximately 60% chance of SVR after 24–48 weeks of treatment. Approximately 50% of patients are eligible for shortened therapy regimens (24–28 weeks). Consider implications before initiating PEG-IFN-α- and RBV-containing regimens.	Strong	
RYR1 and *CACNA1S* genotypes for Potent Volatile Anesthetic Agents and Succinylcholine	Malignant Hyperthermia Susceptible	Halogenated volatile anesthetics or depolarizing Halogenated volatile anesthetics or depolarizing muscle relaxants succinylcholine are relatively contraindicated in persons with MHS. They should not be used, except in extraordinary circumstances in which the benefits outweigh the risks. In general, alternative anesthetics are widely	Strong	(Gonsalves et al., 2019)

Drug/Genotype	Phenotype	Summary of CPIC Therapeutic Recommendations	Level of Recommendations	Reference
		available and effective in patients with MHS		
	Uncertain susceptibility	Clinical findings, family history, further genetic testing and other laboratory data should guide use of halogenated volatile anesthetics or depolarizing muscle relaxants.	Strong	

CPIC notes that evidence for *TYMS* testing is unclear or weak and have assigned *TYMS* a "D" level recommendation. CPIC does not recommend any change in prescription based on *TYMS* genotype (CPIC, 2023a).

American College of Medical Genetics and Genomics (ACMG)

ACMG notes that *CYP2C9* and *VKORC1* testing may be useful for assessing unusual responses to warfarin, but cannot recommend for or against routine genotyping (ACMG, 2007).

American College of Cardiology Foundation (AACF) and the American Heart Association (AHA) Joint Guidelines

A report by the ACCF and the AHA on genetic testing for selection and dosing of clopidogrel provided the following recommendations for practice:

- "Clinicians must be aware that genetic variability in CYP enzymes alter clopidogrel metabolism, which in turn can affect its inhibition of platelet function. Diminished responsiveness to clopidogrel has been associated with adverse patient outcomes in registry experiences and clinical trials."
- "The specific impact of the individual genetic polymorphisms on clinical outcome remains to be determined (e.g., the importance of *CYP2C19**2 versus *3 or *4 for a specific patient), and the frequency of genetic variability differs among ethnic groups."
- "Information regarding the predictive value of pharmacogenomic testing is very limited at this time; resolution of this issue is the focus of multiple ongoing studies."

- "The evidence base is insufficient to recommend either routine genetic or platelet function testing at the present time. There is no information that routine testing improves outcome in large subgroups of patients. In addition, the clinical course of the majority of patients treated with clopidogrel without either genetic testing or functional testing is excellent. Clinical judgment is required to assess clinical risk and variability in patients considered to be at increased risk. Genetic testing to determine if a patient is predisposed to poor clopidogrel metabolism ("poor metabolizers") may be considered before starting clopidogrel therapy in patients believed to be at moderate or high risk for poor outcomes. This might include, among others, patients undergoing elective high-risk PCI procedures (e.g., treatment of extensive and/or very complex disease). If such testing identifies a potential poor metabolizer, other therapies, particularly prasugrel for coronary patients, should be considered. (Holmes et al., 2010).

American Academy of Neurology (AAN)

The AAN published a position paper on the use of opioids for chronic non-cancer pain. Regarding pharmacogenetic testing, the guidelines state "genotyping to determine whether response to opioid therapy can/should be more individualized will require critical original research to determine effectiveness and appropriateness of use" (Franklin, 2014).

American Association for Clinical Chemistry (AACC) Academy Laboratory Medicine Practice Guidelines

AACC Academy issued laboratory medicine practice guidelines on using clinical laboratory tests to monitor drug therapy in pain management. Their guidelines have a total of 26 recommendations and seven expert opinions. Regarding pharmacogenetic testing for pain management, they stated in the recommendation #20 (Level A, II) that: "While the current evidence in the literature doesn't support routine genetic testing for all pain management patients, it should be considered to predict or explain variant pharmacokinetics, and/ or pharmacodynamics of specific drugs as evidenced by repeated treatment failures, and/or adverse drug reactions/toxicity" (AACC, 2017).

American Family Physician (AAFP)

The AAFP has published guidelines on pharmacogenetics: using genetic information to guide drug therapy. CPIC guidelines are cited for many medication/allele combinations in this article. The recommendations by the AAFP are listed in the table below taken from Chang et al. (2015):

Allele	Medications	Test Results and Clinical Implications	Comments
CYP2D6	Codeine, hydrocodone, oxycodone, tramadol	Ultrarapid metabolizer: Avoid codeine because of potential for toxicity Poor metabolizer: Avoid codeine and possibly tramadol because of possible lack of effectiveness	CPIC guidance limits genotype-guided dosing recommendations to codeine. Alternative analgesics not affected by *CYP2D6* variability include morphine, oxymorphone, and nonopioid analgesics. Oxycodone may also have reduced effectiveness in poor *CYP2D6* metabolizers.
CYP2C19	Clopidogrel (Plavix)	Intermediate metabolizer: Use alternative antiplatelet therapy if no contraindications Poor metabolizer: Use alternative antiplatelet therapy if no contraindications	Clopidogrel prescribing information states that *CYP2C19* tests can be used as an aid to determine therapeutic strategy in patients with acute coronary syndromes who are undergoing percutaneous coronary intervention. CPIC guidance limits genotype-guided dosing recommendations to patients undergoing percutaneous coronary intervention for acute coronary syndromes (excluding medical management of acute coronary syndromes, stroke, and peripheral artery disease). ACCF/AHA guidelines state that genotyping may be considered in patients with unstable angina/non-ST segment elevation myocardial infarction (or after percutaneous coronary intervention for acute coronary syndromes) if test results could alter management. Alternative antiplatelet therapy not affected by *CYP2C19* variability

Allele	Medications	Test Results and Clinical Implications	Comments
			includes prasugrel (Effient) and ticagrelor (Brilinta).
CYP2C19	Amitriptyline	Poor metabolizer: Consider 50% reduction in recommended starting dose	CPIC guidance is available for *CYP2D6*- and *CYP2C19*-genotype guided tricyclic antidepressant therapy. Although limited data exist for other tricyclic antidepressants, most supporting evidence of clinically relevant gene-drug effects is for amitriptyline and nortriptyline (Pamelor).
CYP2C19	Citalopram (Celexa), escitalopram (Lexapro)	Ultrarapid metabolizer: Consider alternative Poor metabolizer: Consider 50% starting dose reduction and titrate to response, or use alternative	CPIC guidance is available for *CYP2C19*-genotype guided citalopram and escitalopram therapy. FDA label for citalopram states that 20 mg per day is the maximum recommended dosage for patients older than 60 years, patients with hepatic impairment, and *CYP2C19* poor metabolizers or patients taking cimetidine (Tagamet) or another *CYP2C19* inhibitor.
CYP2C19	Sertraline (Zoloft)	Ultrarapid metabolizer: If patient does not respond to recommended dose, consider alternative Poor metabolizer: Consider 50% dose reduction or alternative	CPIC guidance is available for *CYP2C19*-genotype guided sertraline therapy.
CYP2D6	Amitriptyline, nortriptyline	Ultrarapid metabolizer: Avoid	CPIC guidance is available for CYP2D6- and CYP2C19-genotype

Allele	Medications	Test Results and Clinical Implications	Comments
		because of possible lack of effectiveness Poor metabolizer: Avoid because of possible adverse effects; if use is warranted, consider 50% reduction in recommended starting dose	guided tricyclic antidepressant therapy. Although limited data exist for other tricyclic antidepressants, most supporting evidence of clinically relevant gene-drug effects is for amitriptyline and nortriptyline.
CYP2D6	Aripiprazole (Abilify)	Poor metabolizer: Decrease dose	Quality of supporting evidence is classified as low by PharmGKB FDA label for aripiprazole states that in poor metabolizers, the usual dose should initially be reduced to 50% and then adjusted to achieve a favorable clinical response; in poor metabolizers receiving a strong CYP3A4 inhibitor, the usual dose should be reduced to 25%.
CYP2D6	Atomoxetine (Strattera)	Poor metabolizer: Adjust dose	Quality of supporting evidence is classified as moderate (Level 2a) by PharmGKB. FDA label for atomoxetine states that in poor metabolizers, the initial dosage should be 0.5 mg per kg per day and then increased to the the usual target dosage of 1.2 mg per kg per day only if symptoms do not improve after 4 weeks and the initial dose is well tolerated.
CYP2D6	Paroxetine (Paxil)	Ultrarapid metabolizer: Select alternative because of possible lack of effectiveness.	CPIC guidance is available for CYP2D6-genotype guided paroxetine therapy.

Allele	Medications	Test Results and Clinical Implications	Comments
		Poor metabolizer: Select alternative or if use is warranted, consider 50% starting dose reduction	

Dutch Pharmacogenetics Working Group (DPWG)

The DPWG has published guidelines for the gene-drug interaction of *DPYD* and fluoropyrimidines. Conclusions state that "four variants have sufficient evidence to be implemented into clinical care: DPYD*2A (c.1905+1G>A, IVS14+1G>A), DPYD*13 (c.1679T>G), c.2846A>T and c.1236G>A (in linkage disequilibrium with c.1129–5923C>G). The current guideline only reports recommendations for these four variants; no recommendations are provided for other variants in *DPYD* or other genes" (Lunenburg et al., 2020).

Food and Drug Administration

The FDA published several tables of pharmacogenetic associations with "sufficient scientific evidence to suggest that subgroups of patients with certain genetic variants, or genetic variant-inferred phenotypes (i.e., affected subgroup in the table below), are likely to have altered drug metabolism, and in certain cases, differential therapeutic effects, including differences in risks of adverse events".

The table below lists associations "for which the data support therapeutic management recommendations" (FDA, 2022).

Drug	Gene	Affected Subgroups	Description of Gene-Drug Interaction
Abacavir	*HLA-B*	*57:01 allele positive	Results in higher adverse reaction risk (hypersensitivity reactions). Do not use abacavir in patients positive for HLA-B*57:01.
Abrocitinib	*CYP2C19*	poor metabolizers	Results in higher systemic concentrations and may result in higher adverse reaction risk. Dosage adjustment is recommended. Refer

Drug	Gene	Affected Subgroups	Description of Gene-Drug Interaction
			to FDA labeling for specific dosing recommendations.
Amifampridine	*NAT2*	poor metabolizers	Results in higher systemic concentrations and higher adverse reaction risk. Use lowest recommended starting dosage and monitor for adverse reactions. Refer to FDA labeling for specific dosing recommendations.
Amifampridine Phosphate	*NAT2*	poor metabolizers	Results in higher systemic concentrations. Use lowest recommended starting dosage (15 mg/day) and monitor for adverse reactions.
Amphetamine	*CYP2D6*	poor metabolizers	May affect systemic concentrations and adverse reaction risk. Consider lower starting dosage or use alternative agent.
Aripiprazole	*CYP2D6*	poor metabolizers	Results in higher systemic concentrations and higher adverse reaction risk. Dosage adjustment is recommended. Refer to FDA labeling for specific dosing recommendations.
Aripiprazole Lauroxil	*CYP2D6*	poor metabolizers	Results in higher systemic concentrations. Dosage adjustment is recommended. Refer to FDA labeling for specific dosing recommendations.
Atomoxetine	*CYP2D6*	poor metabolizers	Results in higher systemic concentrations and higher adverse reaction risk. Adjust titration interval and increase dosage if tolerated. Refer to FDA labeling for specific dosing recommendations.

Drug	Gene	Affected Subgroups	Description of Gene-Drug Interaction
Azathioprine	*TPMT* and/or *NUDT15*	intermediate or poor metabolizers	Alters systemic active metabolite concentration and dosage requirements. Results in higher adverse reaction risk (myelosuppression). Consider alternative therapy in poor metabolizers. Dosage reduction is recommended in intermediate metabolizers for NUDT15 or TPMT. Intermediate metabolizers for both genes may require more substantial dosage reductions. Refer to FDA labeling for specific dosing recommendations.
Belinostat	*UGT1A1*	*28/*28 (poor metabolizers)	May result in higher systemic concentrations and higher adverse reaction risk. Reduce starting dose to 750 mg/m2 in poor metabolizers.
Belzutifan	*CYP2C19 and/or UGT2B17*	Poor metabolizers	Results in higher systemic concentrations and may result in higher adverse reaction risk (anemia, hypoxia). Monitor patients who are poor metabolizers for both genes for adverse reactions.
Brexpiprazole	*CYP2D6*	poor metabolizers	Results in higher systemic concentrations. Dosage adjustment is recommended. Refer to FDA labeling for specific dosing recommendations.
Brivaracetam	*CYP2C19*	intermediate or poor metabolizers	Results in higher systemic concentrations and higher adverse reaction risk. Consider dosage reductions in poor metabolizers.
Capecitabine	*DPYD*	intermediate or poor metabolizers	Results in higher adverse reaction risk (severe, life-threatening, or fatal toxicities). No dosage has proven safe in poor metabolizers, and

Drug	Gene	Affected Subgroups	Description of Gene-Drug Interaction
			insufficient data are available to recommend a dosage in intermediate metabolizers. Withhold or discontinue in the presence of early-onset or unusually severe toxicity.
Carbamazepine	*HLA-B*	*15:02 allele positive	Results in higher adverse reaction risk (severe skin reactions). Avoid use unless potential benefits outweigh risks and consider risks of alternative therapies. Patients positive for HLA-B*15:02 may be at increased risk of severe skin reactions with other drugs that are associated with a risk of Stevens Johnson Syndrome/Toxic Epidermal necrolysis (SJS/TEN). Genotyping is not a substitute for clinical vigilance.
Celecoxib	*CYP2C9*	poor metabolizers	Results in higher systemic concentrations. Reduce starting dose to half of the lowest recommended dose in poor metabolizers. Consider alternative therapy in patients with juvenile rheumatoid arthritis.
Citalopram	*CYP2C19*	poor metabolizers	Results in higher systemic concentrations and adverse reaction risk (QT prolongation). The maximum recommended dose is 20 mg.
Clobazam	*CYP2C19*	intermediate or poor metabolizers	Results in higher systemic active metabolite concentrations. Poor metabolism results in higher adverse reaction risk. Dosage adjustment is recommended. Refer to FDA labeling for specific dosing recommendations.

Drug	Gene	Affected Subgroups	Description of Gene-Drug Interaction
Clopidogrel	*CYP2C19*	intermediate or poor metabolizers	Results in lower systemic active metabolite concentrations, lower antiplatelet response, and may result in higher cardiovascular risk. Consider use of another platelet P2Y12 inhibitor.
Clozapine	*CYP2D6*	poor metabolizers	Results in higher systemic concentrations. Dosage reductions may be necessary.
Codeine	*CYP2D6*	ultrarapid metabolizers	Results in higher systemic active metabolite concentrations and higher adverse reaction risk (life-threatening respiratory depression and death). Codeine is contraindicated in children under 12 years of age.
Deutetrabenazine	*CYP2D6*	poor metabolizers	Results in higher systemic concentrations and adverse reaction risk (QT prolongation). The maximum recommended dosage should not exceed 36 mg (maximum single dose of 18 mg).
Dronabinol	*CYP2C9*	intermediate or poor metabolizers	May result in higher systemic concentrations and higher adverse reaction risk. Monitor for adverse reactions.
Eliglustat	*CYP2D6*	ultrarapid, normal, intermediate, or poor metabolizers	Alters systemic concentrations, effectiveness, and adverse reaction risk (QT prolongation). Indicated for normal, intermediate, and poor metabolizer patients. Ultrarapid metabolizers may not achieve adequate concentrations to achieve a therapeutic effect. The recommended dosages are based on CYP2D6 metabolizer status. Coadministration with strong CYP3A

Drug	Gene	Affected Subgroups	Description of Gene-Drug Interaction
			inhibitors is contraindicated in intermediate and poor CYP2D6 metabolizers. Refer to FDA labeling for specific dosing recommendations.
Erdafitinib	*CYP2C9*	*3/*3 (poor metabolizers)	May result in higher systemic concentrations and higher adverse reaction risk. Monitor for adverse reactions.
Flibanserin	*CYP2C19*	poor metabolizers	May result in higher systemic concentrations and higher adverse reaction risk. Monitor patients for adverse reactions.
Flurbiprofen	*CYP2C9*	poor metabolizers	Results in higher systemic concentrations. Use a reduced dosage.
Fluorouracil	*DPYD*	intermediate or poor metabolizer	Results in higher adverse reaction risk (severe, life-threatening, or fatal toxicities). No dosage has proven safe in poor metabolizers and insufficient data are available to recommend a dosage in intermediate metabolizers. Withhold or discontinue in the presence of early-onset or unusually severe toxicity.
Fosphenytoin	*CYP2C9*	Intermediate or poor metabolizers	May result in higher systemic concentrations and higher adverse reaction risk (central nervous system toxicity). Consider starting at the lower end of the dosage range and monitor serum concentrations. Refer to FDA labeling for specific dosing recommendations. Carriers of CYP2C9*3 alleles may be at increased risk of severe cutaneous adverse reactions. Consider avoiding

Drug	Gene	Affected Subgroups	Description of Gene-Drug Interaction
			fosphenytoin as an alternative to carbamazepine in patients who are CYP2C9*3 carriers. Genotyping is not a substitute for clinical vigilance and patient management.
Fosphenytoin	*HLA-B*	*15:02 allele positive	May result in higher adverse reaction risk (severe cutaneous reactions). Patients positive for HLA-B*15:02 may be at increased risk of Stevens Johnson Syndrome/Toxic Epidermal necrolysis (SJS/TEN). Consider avoiding fosphenytoin as an alternative to carbamazepine in patients who are positive for HLA-B*15:02. Genotyping is not a substitute for clinical vigilance and patient management.
Gefitinib	*CYP2D6*	poor metabolizers	Results in higher systemic concentrations and higher adverse reaction risk. Monitor for adverse reactions.
Iloperidone	*CYP2D6*	poor metabolizers	Results in higher systemic concentrations and higher adverse reaction risk (QT prolongation). Reduce dosage by 50%.
Irinotecan	*UGT1A1*	*28/*28 (poor metabolizers)	Results in higher systemic active metabolite concentrations and higher adverse reaction risk (severe neutropenia). Consider reducing the starting dosage by one level and modify the dosage based on individual patient tolerance.
Lofexidine	*CYP2D6*	poor metabolizers	Results in higher systemic concentrations and higher adverse reaction risk. Monitor for orthostatic hypotension and bradycardia.

Drug	Gene	Affected Subgroups	Description of Gene-Drug Interaction
Meclizine	*CYP2D6*	ultrarapid, intermediate, or poor metabolizers	May affect systemic concentrations. Monitor for adverse reactions and clinical effect.
Meloxicam	*CYP2C9*	Poor metabolizers or *3 carriers	Results in higher systemic concentrations. Consider dose reductions in poor metabolizers. Monitor patients for adverse reactions.
Metoclopramide	*CYP2D6*	poor metabolizers	Results in higher systemic concentrations and higher adverse reaction risk. The recommended dosage is lower. Refer to FDA labeling for specific dosing recommendations.
Mercaptopurine	*TPMT* and/or *NUDT15*	intermediate or poor metabolizers	Alters systemic active metabolite concentration and dosage requirements. Results in higher adverse reaction risk (myelosuppression). Initial dosages should be reduced in poor metabolizers; poor metabolizers generally tolerate 10% or less of the recommended dosage. Intermediate metabolizers may require dosage reductions based on tolerability. Intermediate metabolizers for both genes may require more substantial dosage reductions. Refer to FDA labeling for specific dosing recommendations.
Mivacurium	*BCHE*	intermediate or poor metabolizers	Results in higher systemic concentrations and higher adverse reaction risk (prolonged neuromuscular blockade). Avoid use in poor metabolizers.

Drug	Gene	Affected Subgroups	Description of Gene-Drug Interaction
Nateglinide	*CYP2C9*	Poor metabolizers	Results in higher systemic concentrations and may result in higher adverse reaction risk (hypoglycemia). Dosage reduction is recommended. Increase monitoring frequency for adverse reactions. Refer to FDA labeling for specific dosing recommendations.
Oliceridine	*CYP2D6*	Poor metabolizers	Results in higher systemic concentrations and higher adverse reaction risk (respiratory depression and sedation). May require less frequent dosing.
Pantoprazole	*CYP2C19*	poor metabolizers	Results in higher systemic concentrations. Consider dosage reduction in children who are poor metabolizers. No dosage adjustment is needed for adult patients who are poor metabolizers.
Phenytoin	*CYP2C9*	Intermediate or poor metabolizers	May result in higher systemic concentrations and higher adverse reaction risk (central nervous system toxicity). Refer to FDA labeling for specific dosing recommendations. Carriers of CYP2C9*3 alleles may be at increased risk of severe cutaneous adverse reactions. Consider avoiding phenytoin as an alternative to carbamazepine in patients who are CYP2C9*3 carriers. Genotyping is not a substitute for clinical vigilance and patient management.
Phenytoin	*HLA-B*	*15:02 allele positive	May result in higher adverse reaction risk (severe cutaneous reactions). Patients positive for HLA-B*15:02 may be at increased risk of Stevens Johnson Syndrome/Toxic Epidermal

Drug	Gene	Affected Subgroups	Description of Gene-Drug Interaction
			necrolysis (SJS/TEN). Consider avoiding phenytoin as an alternative to carbamazepine in patients who are positive for HLA-B*15:02. Genotyping is not a substitute for clinical vigilance and patient management.
Pimozide	*CYP2D6*	poor metabolizers	Results in higher systemic concentrations. Dosages should not exceed 0.05 mg/kg in children or 4 mg/day in adults who are poor metabolizers and dosages should not be increased earlier than 14 days.
Piroxicam	*CYP2C9*	intermediate or poor metabolizers	Results in higher systemic concentrations. Consider reducing dosage in poor metabolizers.
Pitolisant	*CYP2D6*	Poor metabolizers	Results in higher systemic concentrations. Use lowest recommended starting dosage. Refer to FDA labeling for specific dosing recommendations.
Propafenone	*CYP2D6*	poor metabolizers	Results in higher systemic concentrations and higher adverse reaction risk (arrhythmia). Avoid use in poor metabolizers taking a CYP3A4 inhibitor.
Sacituzumab Govitecan-hziy	*UGT1A1*	*28/*28 (poor metabolizers)	May result in higher systemic concentrations and adverse reaction risk (neutropenia). Monitor for adverse reactions and tolerance to treatment.
Siponimod	*CYP2C9*	intermediate or poor metabolizers	Results in higher systemic concentrations. Adjust dosage based on genotype. Do not use in patients with CYP2C9 *3/*3 genotype. Refer to FDA labeling for specific dosing recommendations.

Drug	Gene	Affected Subgroups	Description of Gene-Drug Interaction
Succinylcholine	*BCHE*	intermediate or poor metabolizers	Results in higher systemic concentrations and higher adverse reaction risk (prolonged neuromuscular blockade). Avoid use in poor metabolizers. May administer test dose to assess sensitivity and administer cautiously via slow infusion.
Tacrolimus	*CYP3A5*	intermediate or normal metabolizers	Results in lower systemic concentrations and lower probability of achieving target concentrations. Measure drug concentrations and adjust dosage based on trough whole blood tacrolimus concentrations.
Tetrabenazine	*CYP2D6*	poor metabolizers	Results in higher systemic concentrations. The maximum recommended single dose is 25 mg and should not exceed 50 mg/day.
Thioguanine	*TPMT* and/or *NUDT15*	intermediate or poor metabolizers	Alters systemic active metabolite concentration and dosage requirements. Results in higher adverse reaction risk (myelosuppression). Initial dosages should be reduced in poor metabolizers; poor metabolizers generally tolerate 10% or less of the recommended dosage. Intermediate metabolizers may require dosage reductions based on tolerability. Intermediate metabolizers for both genes may require more substantial dosage reductions. Refer to FDA labeling for specific dosing recommendations.
Thioridazine	*CYP2D6*	poor metabolizers	Results in higher systemic concentrations and higher adverse

Drug	Gene	Affected Subgroups	Description of Gene-Drug Interaction
			reaction risk (QT prolongation). Predicted effect based on experience with CYP2D6 inhibitors. Contraindicated in poor metabolizers.
Tramadol	*CYP2D6*	Ultrarapid metabolizers	Results in higher systemic and breast milk active metabolite concentrations, which may result in respiratory depression and death. Contraindicated in children under 12 and in adolescents following tonsillectomy/adenoidectomy. Breastfeeding is not recommended during treatment.
Valbenazine	*CYP2D6*	poor metabolizers	Results in higher systemic active metabolite concentrations and higher adverse reaction risk (QT prolongation). Dosage reductions may be necessary.
Venlafaxine	*CYP2D6*	poor metabolizers	Alters systemic parent drug and metabolite concentrations. Consider dosage reductions.
Vortioxetine	*CYP2D6*	poor metabolizers	Results in higher systemic concentrations. The maximum recommended dose is 10 mg.
Warfarin	*CYP2C9*	intermediate or poor metabolizers	Alters systemic concentrations and dosage requirements. Select initial dosage, taking into account clinical and genetic factors. Monitor and adjust dosages based on INR.
Warfarin	*CYP4F2*	V433M variant carriers	May affect dosage requirements. Monitor and adjust doses based on INR.
Warfarin	*VKORC1*	-1639G>A variant carriers	Alters dosage requirements. Select initial dosage, taking into account clinical and genetic factors. Monitor and adjust dosages based on INR.

The table below lists associations "for which the data indicate a potential impact on safety or response" (FDA, 2022).

Drug	Gene	Affected Subgroups	Description of Gene-Drug Interaction
Allopurinol	*HLA-B*	*58:01 allele positive	Results in higher adverse reaction risk (severe skin reactions).
Carbamazepine	*HLA-A*	*31:01 allele positive	Results in higher adverse reaction risk (severe skin reactions). Consider risk and benefit of carbamazepine use in patients positive for HLA-A*31:01. Genotyping is not a substitute for clinical vigilance.
Carvedilol	*CYP2D6*	poor metabolizers	Results in higher systemic concentrations and higher adverse reaction risk (dizziness).
Cevimeline	*CYP2D6*	poor metabolizers	May result in higher adverse reaction risk. Use with caution.
Codeine	*CYP2D6*	poor metabolizers	Results in lower systemic active metabolite concentrations and may result in reduced efficacy.
Efavirenz	*CYP2B6*	poor metabolizers	Results in higher systemic concentrations and higher adverse reaction risk (QT prolongation).
Isoniazid	Nonspecific (NAT)	poor metabolizers	May result in higher systemic concentrations and adverse reaction risk.
Lapatinib	*HLA-DRB1*	*07:01 allele positive	Results in higher adverse reaction risk (hepatotoxicity). Monitor liver function tests regardless of genotype.
Lapatinib	*HLA-DQA1*	*02:01 allele positive	Results in higher adverse reaction risk (hepatotoxicity). Monitor liver function tests regardless of genotype.
Mavacamten	*CYP2C19*	Intermediate or poor metabolizers	Results in higher systemic concentrations and may have higher adverse reaction risk (heart

Drug	Gene	Affected Subgroups	Description of Gene-Drug Interaction
			failure). Dosage is based on individual response. The dose titration and monitoring schedule accounts for differences due to CYP2C19 genetic variation, so adjustments based on CYP2C19 genotype are not necessary. Refer to FDA labeling for specific dosing recommendations and monitoring.
Nilotinib	*UGT1A1*	*28/*28 (poor metabolizers)	Results in higher adverse reaction risk (hyperbilirubinemia).
Oxcarbazepine	*HLA-B*	*15:02 allele positive	Results in higher adverse reaction risk (severe skin reactions). Patients positive for HLA-B*15:02 may be at increased risk of severe skin reactions with other drugs that are associated with a risk of Stevens Johnson Syndrome/Toxic Epidermal necrolysis (SJS/TEN). Genotyping is not a substitute for clinical vigilance.
Pazopanib	*HLA-B*	*57:01 allele positive	May result in higher adverse reaction risk (liver enzyme elevations). Monitor liver function tests regardless of genotype.
Pazopanib	*UGT1A1*	*28/*28 (poor metabolizers)	Results in higher adverse reaction risk (hyperbilirubinemia).
Perphenazine	*CYP2D6*	poor metabolizers	Results in higher systemic concentrations and higher adverse reaction risk.
Procainamide	Nonspecific (NAT)	poor metabolizers	Alters systemic parent drug and metabolite concentrations. May result in higher adverse reaction risk.
Simvastatin	*SLCO1B1*	521 TC or 521 CC (intermediate	Results in higher systemic concentrations and higher adverse reaction risk (myopathy). The risk

Drug	Gene	Affected Subgroups	Description of Gene-Drug Interaction
		or poor function transporters)	of adverse reaction (myopathy) is higher for patients on 80 mg than for those on lower doses.
Sulfamethoxazole and Trimethoprim	Nonspecific (NAT)	poor metabolizers	May result in higher adverse reaction risk.
Sulfasalazine	Nonspecific (NAT)	poor metabolizers	Results in higher systemic metabolite concentrations and higher adverse reaction risk.
Tolterodine	*CYP2D6*	poor metabolizers	Results in higher systemic concentrations and higher adverse reaction risk (QT prolongation).
Tramadol	*CYP2D6*	poor metabolizers	Results in lower systemic active metabolite concentrations and may result in reduced efficacy.
Voriconazole	*CYP2C19*	Intermediate or poor metabolizers	Results in higher systemic concentrations and may result in higher adverse reaction risk.

The International Society of Psychiatric Genetics

The International Society of Psychiatric Genetics (ISPG) released recommendations on the use of pharmacogenetic testing to guide psychiatric treatment. ISPG recommends that pharmacogenetic testing should be used as a decision-support tool. *HLA-A* and *HLA-B* testing is recommended before the use of carbamazepine and oxcarbazepine. *CYP2C19* and *CYP2D6* testing would be beneficial for those who experienced an inadequate response or adverse reaction to a previous antidepressant or antipsychotic medication (ISPG, 2019).

The American Academy of Child and Adolescent Psychiatry

AACAP does not recommend the use of pharmacogenetic testing to select psychotropic medications for children and adolescents (AACAP, 2020)

Association for Molecular Pathology PGx Working Group (AMP)

AMP released clinical practice guidelines to define a minimum set of *CYP2C19* allele variants that should be included in the pharmacogenomic genotyping assay. Tier 1 represents alleles that have been shown to affect drug response and should be included, while Tier 2 represents alleles which meet at least one but not all the criteria

for inclusion in Tier 1 and are considered optional for inclusion in expanded clinical genotyping panels. Those in Tier 1 include alleles *2, *3, and *17. The following *CYP2C19* alleles were recommended as Tier 2: *4A, *4B, *5, *6, *7, *8, *9, *10, and *35 (Pratt et al., 2018). Regarding *CYP2C9* variant alleles, Tier 1 alleles include *CYP2C9* *2, *3, *5, *6, *8, and *11. The following *CYP2C9* alleles are recommended for inclusion in Tier 2: CYP2C9*12, *13, and *15 (Pratt et al., 2019). For testing genes and alleles specific to warfarin, AMP recommends including *VKORC1* c.-1639G>A in Tier 1 and *VKORC1* c.196G>A and c.106G>A in Tier 2 (Pratt et al., 2020). In a joint recommendation endorsed by the AMP, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, and the European Society for Pharmacogenomics and Personalized Therapy, *CYP2D6* variant alleles were elucidated. Tier 1 alleles include *CYP2D6* *2 to *6, *9, *10, *17, *29, and *41. Tier 2 *CYP2D6* alleles include *CYP2D6* *7, *8, *12, *14, *15, *21, *31, *40, *42, *49, *56, and *59, and hybrid genes containing portions of *CYP2D6* and *CYP2D7* (Pratt et al., 2021). These recommendations should help to standardize testing and genotyping concordance among laboratories.

European Medicines Agency

EMA released recommendations on *DPD* testing before treatment with fluorouracil, capecitabine, tegafur, and flucytosine. EMA recommends testing for the lack of *DPD* before starting cancer treatment with fluorouracil, capecitabine, or tegafur. Patients who completely lack *DPD* should not be given these medications. For patients with partial deficiency, the physician may consider beginning treatment at a lower dose and terminating treatment if severe side effects occur. These recommendations do not apply to fluorouracil medications used for skin conditions or flucytosine used for fungal infection (EMA, 2020).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Diagnostic genotyping tests for certain drug metabolizing enzymes are FDA-approved. Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

Currently, there are over 14 other FDA-approved tests for the drug metabolizing enzymes that are nucleic acid-based tests including xTAG *CYP2D6* Kit v3 and XTAG *CYP2C19* KIT V3 (Luminex Molecular Diagnostics, Inc), Spartan RX *CYP2C19* Test System (Spartan Bioscience, Inc), Verigene *CYP2C19* Nucleic Acid Test (Nanosphere, Inc), INFINITI *CYP2C19* Assay (AutoGenomics, Inc), Invader *UGT1A1* (Third Wave Technologies Inc.), eSensor Warfarin Sensitivity Saliva Test (GenMark Diagnostics), eQ-PCR LC Warfarin Genotyping kit (TrimGen Corporation), eSensor Warfarin Sensitivity Test and XT-8 Instrument (Osmetech Molecular Diagnostics), Gentris Rapid Genotyping Assay-*CYP2C9*&VKORCI (ParagonDx, LLC), INFINITI 2C9 & *VKORC1* Multiplex Assay for Warfarin (AutoGenomics, Inc), Verigene Warfarin Metabolism Nucleic Acid Test and Verigene System (Nanosphere, Inc), TruDiagnosis System (Akonni Systems, Inc), Roche AmpliChip CYP450 microassay (Roche Molecular Systems, Inc) (FDA, 2021a).

FDA Notes

The Office of Clinical Pharmacology within FDA includes The Genomics and Targeted Therapy Group responsible for applying pharmacogenomics and other biomarkers in drug development and clinical practice. The FDA scientists review current pharmacogenomic information and ensure that pharmacogenomic strategies are utilized appropriately in all phases of drug development (FDA, 2022).

The current list of pharmacogenomic biomarkers in drug labeling by FDA contain numerous medications that have genotypes related to metabolism dosage recommendations or warnings. These medications are involved in different therapeutic areas and the list includes the following genes and medications:

CYP1A2: Rucaparib

CYP2B6: Efavirenz, Prasugrel, Ospemifene

CYP2C19 contains 22 different medications: Clopidogrel, Prasugrel, Ticagrelor, Lansoprazole, Omeprazole, Esomeprazole, Rabeprazole, Pantoprazole, Dexlansoprazole, Flibanserin, Drospirenone and Ethinyl Estradiol, Voriconazole, Lacosamide, Brivaracetam, Clobazam, Phenytoin, Diazepam, Citalopram, Escitalopram, Doxepin, Formoterol, Carisoprodol

CYP2C9 contains 15 different medications: Prasugrel, Dronabinol, Flibanserin, Warfarin, Phenytoin, Celecoxib, Piroxicam, Flurbiprofen, Lesinurad, Avatrombopag, Erdafitinib, Ospemifene, Siponimod, Meloxicam, Rimegepant

CYP2D6 contains 70 different medications: Tramadol, Metoprolol, Nebivolol, Propafenone, Propranolol, Ondansetron, Palonosetron, Flibanserin, Eliglustat, Deutetrabenazine, Dextromethorphan and Quinidine, Galantamine, Tetrabenazine, Valbenazine, Rucaparib, Aripiprazole, Aripiprazole Lauroxil, Atomoxetine, Brexpiprazole, Cariprazine, Citalopram, Clozapine, Desvenlafaxine, Doxepin, Escitalopram, Fluoxetine, Fluvoxamine, Iloperidone, Modafinil, Paroxetine, Perphenazine, Risperidone, Venlafaxine, Vortioxetine, Arformoterol, Formoterol, Umeclidinium, Darifenacin, Mirabegron, Tolterodine, Amphetamine, Donepezil, Fesoterodine, Gefitinib, Metoclopramide, Paliperidone, Tamoxifen, Carvedilol, Amitriptyline, Amoxapine, Clomipramine, Codeine, Desipramine, Duloxetine, Imipramine, Meclizine, Metoclopramide, Nefazodone, Nortriptyline, Pimozide, Protriptyline, Quinine Sulfate, Tamsulosin, Thioridazine, Trimipramine, Pitolisant, Upadacitinib, Bupropion.

CYP3A5: Prasugrel

TPMT: Thioguanine, Azathioprine, Mercaptopurine, Cisplatin

NUDT15: Thioguanine, Azathioprine, Mercaptopurine

UGT1A1: Arformoterol, Belinostat, Binimetinib, Dolutegravir, Indacaterol, Irinotecan, Nilotinib, Pazopanib, Raltegravir, Sacituzumab Govitecan-hziy (FDA, 2021b).

FDA Recommendations

The FDA package insert for Plavix (clopidogrel) carries the following "Black Box" warning: "The effectiveness of Plavix results from its antiplatelet activity which is dependent on its conversion to an active metabolite by the cytochrome P450 (CYP) system, principally *CYP2C19*. Plavix at recommended doses forms less of the active metabolite and so has a reduced effect on platelet activity in patients who are homozygous for nonfunctional alleles of the *CYP2C19* gene, (termed "*CYP2C19* poor metabolizers"). Tests are available to identify patients who are *CYP2C19* poor metabolizers. Consider another platelet P2Y12 inhibitor in patients identified as *CYP2C19* poor metabolizers." (FDA, 2016)

The FDA package insert for Xenazine (tetrabenazine) indicates, "Patients who require doses of Xenazine greater than 50 mg per day should be first tested and genotyped to determine if they are poor metabolizers (PMs) or extensive metabolizers (EMs) by their ability to express the drug metabolizing enzyme, *CYP2D6*. The dose of XENAZINE should then be individualized accordingly to their status as PMs or EMs. (FDA, 2008)

The Coumadin (warfarin) highlights of prescription information notes that "The appropriate initial dosing of COUMADIN varies widely for different patients. Not all factors responsible for warfarin dose variability are known, and the initial dose is influenced by: Genetic factors (*CYP2C9* and *VKORC1* genotypes)." Although dosage suggestions based on *CYP2C9* and *VKORC1* genotypes are provided in the package insert, the requirement for genetic testing is not included (FDA)

The eligibility and dosing of Eliglustat is dependent on cytochrome P450 *CYP2D6* genotype as eliglustat is extensively metabolized by *CYP2D6*. The FDA contraindicates this medication in the following patients due "to the risk of cardiac arrhythmias from prolongation of the PR, QTc, and/or QRS cardiac Intervals":

EMs of *CYP2D6*

- Taking a strong or moderate CYP2D6 inhibitor concomitantly with a strong or moderate CYP3A inhibitor
- Moderate or severe hepatic impairment
- Mild hepatic impairment and taking a strong or moderate CYP2D6 inhibitor

IMs

- Taking a strong or moderate CYP2D6 inhibitor concomitantly with a strong or moderate CYP3A inhibitor
- Taking a strong CYP3A inhibitor
- Any degree of hepatic impairment

PMs

- Taking a strong CYP3A inhibitor
- Any degree of hepatic impairment (FDA, 2014)

The FDA also includes a warning for irinotecan's interaction with *UGT1A1*, stating "When administered in combination with other agents, or as a single-agent, a reduction in the starting dose by at least one level of CAMPTOSAR [irinotecan] should be considered for patients known to be homozygous for the *UGT1A1**28 allele" (FDA, 2021b).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81220	CFTR (cystic fibrosis transmembrane conductance regulator) (eg, cystic fibrosis) gene analysis; common variants (eg, ACMG/ACOG guidelines)
81225	CYP2C19 (cytochrome P450, family 2, subfamily C, polypeptide 19) (eg, drug metabolism), gene analysis, common variants (eg, *2, *3, *4, *8, *17)
81226	CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6) (eg, drug metabolism), gene analysis, common variants (eg, *2, *3, *4, *5, *6, *9, *10, *17, *19, *29, *35, *41, *1XN, *2XN, *4XN)
81227	CYP2C9 (cytochrome P450, family 2, subfamily C, polypeptide 9) (eg, drug metabolism), gene analysis, common variants (eg, *2, *3, *5, *6)
81230	CYP3A4 (cytochrome P450 family 3 subfamily A member 4) (eg, drug metabolism), gene analysis, common variant(s) (eg, *2, *22)
81231	CYP3A5 (cytochrome P450 family 3 subfamily A member 5) (eg, drug metabolism), gene analysis, common variants (eg, *2, *3, *4, *5, *6, *7)
81232	DPYD (dihydropyrimidine dehydrogenase) (eg, 5-fluorouracil/5-FU and capecitabine drug metabolism), gene analysis, common variant(s) (eg, *2A, *4, *5, *6)
81247	G6PD (glucose-6-phosphate dehydrogenase) (eg, hemolytic anemia, jaundice), gene analysis; common variant(s) (eg, A, A-)
81283	IFNL3 (interferon, lambda 3) (eg, drug response), gene analysis, rs12979860 variant
81291	MTHFR (5,10-methylenetetrahydrofolate reductase) (eg, hereditary hypercoagulability) gene analysis, common variants (eg, 677T, 1298C)
81306	NUDT15 (nudix hydrolase 15) (eg, drug metabolism) gene analysis, common variant(s) (eg, *2, *3, *4, *5, *6)
81328	SLCO1B1 (solute carrier organic anion transporter family, member 1B1) (eg, adverse drug reaction), gene analysis, common variant(s) (eg, *5)
81335	TPMT (thiopurine S-methyltransferase) (eg, drug metabolism), gene analysis, common variants (eg, *2, *3)
81346	TYMS (thymidylate synthetase) (eg, 5-fluorouracil/5-FU drug metabolism), gene analysis, common variant(s) (eg, tandem repeat variant)
81350	UGT1A1 (UDP glucuronosyltransferase 1 family, polypeptide A1) (eg, drug metabolism, hereditary unconjugated hyperbilirubinemia [Gilbert syndrome]) gene analysis, common variants (eg, *28, *36, *37)
81355	VKORC1 (vitamin K epoxide reductase complex, subunit 1) (eg, warfarin metabolism), gene analysis, common variant(s) (eg, -1639G>A, c.173+1000C>T)

CPT	Code Description
81381	HLA Class I typing, high resolution (ie, alleles or allele groups); one allele or allele group (eg, B*57:01P), each
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons)
81418	Drug metabolism (eg, pharmacogenomics) genomic sequence analysis panel, must include testing of at least 6 genes, including CYP2C19, CYP2D6, and CYP2D6 duplication/deletion analysis
81479	Unlisted molecular pathology procedure
0029U	Drug metabolism (adverse drug reactions and drug response), targeted sequence analysis (ie, CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4, CYP3A5, CYP4F2, SLCO1B1, VKORC1 and rs12777823) Proprietary test: Focused Pharmacogenomics Panel Lab/Manufacturer: Mayo Clinic
0030U	Drug metabolism (warfarin drug response), targeted sequence analysis (ie, CYP2C9, CYP4F2, VKORC1, rs12777823) Proprietary test: Warfarin Response Genotype Lab/Manufacturer: Mayo Clinic
0031U	CYP1A2 (cytochrome P450 family 1, subfamily A, member 2) (eg, drug metabolism) gene analysis, common variants (ie, *1F, *1K, *6, *7) Proprietary test: Cytochrome P450 1A2 Genotype Lab/Manufacturer: Mayo Clinic
0032U	COMT (catechol-O-methyltransferase)(eg, drug metabolism) gene analysis, c.472G>A (rs4680) variant Proprietary test: Catechol-O-Methyltransferase (COMT) Genotype Lab/Manufacturer: Mayo Clinic
0033U	HTR2A (5-hydroxytryptamine receptor 2A), HTR2C (5-hydroxytryptamine receptor 2C) (eg, citalopram metabolism) gene analysis, common variants (ie, HTR2A rs7997012 [c.614-2211T>C], HTR2C rs3813929 [c.-759C>T] and rs1414334 [c.551-3008C>G]) Proprietary test: Serotonin Receptor Genotype (HTR2A and HTR2C) Lab/Manufacturer: Mayo Clinic
0034U	TPMT (thiopurine S-methyltransferase), NUDT15 (nudix hydroxylase **15**)(eg, thiopurine metabolism) gene analysis, common variants (ie, TPMT *2, *3A, *3B, *3C, *4, *5, *6, *8, ***12**; NUDT15 *3, *4, *5)

CPT	Code Description
	Proprietary test: Thiopurine Methyltransferase (TPMT) and Nudix Hydrolase (NUDT15) Genotyping Lab/Manufacturer: Mayo Clinic
0070U	CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6) (eg, drug metabolism) gene analysis, common and select rare variants (ie, *2, *3, *4, *4N, *5, *6, *7, *8, *9, *10, *11, *12, *13, *14A, *14B, *15, *17, *29, *35, *36, *41, *57, *61, *63, *68, *83, *xN) Proprietary test: CYP2D6 Common Variants and Copy Number Lab/Manufacturer: Mayo Clinic
0071U	CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6) (eg, drug metabolism) gene analysis, full gene sequence (List separately in addition to code for primary procedure) Proprietary test: CYP2D6 Full Gene Sequencing Lab/Manufacturer: Mayo Clinic
0072U	CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6) (eg, drug metabolism) gene analysis, targeted sequence analysis (ie, CYP2D6-2D7 hybrid gene) (List separately in addition to code for primary procedure) Proprietary test: CYP2D6-2D7 Hybrid Gene Targeted Sequence Analysis Lab/Manufacturer: Mayo Clinic
0073U	CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6) (eg, drug metabolism) gene analysis, targeted sequence analysis (ie, CYP2D7-2D6 hybrid gene) (List separately in addition to code for primary procedure) Proprietary test: CYP2D7-2D6 Hybrid Gene Targeted Sequence Analysis Lab/Manufacturer: Mayo Clinic
0074U	CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6) (eg, drug metabolism) gene analysis, targeted sequence analysis (ie, non-duplicated gene when duplication/multiplication is trans) (List separately in addition to code for primary procedure) Proprietary test: CYP2D6 trans-duplication/multiplication non-duplicated gene targeted sequence analysis Lab/Manufacturer: Mayo Clinic
0075U	CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6) (eg, drug metabolism) gene analysis, targeted sequence analysis (ie, 5' gene

CPT	Code Description
	duplication/multiplication) (List separately in addition to code for primary procedure) Proprietary test: CYP2D6 5′ gene duplication/multiplication targeted sequence analysis Lab/Manufacturer: Mayo Clinic
0076U	CYP2D6 (cytochrome P450, family 2, subfamily D, polypeptide 6) (eg, drug metabolism) gene analysis, targeted sequence analysis (ie, 3′ gene duplication/ multiplication) (List separately in addition to code for primary procedure) Proprietary test: CYP2D6 3′ gene duplication/multiplication targeted sequence analysis Lab/Manufacturer: Mayo Clinic
0169U	NUDT15 (nudix hydrolase 15) and TPMT (thiopurine S-methyltransferase) (eg, drug metabolism) gene analysis, common variants Proprietary test: NT (NUDT15 and TPMT) genotyping panel Lab/Manufacturer: RPRD Diagnostics
0286U	CEP72 (centrosomal protein, 72-KDa), NUDT15 (nudix hydrolase 15) and TPMT (thiopurine S-methyltransferase) (eg, drug metabolism) gene analysis, common variants Proprietary test: CNT (CEP72, TPMT and NUDT15) genotyping panel Lab/Manufacturer: RPRD Diagnostics
0345U	Psychiatry (eg, depression, anxiety, attention deficit hyperactivity disorder [ADHD]), genomic analysis panel, variant analysis of 15 genes, including deletion/duplication analysis of CYP2D6 Proprietary test: GeneSight® Psychotropic Lab/Manufacturer: Assurex Health, Inc
0347U	Drug metabolism or processing (multiple conditions), whole blood or buccal specimen, DNA analysis, 16 gene report, with variant analysis and reported phenotypes Proprietary test: RightMed® PGx16 Test Lab/Manufacturer: OneOme®
0348U	Drug metabolism or processing (multiple conditions), whole blood or buccal specimen, DNA analysis, 25 gene report, with variant analysis and reported phenotypes

CPT	Code Description
	Proprietary test: RightMed® Comprehensive Test Exclude F2 and F5 Lab/Manufacturer: OneOme®
0349U	Drug metabolism or processing (multiple conditions), whole blood or buccal specimen, DNA analysis, 27 gene report, with variant analysis, including reported phenotypes and impacted gene-drug interactions Proprietary test: RightMed® Comprehensive Test Lab/Manufacturer: OneOme®
0350U	Drug metabolism or processing (multiple conditions), whole blood or buccal specimen, DNA analysis, 27 gene report, with variant analysis and reported phenotypes Proprietary test: RightMed® Gene Report Lab/Manufacturer: OneOme®
0380U	Drug metabolism (adverse drug reactions and drug response), targeted sequence analysis, 20 gene variants and CYP2D6 deletion or duplication analysis with reported genotype and phenotype Proprietary test: PersonalisedRX Lab/Manufacturer: Lab Genomics LLC
0392U	Drug metabolism (depression, anxiety, attention deficit hyperactivity disorder [ADHD]), gene-drug interactions, variant analysis of 16 genes, including deletion/duplication analysis of CYP2D6, reported as impact of gene-drug interaction for each drug Proprietary test: Medication Management Neuropsychiatric Panel Lab/Manufacturer: RCA Laboratory Services LLC d/b/a GENETWORx
0411U	Psychiatry (eg, depression, anxiety, attention deficit hyperactivity disorder [ADHD]), genomic analysis panel, variant analysis of 15 genes, including deletion/duplication analysis of CYP2D6 Proprietary test: IDgenetix® Lab/Manufacturer: Castle Biosciences, Inc
0419U	Neuropsychiatry (eg, depression, anxiety), genomic sequence analysis panel, variant analysis of 13 genes, saliva or buccal swab, report of each gene phenotype Proprietary test: Tempus nP Lab/Manufacturer: Tempus Labs, Inc

CPT	Code Description
0423U	Psychiatry (eg, depression, anxiety), genomic analysis panel, including variant analysis of 26 genes, buccal swab, report including metabolizer status and risk of drug toxicity by condition. Proprietary test: Genomind® Pharmacogenetics Report - Full Lab/Manufacturer: Genomind®, Inc
0434U	Drug metabolism (adverse drug reactions and drug response), genomic analysis panel, variant analysis of 25 genes with reported phenotypes. Proprietary test: RightMed® Gene Test Exclude F2 and F5 Lab/Manufacturer: OneOme® LLC
0437U	Psychiatry (anxiety disorders), mRNA, gene expression profiling by RNA sequencing of 15 biomarkers, whole blood, algorithm reported as predictive risk score. Proprietary test: MindX One™ Blood Test – Anxiety Lab/Manufacturer: MindX Sciences
0438U	Drug metabolism (adverse drug reactions and drug response), buccal specimen, gene-drug interactions, variant analysis of 33 genes, including deletion/duplication analysis of CYP2D6, including reported phenotypes and impacted gene- drug interactions. Proprietary test: EffectiveRX™ Comprehensive Panel Lab/Manufacturer: RCA Laboratory Services LLC d/b/a GENETWORx
0460U	Oncology, whole blood or buccal, DNA single-nucleotide polymorphism (SNP) genotyping by real-time PCR of 24 genes, with variant analysis and reported phenotypes Proprietary test: RightMed® Oncology Gene Report Lab/Manufacturer: OneOme® LLC
0461U	Oncology, pharmacogenomic analysis of single-nucleotide polymorphism (SNP) genotyping by real-time PCR of 24 genes, whole blood or buccal swab, with variant analysis, including impacted gene-drug interactions and reported phenotypes Proprietary test: RightMed® Oncology Medication Report Lab/Manufacturer: OneOme® LLC
G9143	Warfarin responsiveness testing by genetic technique using any method, any number of specimen(s)

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

AACAP. (2020). Clinical Use of Pharmacogenetic Tests in Prescribing Psychotropic Medications for Children and Adolescents. https://www.aacap.org/aacap/Policy_Statements/2020/Clinical-Use-Pharmacogenetic-Tests-Prescribing-Psychotropic-Medications-for-Children-Adolescents.aspx

AACC. (2017, 01/01/2017). *Using Clinical Laboratory Tests to Monitor Drug Therapy in Pain Management Patients*. https://www.aacc.org/science-and-practice/practice-guidelines/using-clinical-laboratory-tests-to-monitor-drug-therapy-in-pain-management-patients

ACMG. (2007). *Pharmacogenetic testing of CYP2C9 and VKORC1 alleles for warfarin*. http://www.acmg.net/PDFLibrary/CYP2C9-VKORC1-Parmacogenetic-Testing.pdf

Ahern, T. P., Hertz, D. L., Damkier, P., Ejlertsen, B., Hamilton-Dutoit, S. J., Rae, J. M., Regan, M. M., Thompson, A. M., Lash, T. L., & Cronin-Fenton, D. P. (2017). Cytochrome P-450 2D6 (CYP2D6) Genotype and Breast Cancer Recurrence in Tamoxifen-Treated Patients: Evaluating the Importance of Loss of Heterozygosity. *Am J Epidemiol*, *185*(2), 75-85. https://doi.org/10.1093/aje/kww178

Aka, I., Bernal, C. J., Carroll, R., Maxwell-Horn, A., Oshikoya, K. A., & Van Driest, S. L. (2017). Clinical Pharmacogenetics of Cytochrome P450-Associated Drugs in Children. *J Pers Med*, *7*(4). https://doi.org/10.3390/jpm7040014

Amstutz, U., Henricks, L. M., Offer, S. M., Barbarino, J., Schellens, J. H. M., Swen, J. J., Klein, T. E., McLeod, H. L., Caudle, K. E., Diasio, R. B., & Schwab, M. (2018). Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing: 2017 Update. *Clin Pharmacol Ther*, *103*(2), 210-216. https://doi.org/10.1002/cpt.911

Bains, R. K. (2013). African variation at Cytochrome P450 genesEvolutionary aspects and the implications for the treatment of infectious diseases. *Evolution, Medicine, and Public Health*, *2013*(1), 118-134. https://doi.org/10.1093/emph/eot010

Bell, G. C., Caudle, K. E., Whirl-Carrillo, M., Gordon, R. J., Hikino, K., Prows, C. A., Gaedigk, A., Agundez, J., Sadhasivam, S., Klein, T. E., & Schwab, M. (2016). Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for CYP2D6 genotype and use of ondansetron and tropisetron. *Clin Pharmacol Ther*, *102*(2), 213-218. https://doi.org/10.1002/cpt.598

Benitez, J., Cool, C. L., & Scotti, D. J. (2018). Use of combinatorial pharmacogenomic guidance in treating psychiatric disorders. *Per Med*, *15*(6), 481-494. https://doi.org/10.2217/pme-2018-0074

Birdwell, K. A., Decker, B., Barbarino, J. M., Peterson, J. F., Stein, C. M., Sadee, W., Wang, D., Vinks, A. A., He, Y., Swen, J. J., Leeder, J. S., van Schaik, R., Thummel, K. E., Klein, T. E., Caudle, K. E., & MacPhee, I. A. (2015). Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for CYP3A5 Genotype and Tacrolimus Dosing. *Clin Pharmacol Ther*, *98*(1), 19-24. https://doi.org/10.1002/cpt.113

Bousman, C. A., Stevenson, J. M., Ramsey, L. B., Sangkuhl, K., Hicks, J. K., Strawn, J. R., Singh, A. B., Ruaño, G., Mueller, D. J., Tsermpini, E. E., Brown, J. T., Bell, G. C., Leeder, J. S., Gaedigk, A., Scott, S. A., Klein, T. E., Caudle, K. E., & Bishop, J. R. (2023). Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6, CYP2C19, CYP2B6, SLC6A4, and HTR2A Genotypes and Serotonin Reuptake Inhibitor Antidepressants. *Clin Pharmacol Ther*, *114*(1), 51-68. https://doi.org/10.1002/cpt.2903

Braten, L. S., Haslemo, T., Jukic, M. M., Ingelman-Sundberg, M., Molden, E., & Kringen, M. K. (2020). Impact of CYP2C19 genotype on sertraline exposure in 1200 Scandinavian patients. *Neuropsychopharmacology*, *45*(3), 570-576. https://doi.org/10.1038/s41386-019-0554-x

Brown, J. T., Bishop, J. R., Sangkuhl, K., Nurmi, E. L., Mueller, D. J., Dinh, J. C., Gaedigk, A., Klein, T. E., Caudle, K. E., McCracken, J. T., de Leon, J., & Leeder, J. S. (2019). Clinical Pharmacogenetics Implementation Consortium Guideline for Cytochrome P450 (CYP)2D6 Genotype and Atomoxetine Therapy. *Clin Pharmacol Ther*, *106*(1), 94-102. https://doi.org/10.1002/cpt.1409

Cacabelos, R., Martinez, R., Fernandez-Novoa, L., Carril, J. C., Lombardi, V., Carrera, I., Corzo, L., Tellado, I., Leszek, J., McKay, A., & Takeda, M. (2012). Genomics of Dementia: APOE- and CYP2D6-Related Pharmacogenetics. *Int J Alzheimers Dis*, *2012*, 518901. https://doi.org/10.1155/2012/518901

Castro-Rojas, C. A., Esparza-Mota, A. R., Hernandez-Cabrera, F., Romero-Diaz, V. J., Gonzalez-Guerrero, J. F., Maldonado-Garza, H., Garcia-Gonzalez, I. S., Buenaventura-Cisneros, S., Sanchez-Lopez, J. Y., Ortiz-Lopez, R., Camacho-Morales, A., Barboza-Quintana, O., & Rojas-Martinez, A. (2017). Thymidylate synthase gene variants as predictors of clinical response and toxicity to fluoropyrimidine-based chemotherapy for colorectal cancer. *Drug Metab Pers Ther*, *32*(4), 209-218. https://doi.org/10.1515/dmpt-2017-0028

Caudle, K. E., Rettie, A. E., Whirl-Carrillo, M., Smith, L. H., Mintzer, S., Lee, M. T., Klein, T. E., & Callaghan, J. T. (2014). Clinical pharmacogenetics implementation consortium guidelines for CYP2C9 and HLA-B genotypes and phenytoin dosing. *Clin Pharmacol Ther*, *96*(5), 542-548. https://doi.org/10.1038/clpt.2014.159

Caudle, K. E., Sangkuhl, K., Whirl-Carrillo, M., Swen, J. J., Haidar, C. E., Klein, T. E., Gammal, R. S., Relling, M. V., Scott, S. A., Hertz, D. L., Guchelaar, H. J., & Gaedigk, A. (2020). Standardizing CYP2D6 Genotype to Phenotype Translation: Consensus Recommendations from the Clinical Pharmacogenetics Implementation Consortium and Dutch Pharmacogenetics Working Group. *Clin Transl Sci*, *13*(1), 116-124. https://doi.org/10.1111/cts.12692

Chang, K. L., Weitzel, K., & Schmidt, S. (2015). Pharmacogenetics: Using Genetic Information to Guide Drug Therapy. *Am Fam Physician*, *92*(7), 588-594. https://www.aafp.org/afp/2015/1001/p588.html

Clancy, J. P., Johnson, S. G., Yee, S. W., McDonagh, E. M., Caudle, K. E., Klein, T. E., Cannavo, M., & Giacomini, K. M. (2014). Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for ivacaftor therapy in the context of CFTR genotype. *Clin Pharmacol Ther*, *95*(6), 592-597. https://doi.org/10.1038/clpt.2014.54

Cooper-DeHoff, R. M., Niemi, M., Ramsey, L. B., Luzum, J. A., Tarkiainen, E. K., Straka, R. J., Gong, L., Tuteja, S., Wilke, R. A., Wadelius, M., Larson, E. A., Roden, D. M., Klein, T. E., Yee, S. W., Krauss, R. M., Turner, R. M., Palaniappan, L., Gaedigk, A., Giacomini, K. M., . . . Voora, D. (2022). The Clinical Pharmacogenetics Implementation Consortium Guideline for SLCO1B1, ABCG2, and CYP2C9 genotypes and Statin-Associated Musculoskeletal Symptoms. *Clin Pharmacol Ther*, *111*(5), 1007-1021. https://doi.org/10.1002/cpt.2557

CPIC. (2023a, 06/15/2021). *Genes-Drugs*. Retrieved 02/05/2023 from https://cpicpgx.org/genes-drugs/

CPIC. (2023b). *What is CPIC?* https://cpicpgx.org/

Crews, K. R., Monte, A. A., Huddart, R., Caudle, K. E., Kharasch, E. D., Gaedigk, A., Dunnenberger, H. M., Leeder, J. S., Callaghan, J. T., Samer, C. F., Klein, T. E., Haidar, C. E., Van Driest, S. L., Ruano, G., Sangkuhl, K., Cavallari, L. H., Müller, D. J., Prows, C. A., Nagy, M., . . . Skaar, T. C. (2021). Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for CYP2D6, OPRM1, and COMT genotype and select opioid therapy. *Clinical Pharmacology & Therapeutics*, *n/a*(n/a). https://doi.org/https://doi.org/10.1002/cpt.2149

Desta, Z., Gammal, R. S., Gong, L., Whirl-Carrillo, M., Gaur, A. H., Sukasem, C., Hockings, J., Myers, A., Swart, M., Tyndale, R. F., Masimirembwa, C., Iwuchukwu, O. F., Chirwa, S., Lennox, J., Gaedigk, A., Klein, T. E., & Haas, D. W. (2019). Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2B6 and Efavirenz-Containing Antiretroviral Therapy. *Clin Pharmacol Ther*, *106*(4), 726-733. https://doi.org/10.1002/cpt.1477

EMA. (2020). Test before fluorouracil, capecitabine, tegafur or flucytosine therapy. *Reactions Weekly*, *1804*(1), 6-6. https://doi.org/10.1007/s40278-020-78435-3

FDA. *HIGHLIGHTS OF PRESCRIBING INFORMATION*. https://www.accessdata.fda.gov/drugsatfda_docs/label/2011/009218s107lbl.pdf

FDA. (2005). *510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION DECISION SUMMARY ASSAY ONLY TEMPLATE*. https://www.accessdata.fda.gov/cdrh_docs/reviews/K043576.pdf
FDA. (2008). *HIGHLIGHTS OF PRESCRIBING INFORMATION*. https://www.lundbeck.com/upload/us/files/pdf/Products/Xenazine_PI_US_EN.pdf
FDA. (2014). *HIGHLIGHTS OF PRESCRIBING INFORMATION*. https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/205494s003lbl.pdf
FDA. (2016). *SUPPLEMENT APPROVAL*. https://www.accessdata.fda.gov/drugsatfda_docs/appletter/2016/020839Orig1s062,s064ltr.pdf
FDA. (2021a). *Nucleic Acid Based Tests*. Retrieved 1/20/2021 from https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm
FDA. (2021b). Table of Pharmacogenomic Biomarkers in Drug Labeling. https://www.fda.gov/drugs/science-and-research-drugs/table-pharmacogenomic-biomarkers-drug-labeling
FDA. (2022, 11/08/2021). *Table of Pharmacogenetic Associations*. https://www.fda.gov/medical-devices/precision-medicine/table-pharmacogenetic-associations
FDA. (2023). FDA Converts Novel Alzheimer's Disease Treatment to Traditional Approval. https://www.fda.gov/news-events/press-announcements/fda-converts-novel-alzheimers-disease-treatment-traditional-approval
Franklin, G. M. (2014). Opioids for chronic noncancer pain: a position paper of the American Academy of Neurology. *Neurology*, *83*(14), 1277-1284. https://doi.org/10.1212/wnl.0000000000000839
Galli, M., Benenati, S., Capodanno, D., Franchi, F., Rollini, F., D'Amario, D., Porto, I., & Angiolillo, D. J. (2021). Guided versus standard antiplatelet therapy in patients undergoing percutaneous coronary intervention: a systematic review and meta-analysis. *The Lancet*, *397*(10283), 1470-1483.
Gammal, R. S., Court, M. H., Haidar, C. E., Iwuchukwu, O. F., Gaur, A. H., Alvarellos, M., Guillemette, C., Lennox, J. L., Whirl-Carrillo, M., Brummel, S. S., Ratain, M. J., Klein, T. E., Schackman, B. R., Caudle, K. E., & Haas, D. W. (2016). Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for UGT1A1 and Atazanavir Prescribing. *Clin Pharmacol Ther*, *99*(4), 363-369. https://doi.org/10.1002/cpt.269
Gammal, R. S., Pirmohamed, M., Somogyi, A. A., Morris, S. A., Formea, C. M., Elchynski, A. L., Oshikoya, K. A., McLeod, H. L., Haidar, C. E., Whirl-Carrillo, M., Klein, T. E., Caudle, K. E., & Relling, M. V. (2023). Expanded Clinical Pharmacogenetics Implementation Consortium Guideline for Medication Use in the Context of G6PD Genotype. *Clin Pharmacol Ther*, *113*(5), 973-985. https://doi.org/10.1002/cpt.2735

Ghanbarian, S., Wong, G. W. K., Bunka, M., Edwards, L., Cressman, S., Conte, T., Price, M., Schuetz, C., Riches, L., Landry, G., Erickson, D., McGrail, K., Peterson, S., Vijh, R., Hoens, A. M., Austin, J., & Bryan, S. (2023). Cost-effectiveness of pharmacogenomic-guided treatment for major depression. *Cmaj*, *195*(44), E1499-e1508. https://doi.org/10.1503/cmaj.221785

Goetz, M. P., Sangkuhl, K., Guchelaar, H. J., Schwab, M., Province, M., Whirl-Carrillo, M., Symmans, W. F., McLeod, H. L., Ratain, M. J., Zembutsu, H., Gaedigk, A., van Schaik, R. H., Ingle, J. N., Caudle, K. E., & Klein, T. E. (2018). Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6 and Tamoxifen Therapy. *Clin Pharmacol Ther*, *103*(5), 770-777. https://doi.org/10.1002/cpt.1007

Gonsalves, S. G., Dirksen, R. T., Sangkuhl, K., Pulk, R., Alvarellos, M., Vo, T., Hikino, K., Roden, D., Klein, T. E., Poler, S. M., Patel, S., Caudle, K. E., Gordon, R., Brandom, B., & Biesecker, L. G. (2019). Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for the Use of Potent Volatile Anesthetic Agents and Succinylcholine in the Context of RYR1 or CACNA1S Genotypes. *Clin Pharmacol Ther*, *105*(6), 1338-1344. https://doi.org/10.1002/cpt.1319

Greden, J. F., Parikh, S. V., Rothschild, A. J., Thase, M. E., Dunlop, B. W., DeBattista, C., Conway, C. R., Forester, B. P., Mondimore, F. M., Shelton, R. C., Macaluso, M., Li, J., Brown, K., Gilbert, A., Burns, L., Jablonski, M. R., & Dechairo, B. (2019). Impact of pharmacogenomics on clinical outcomes in major depressive disorder in the GUIDED trial: A large, patient- and rater-blinded, randomized, controlled study. *Journal of Psychiatric Research*. https://doi.org/https://doi.org/10.1016/j.jpsychires.2019.01.003

GTR. (2017). OneOme RightMed pharmacogenomic test. https://www.ncbi.nlm.nih.gov/gtr/tests/552110.3/performance-characteristics/

Hershfield, M. S., Callaghan, J. T., Tassaneeyakul, W., Mushiroda, T., Thorn, C. F., Klein, T. E., & Lee, M. T. (2013). Clinical Pharmacogenetics Implementation Consortium guidelines for human leukocyte antigen-B genotype and allopurinol dosing. *Clin Pharmacol Ther*, *93*(2), 153-158. https://doi.org/10.1038/clpt.2012.209

Hicks, J. K., Sangkuhl, K., Swen, J. J., Ellingrod, V. L., Muller, D. J., Shimoda, K., Bishop, J. R., Kharasch, E. D., Skaar, T. C., Gaedigk, A., Dunnenberger, H. M., Klein, T. E., Caudle, K. E., & Stingl, J. C. (2016). Clinical pharmacogenetics implementation consortium guideline (CPIC) for CYP2D6 and CYP2C19 genotypes and dosing of tricyclic antidepressants: 2016 update. *Clin Pharmacol Ther*, *102*(1), 37-44. https://doi.org/10.1002/cpt.597

Holmes, D. R., Jr., Dehmer, G. J., Kaul, S., Leifer, D., O'Gara, P. T., & Stein, C. M. (2010). ACCF/AHA clopidogrel clinical alert: approaches to the FDA "boxed warning": a report of the American College of Cardiology Foundation Task Force on clinical expert consensus documents and the American Heart Association endorsed by the Society for Cardiovascular Angiography and Interventions and the Society of

Thoracic Surgeons. *J Am Coll Cardiol*, *56*(4), 321-341. https://doi.org/10.1016/j.jacc.2010.05.013
ISPG. (2019). A Statement from the International Society of Psychiatric Genetics. https://ispg.net/genetic-testing-statement/
Johnson, J. A., Caudle, K. E., Gong, L., Whirl-Carrillo, M., Stein, C. M., Scott, S. A., Lee, M. T., Gage, B. F., Kimmel, S. E., Perera, M. A., Anderson, J. L., Pirmohamed, M., Klein, T. E., Limdi, N. A., Cavallari, L. H., & Wadelius, M. (2017). Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Pharmacogenetics-Guided Warfarin Dosing: 2017 Update. *Clin Pharmacol Ther*, *102*(3), 397-404. https://doi.org/10.1002/cpt.668
Kapur, B. M., Lala, P. K., & Shaw, J. L. (2014). Pharmacogenetics of chronic pain management. *Clin Biochem*, *47*(13-14), 1169-1187. https://doi.org/10.1016/j.clinbiochem.2014.05.065
Karnes, J. H., Rettie, A. E., Somogyi, A. A., Huddart, R., Fohner, A. E., Formea, C. M., Ta Michael Lee, M., Llerena, A., Whirl-Carrillo, M., Klein, T. E., Phillips, E. J., Mintzer, S., Gaedigk, A., Caudle, K. E., & Callaghan, J. T. (2021). Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2C9 and HLA-B Genotypes and Phenytoin Dosing: 2020 Update. *Clinical Pharmacology & Therapeutics*, *109*(2), 302-309. https://doi.org/https://doi.org/10.1002/cpt.2008
Kekic, A., Seetharam, M., Singh, P., Kunze, K., Golafshar, M. A., Azevedo, C., Welp, S., Bofferding, A., Koep, T., Ross, J., Nelson, E., Grilli, C., & Samadder, J. (2020). Integrating pharmacogenomics panel testing for supportive care medications in patients with solid tumors. *Journal of Clinical Oncology*, *38*(15_suppl), e24114-e24114. https://doi.org/10.1200/JCO.2020.38.15_suppl.e24114
Kelly, L. E., Rieder, M., van den Anker, J., Malkin, B., Ross, C., Neely, M. N., Carleton, B., Hayden, M. R., Madadi, P., & Koren, G. (2012). More codeine fatalities after tonsillectomy in North American children. *Pediatrics*, *129*(5), e1343-1347. https://doi.org/10.1542/peds.2011-2538
Krishnamurthi, S., & Kamath, S. (2024, May 8). *Clinical presentation and risk factors for chemotherapy-associated diarrhea, constipation, and intestinal perforation*. https://www.uptodate.com/contents/clinical-presentation-and-risk-factors-for-chemotherapy-associated-diarrhea-constipation-and-intestinal-perforation
Lee, C. R., Luzum, J. A., Sangkuhl, K., Gammal, R. S., Sabatine, M. S., Stein, C. M., Kisor, D. F., Limdi, N. A., Lee, Y. M., Scott, S. A., Hulot, J. S., Roden, D. M., Gaedigk, A., Caudle, K. E., Klein, T. E., Johnson, J. A., & Shuldiner, A. R. (2022). Clinical Pharmacogenetics Implementation Consortium Guideline for CYP2C19 Genotype and Clopidogrel Therapy: 2022 Update. *Clin Pharmacol Ther*. https://doi.org/10.1002/cpt.2526
Lima, J. J., Thomas, C. D., Barbarino, J., Desta, Z., Van Driest, S. L., El Rouby, N., Johnson, J. A., Cavallari, L. H., Shakhnovich, V., Thacker, D. L., Scott, S. A., Schwab, M., Uppugunduri, C. R. S., Formea, C. M., Franciosi, J. P., Sangkuhl, K., Gaedigk, A., Klein,

T. E., Gammal, R. S., & Furuta, T. (2020). Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2C19 and Proton Pump Inhibitor Dosing. *Clin Pharmacol Ther*. https://doi.org/10.1002/cpt.2015

Lunenburg, C., van der Wouden, C. H., Nijenhuis, M., Crommentuijn-van Rhenen, M. H., de Boer-Veger, N. J., Buunk, A. M., Houwink, E. J. F., Mulder, H., Rongen, G. A., van Schaik, R. H. N., van der Weide, J., Wilffert, B., Deneer, V. H. M., Swen, J. J., & Guchelaar, H. J. (2020). Dutch Pharmacogenetics Working Group (DPWG) guideline for the gene-drug interaction of DPYD and fluoropyrimidines. *Eur J Hum Genet*. https://doi.org/10.1038/s41431-019-0540-0

Martin, M. A., Hoffman, J. M., Freimuth, R. R., Klein, T. E., Dong, B. J., Pirmohamed, M., Hicks, J. K., Wilkinson, M. R., Haas, D. W., & Kroetz, D. L. (2014). Clinical Pharmacogenetics Implementation Consortium Guidelines for HLA-B Genotype and Abacavir Dosing: 2014 update. *Clin Pharmacol Ther*, *95*(5), 499-500. https://doi.org/10.1038/clpt.2014.38

Mayo. (2023). Focused Pharmacogenomics Panel, Varies. https://www.mayocliniclabs.com/test-catalog/Overview/610057

Moriyama, B., Obeng, A. O., Barbarino, J., Penzak, S. R., Henning, S. A., Scott, S. A., Agundez, J., Wingard, J. R., McLeod, H. L., Klein, T. E., Cross, S. J., Caudle, K. E., & Walsh, T. J. (2017). Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for CYP2C19 and Voriconazole Therapy. *Clin Pharmacol Ther*, *102*(1), 45-51. https://doi.org/10.1002/cpt.583

Muir, A. J., Gong, L., Johnson, S. G., Lee, M. T., Williams, M. S., Klein, T. E., Caudle, K. E., & Nelson, D. R. (2014). Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for IFNL3 (IL28B) genotype and PEG interferon-alpha-based regimens. *Clin Pharmacol Ther*, *95*(2), 141-146. https://doi.org/10.1038/clpt.2013.203

Myers, R. H., Schaefer, E. J., Wilson, P. W., D'Agostino, R., Ordovas, J. M., Espino, A., Au, R., White, R. F., Knoefel, J. E., Cobb, J. L., McNulty, K. A., Beiser, A., & Wolf, P. A. (1996). Apolipoprotein E epsilon4 association with dementia in a population-based study: The Framingham study. *Neurology*, *46*(3), 673-677. https://doi.org/10.1212/wnl.46.3.673

Myriad. (2016). *What is the difference between traditional "single-gene" testing and the GeneSight® test's proprietary CPGx® approach?* https://s3.amazonaws.com/myriad-web/Managed+Care/CPGx-approach.pdf

Myriad. (2019). *GeneSight*. https://s3.amazonaws.com/myriad-web/Managed+Care/GeneSight-ExecutiveSummary.pdf

Myriad. (2022). Myriad Genetics Announces Upgrades to the GeneSight® Test. https://genesight.com/news-and-press/myriad-genetics-announces-upgrades-to-the-genesight-test/

OneOme. (2021). The RightMed® Test. https://oneome.com/rightmed-test/#genes-covered

Oslin, D. W., Lynch, K. G., Shih, M. C., Ingram, E. P., Wray, L. O., Chapman, S. R., Kranzler, H. R., Gelernter, J., Pyne, J. M., Stone, A., DuVall, S. L., Lehmann, L. S., Thase, M. E., Aslam, M., Batki, S. L., Bjork, J. M., Blow, F. C., Brenner, L. A., Chen, P., . . . Wood, A. E. (2022). Effect of Pharmacogenomic Testing for Drug-Gene Interactions on Medication Selection and Remission of Symptoms in Major Depressive Disorder: The PRIME Care Randomized Clinical Trial. *Jama*, *328*(2), 151-161. https://doi.org/10.1001/jama.2022.9805

Phillips, E. J., Sukasem, C., Whirl-Carrillo, M., Muller, D. J., Dunnenberger, H. M., Chantratita, W., Goldspiel, B., Chen, Y. T., Carleton, B. C., George, A. L., Jr., Mushiroda, T., Klein, T., Gammal, R. S., & Pirmohamed, M. (2018). Clinical Pharmacogenetics Implementation Consortium Guideline for HLA Genotype and Use of Carbamazepine and Oxcarbazepine: 2017 Update. *Clin Pharmacol Ther*, *103*(4), 574-581. https://doi.org/10.1002/cpt.1004

Plumpton, C. O., Pirmohamed, M., & Hughes, D. A. (2019). Cost-Effectiveness of Panel Tests for Multiple Pharmacogenes Associated With Adverse Drug Reactions: An Evaluation Framework. *Clin Pharmacol Ther*, *105*(6), 1429-1438. https://doi.org/10.1002/cpt.1312

Pratt, V. M., Cavallari, L. H., Del Tredici, A. L., Gaedigk, A., Hachad, H., Ji, Y., Kalman, L. V., Ly, R. C., Moyer, A. M., Scott, S. A., van Schaik, R. H. N., Whirl-Carrillo, M., & Weck, K. E. (2021). Recommendations for Clinical CYP2D6 Genotyping Allele Selection: A Joint Consensus Recommendation of the Association for Molecular Pathology, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, and the European Society for Pharmacogenomics and Personalized Therapy. *J Mol Diagn*, *23*(9), 1047-1064. https://doi.org/10.1016/j.jmoldx.2021.05.013

Pratt, V. M., Cavallari, L. H., Del Tredici, A. L., Hachad, H., Ji, Y., Kalman, L. V., Ly, R. C., Moyer, A. M., Scott, S. A., Whirl-Carrillo, M., & Weck, K. E. (2020). Recommendations for Clinical Warfarin Genotyping Allele Selection: A Report of the Association for Molecular Pathology and the College of American Pathologists. *J Mol Diagn*, *22*(7), 847-859. https://doi.org/10.1016/j.jmoldx.2020.04.204

Pratt, V. M., Cavallari, L. H., Del Tredici, A. L., Hachad, H., Ji, Y., Moyer, A. M., Scott, S. A., Whirl-Carrillo, M., & Weck, K. E. (2019). Recommendations for Clinical CYP2C9 Genotyping Allele Selection: A Joint Recommendation of the Association for Molecular Pathology and College of American Pathologists. *The Journal of Molecular Diagnostics*, *21*(5), 746-755. https://doi.org/10.1016/j.jmoldx.2019.04.003

Pratt, V. M., Del Tredici, A. L., Hachad, H., Ji, Y., Kalman, L. V., Scott, S. A., & Weck, K. E. (2018). Recommendations for Clinical CYP2C19 Genotyping Allele Selection: A Report of the Association for Molecular Pathology. *The Journal of Molecular Diagnostics*, *20*(3), 269-276. https://doi.org/https://doi.org/10.1016/j.jmoldx.2018.01.011

Relling, M. V., Gardner, E. E., Sandborn, W. J., Schmiegelow, K., Pui, C. H., Yee, S. W., Stein, C. M., Carrillo, M., Evans, W. E., & Klein, T. E. (2013). Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. *Clin Pharmacol Ther*, *89*(3), 387-391. https://doi.org/10.1038/clpt.2010.320

Relling, M. V., Schwab, M., Whirl-Carrillo, M., Suarez-Kurtz, G., Pui, C. H., Stein, C. M., Moyer, A. M., Evans, W. E., Klein, T. E., Antillon-Klussmann, F. G., Caudle, K. E., Kato, M., Yeoh, A. E. J., Schmiegelow, K., & Yang, J. J. (2018). Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for thiopurine dosing based on TPMT and NUDT15 genotypes: 2018 update. *Clin Pharmacol Ther*. https://doi.org/10.1002/cpt.1304

Roscizewski, L., Henneman, A., & Snyder, T. (2021). Effect of pharmacogenomic testing on pharmacotherapy decision making in patients with symptoms of depression in an interprofessional primary care clinic. *J Am Pharm Assoc (2003)*. https://doi.org/10.1016/j.japh.2021.10.033

Saito, Y., Stamp, L. K., Caudle, K. E., Hershfield, M. S., McDonagh, E. M., Callaghan, J. T., Tassaneeyakul, W., Mushiroda, T., Kamatani, N., Goldspiel, B. R., Phillips, E. J., Klein, T. E., & Lee, M. T. (2016). Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for human leukocyte antigen B (HLA-B) genotype and allopurinol dosing: 2015 update. *Clin Pharmacol Ther*, *99*(1), 36-37. https://doi.org/10.1002/cpt.161

Sema4. (2022). Comprehensive Pharmacogenetic Genotyping Panel. https://sema4.com/products/test-catalog/comprehensive-pharmacogenetic-genotyping-panel/#

Sherva, R., & Kowall, N. W. (2022, May 19). *Genetics of Alzheimer disease*. https://www.uptodate.com/contents/genetics-of-alzheimer-disease

Stevenson, J. M., Alexander, G. C., Palamuttam, N., & Mehta, H. B. (2021). Projected Utility of Pharmacogenomic Testing Among Individuals Hospitalized With COVID-19: A Retrospective Multicenter Study in the United States. *Clin Transl Sci*, *14*(1), 153-162. https://doi.org/10.1111/cts.12919

Suchowersky, O. (2023, October 25). *Huntington disease: Management*. https://www.uptodate.com/contents/huntington-disease-management

Swen, J. J., van der Wouden, C. H., Manson, L. E., Abdullah-Koolmees, H., Blagec, K., Blagus, T., Böhringer, S., Cambon-Thomsen, A., Cecchin, E., Cheung, K. C., Deneer, V. H., Dupui, M., Ingelman-Sundberg, M., Jonsson, S., Joefield-Roka, C., Just, K. S., Karlsson, M. O., Konta, L., Koopmann, R., . . . Guchelaar, H. J. (2023). A 12-gene pharmacogenetic panel to prevent adverse drug reactions: an open-label, multicentre, controlled, cluster-randomised crossover implementation study. *Lancet*, *401*(10374), 347-356. https://doi.org/10.1016/s0140-6736(22)01841-4

Tantisira, K., & Weiss, S. T. (2023, September 6). *Overview of pharmacogenomics*. https://www.uptodate.com/contents/overview-of-pharmacogenomics

Tantry, U., Hennekens, C., Zehnder, J., & Gurbel, P. (2021, 11/09/2021). *Clopidogrel resistance and clopidogrel treatment failure*. Wolters Kluwer. https://www.uptodate.com/contents/clopidogrel-resistance-and-clopidogrel-treatment-failure

Theken, K. N., Lee, C. R., Gong, L., Caudle, K. E., Formea, C. M., Gaedigk, A., Klein, T. E., Agúndez, J. A. G., & Grosser, T. (2020). Clinical Pharmacogenetics Implementation Consortium Guideline (CPIC) for CYP2C9 and Nonsteroidal Anti-Inflammatory Drugs. *Clin Pharmacol Ther*, *108*(2), 191-200. https://doi.org/10.1002/cpt.1830

Ting, S., & Schug, S. (2016). The pharmacogenomics of pain management: prospects for personalized medicine. *J Pain Res*, *9*, 49-56. https://doi.org/10.2147/jpr.s55595

Tuteja, S., Glick, H., Matthai, W., Nachamkin, I., Nathan, A., Monono, K., Carcuffe, C., Maslowski, K., Chang, G., Kobayashi, T., Anwaruddin, S., Hirshfeld, J., Jr., Wilensky, R. L., Herrmann, H. C., Kolansky, D. M., Rader, D. J., & Giri, J. (2020). Prospective CYP2C19 Genotyping to Guide Antiplatelet Therapy Following Percutaneous Coronary Intervention: A Pragmatic Randomized Clinical Trial. *Circ Genom Precis Med*. https://doi.org/10.1161/circgen.119.002640

van der Wouden, C. H., van Rhenen, M. H., Jama, W. O. M., Ingelman-Sundberg, M., Lauschke, V. M., Konta, L., Schwab, M., Swen, J. J., & Guchelaar, H. J. (2019). Development of the PGx-Passport: A Panel of Actionable Germline Genetic Variants for Pre-Emptive Pharmacogenetic Testing. *Clin Pharmacol Ther*, *106*(4), 866-873. https://doi.org/10.1002/cpt.1489

Viatte, S. (2023, September 8). *Human leukocyte antigens (HLA): A roadmap*. https://www.uptodate.com/contents/human-leukocyte-antigens-hla-a-roadmap

Precision Genetic Test Management Reimbursement Policy

Policy Number: AHS – R2183 – Precision Genetic Test Management Reimbursement Policy	Initial Policy Effective Date: 01/01/2026

POLICY SPECIFIC DEFINITIONS | REIMBURSEMENT GUIDELINES | CLAIM SUBMISSION REQUIREMENTS | OBTAINING A PALMETTO DEX Z-CODE® |

I. Policy Specific Definitions

Molecular Pathology: Molecular pathology procedures are medical laboratory procedures that involve the analysis of nucleic acids or proteins in the tissue, blood, or other bodily fluids. These molecular pathology procedures can detect genetic, genomic, or other molecular abnormalities, with results being used to assist with diagnosis and/or prognosis or to help guide treatment.

Molecular Diagnostic Test (MDT): MDTs involve the detection or identification of nucleic acid(s) (deoxyribonucleic acid/ribonucleic acid [DNA/RNA]), proteins, chromosomes, enzymes, cancer chemotherapy sensitivity, and/or other metabolite(s). MDTs may include multiple components, may analyze or identify a single mutation or multiple mutations, and may rely upon an algorithm or other form of data evaluation/derivation. MDTs are laboratory developed tests that may or may not have Food and Drug Administration (FDA) approval or clearance.

II. Reimbursement Guidelines

Providers are required to submit the appropriate DEX Z-Code® and associated CPT® code for a molecular pathology service and molecular diagnostic test submitted for reimbursement consideration. The DEX Z-Code® is a unique test identifier that, when reported in conjunction with the appropriate CPT®/HCPCS code, enables identification of the exact test that was performed and more efficient reimbursement determinations. Only Z-Code® and associated CPT® codes with a "Successful" technical assessment outcome and the appropriate intended use will be considered for reimbursement.

Providers must report molecular pathology and molecular diagnostic tests with the CPT® and/or HCPCS code(s) that was assigned through the Palmetto DEX Z-Code® registry.

For tests that may involve multiple genes, but produce a single result, only one Z-Code® should be submitted with a CPT® code. Claims with the same DEX Z-Code® associated with multiple CPT® codes for the same patient and date of service will be denied.

III. Claim Submission Requirements

To report an MDT service, please submit the following claim information:

- Select appropriate Molecular Pathology CPT® code
- Enter 1 unit of service (UOS)
- Enter the appropriate DEX Z-Code® identifier adjacent to the CPT® code in the comment/narrative field for the following professional claim field/types:
  - Loop 2400 or SV-101-7 for the 5010A1 837P
  - Box 19 for paper claim
- Enter the appropriate DEX Z-Code® identifier adjacent to the CPT® code in the comment/narrative field for the following Facility claim field/types:
  - Line SV-202-7 for 837I electronic claim
  - Block 80 for the UB04 claim form

For information pertaining to Medicare codes, coverage, and Palmetto LCD, visit:

LCD - MolDX: Molecular Diagnostic Tests (MDT) (L35025)

Article - Billing and Coding: MolDX: Molecular Diagnostic Tests (MDT) (A56853)*

*Note that the list of procedure codes within this link is for informational purposes only and is not inclusive of all procedure codes enforced within this reimbursement policy.

IV. Obtaining a Palmetto DEX Z-Code®

Before submitting claims for an MDT, laboratories must register and receive a DEX Z-Code® and appropriate CPT®/HCPCS code.

- To register with the DEX® Diagnostics Exchange Registry, visit https//app.dexzcodes.com
  - Select the appropriate organization type under the **Register My Organization** header and provide the requested information
- To add a test to the registry and receive a DEX Z-Code® and appropriate CPT®/HCPCS assignment:
  - After logging into the registry, select the **Add Test** button from either the Catalog Overview or My Diagnostics Exchange tab.
  - Complete all required fields and submit your test for review.

For more information, visit http://www.dexzcodes.com.

Pre-Implantation Genetic Testing

Policy Number: AHS – M2039 – Pre-Implantation Genetic Testing	Prior Policy Name and Number, as applicable:
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Preimplantation genetic testing (PGT) involves the biopsy of a single cell, or a few cells of embryos to facilitate genetic testing for various genetic conditions. These conditions range from aneuploidies to monogenic disorders to structural deformities of the chromosomes themselves (Schattman & Xu, 2024).

II. Related Policies

Policy Number	Policy Title
AHS-M2017	Genetic Testing for Cystic Fibrosis
AHS-M2145	General Genetic Testing, Germline Disorders
AHS-M2146	General Genetic Testing, Somatic Disorders
AHS-M2179	Prenatal Screening (Genetic)

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Genetic counseling **IS REQUIRED** for individuals contemplating preimplantation genetic testing.

2) Preimplantation genetic testing for specific mutation(s) or chromosomal changes that have been associated with a specific disorder **MEETS COVERAGE CRITERIA** when one of the following conditions is met:

   a) Both biological parents are known carriers of an early-onset, autosomal recessive disorder.

b) One biological parent is a known carrier of an early-onset, autosomal recessive disorder and the other biological parent is unavailable for testing.

c) One biological parent is a known carrier of an early-onset, autosomal recessive disorder and together, the biological parents have produced previous offspring affected with the disorder.

d) One biological parent is a known carrier of an early-onset, autosomal dominant disorder.

e) One biological parent is a known carrier of an early-onset, X-linked disorder.

f) One biological parent carries a balanced or unbalanced chromosomal translocation.

3) In the absence of an early-onset, sex-linked disorder, preimplantation genetic testing for sex selection **DOES NOT MEET COVERAGE CRITERIA**.

4) Preimplantation genetic testing **DOES NOT MEET COVERAGE CRITERIA** for any of the following situations:

a) Preimplantation genetic testing for adult-onset disorders.

b) Preimplantation HLA genotyping for purposes of identifying potential tissue or organ donors.

c) Routine preimplantation screening for chromosomal abnormalities, including testing based on advanced maternal age.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

5) For all other situations not described above, preimplantation genetic testing **DOES NOT MEET COVERAGE CRITERIA**.

IV. Table of Terminology

Term	Definition
aCGH	Array-based comparative genomic hybridization
ACMG	American College of Medical Genetics and Genomics
AD	Autosomal dominant
AR	Autosomal recessive
ART	Assisted reproductive technology
ASRM	American Society of Reproductive Medicine

BFS	British Fertility Society
BMI	Body mass index
cLBR	Cumulative live birth rate
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid
DNA	Deoxyribose nucleic acid
ESHRE	European Society of Human Reproduction and Embryology
HLA	Human leukocyte antigen
ISAR	Indian Society for Assisted Reproduction
IVF	In vitro fertilization
LDTs	Laboratory-developed tests
NGS	Next-generation sequencing
PCR	Polymerase chain reaction
PGD	Preimplantation genetic diagnosis
PGD-A	Preimplantation genetic diagnosis for aneuploidy testing
PGDIS	Preimplantation Genetic Diagnosis International Society
PGS	Preimplantation genetic screening
PGT	Preimplantation genetic testing
PGT-A	Preimplantation genetic test for aneuploidy
PGT-M	Preimplantation genetic testing for monogenic defects
PGT-SR	Preimplantation genetic testing-structural rearrangements
qPCR	Quantitative real-time PCR
RPL	Recurrent pregnancy loss
SNP	Single nucleotide polymorphism
CCMG	Canadian College of Medical Geneticists
XL	X-linked

V. Scientific Background

Preimplantation genetic testing (PGT) in conjunction with assisted reproductive technology (ART) was developed to allow reproductive partners at risk of transmitting a genetic condition to their offspring to have an unaffected child without facing prenatal diagnosis and termination of pregnancy (PGDIS, 2008). Initially offered for diagnosis in couples at-risk for single gene genetic disorders, such as cystic fibrosis, spinal muscular atrophy and Huntington disease, preimplantation genetic diagnosis (PGD) has most frequently been employed in assisted reproduction as preimplantation

genetic screening (PGS) for detection of chromosome aneuploidy from advancing maternal age or structural chromosome rearrangements. The Preimplantation Genetic Diagnosis International Society (PGDIS) estimates that nearly 80% of PGT cycles have been performed for aneuploidy screening, 12% for single gene disorders, 6% for chromosome rearrangements and 2% for sibling human leukocyte antigen (HLA) matching (PGDIS, 2008). Both this and the European Society of Human Reproduction and Embryology (ESHRE) surveys confirm that aneuploidy testing is the major indication for PGT (Stern, 2014).

Embryonic genetic material used for PGT can be obtained from any of three sources: polar bodies from oocytes, blastomeres from day two or three, or trophectoderm cells from blastocysts (K. L. Scott et al., 2013). Polar bodies are typically analyzed if the embryo cannot be biopsied. However, polar body analysis is only useful for finding maternally inherited mutations or a cell division error during oocyte development. Furthermore, since genetic changes occur after the polar body develops, test results are of limited use. Additionally, as many as 30% of oocytes will not fertilize successfully, causing the test to fail (Schattman & Xu, 2024).

Blastomeres from day two or three (cleavage stage) were once the preferred practice in in-vitro-fertilization (IVF) as more embryos survived in culture by day three compared to days five or six (blastocyst stage). Despite the greater survival rate of day three embryos, these embryos were found to have a lower survival rate in a sustained implantation compared to day five embryos. Overall, trophectoderm biopsy on day five is preferable as it has no measurable impact on embryo development (Scott et al., 2013). Up to two or three dozen cells can be removed without disrupting development; although, common practice is to remove five to eight cells. Day five and later embryos also provide more DNA for testing compared to other stages of development (Schattman & Xu, 2024). Improved results have been seen with decreasing use of day three blastomere biopsy in favor of day five trophectoderm biopsy (K. L. Scott et al., 2013).

Pre-implantation genetic screening (PGS) is emerging as one of the most valuable tools to enhance pregnancy success with assisted reproductive technologies by assessing embryos for aneuploidy (Brezina et al., 2016).

As the genetic basis of more disorders are identified, increasing demand for and acceptance of the use of PGT for adult-onset disorders, such as Huntington disease, hereditary breast and ovarian cancer and Alzheimer disease, have occurred. Using PGD to screen embryos for diseases or mutations that confer an increased risk for developing a particular disease raises issues of how to weigh the benefits of PGD to the future child against the risks of PGD and ART (Stern, 2014). The Ethics committee

for the American Society of Reproductive Medicine (ASRM) found that PGD for adult-onset conditions is ethically justifiable when the conditions are serious and when there are no known interventions for the conditions or the available interventions are either inadequately effective or significantly burdensome (ASRM, 2013). The use of PGT for nonmedical sex selection or family balancing continues to be controversial, and the ethics committee has stated that it is acceptable for facilities to offer this service; however, employees wishing to decline participation in these procedures should be allowed to do so (ASRM, 2015).

Women recommended for pre-implantation genetic diagnosis for aneuploidy testing (PGD-A) or PGT for aneuploidy (PGT-A) are those of advanced reproductive age with a history of recurrent miscarriages and/or IVF failures; PGD-A is currently performed on trophectoderm biopsies by 24 different chromosome screening techniques (Vaiarelli et al., 2016). Trophectoderm biopsies are a safe and extensively validated approach with a low margin of error and miscarriage rate as well as a suspected high sustained pregnancy rate (Vaiarelli et al., 2016). However, Alteri et al. (2019) states that while PGT-A allows for an increased implantation rate, current data does not show an increase in successful pregnancy rates. Researchers agree that this technology is imperfect as Ledger (2019) reports that PGD-A can incorrectly designate an euploid embryo as an aneuploid embryo, leading to the unnecessary waste of embryos. These researchers also suggest that this type of screening may only be necessary in women between the ages of 35 and 44, as embryonic aneuploidy rates are low below 37 years of age and "costly screening for aneuploid seems pointless for women over 44 years of age, as almost all embryos are aneuploid" (Ledger, 2019).

The development of whole genome amplification and genomic tools, such as single nucleotide polymorphism (SNP) microarrays and comparative genomic hybridization microarrays, has led to faster, more accurate diagnoses that lead to improved pregnancy and live birth rates (Sullivan-Pyke & Dokras, 2018). Next-generation sequencing has also been used to distinguish between normal and abnormal embryos (García-Herrero et al., 2019). PGD-polymerase chain reaction (PCR) is often used to amplify the obtained DNA from the blastomere biopsy for further analysis (Feldman et al., 2017). Fluorescence In-situ Hybridization (FISH) has also been used for PGD and is an efficient method that may help to decrease IVF failure in infertile patients (Montazeri et al., 2018).

Other researchers are attempting to develop a non-invasive pre-implantation genetic testing technique. Farra et al. (2018) state that circulating cell-free embryonic DNA can be obtained from used culture media from blastocysts and in blastocoel fluid; this can then be used as a non-invasive method to evaluate genetic embryonic properties.

Proprietary Testing

Companies, such as Natera, have developed a preimplantation genetic test for monogenic/single gene conditions called Spectrum™ that includes PGT-A, PGT-M, and PGT-SR. This PGT-A uses SNP microarray technology. "Spectrum's SNP microarray platform typically yields >99% accuracy and allows for simultaneous PGT-M and/or PGT-SR with PGT-A" (Natera, 2022). Simon et al. (2018) studied IVF outcomes with this test when measuring PGT-A and euploid embryo transfer in day five or six embryos. An implantation rate and live birth rate of 69.9% and 64.5%, respectively, was identified (Simon et al., 2018). The authors concluded that "SNP-based PGT-A can mitigate the negative effects of maternal age on IVF outcomes in cycles with transfer, and that pregnancy outcomes from SET [single embryo transfer] cycles are not significantly different from those of double-embryo transfer cycles, and support the use of SET when transfers are combined with SNP-based PGT-A" (Simon et al., 2018).

iGLS Reproductive Genetics developed a PGT-A that uses next generation sequencing (NGS) to analyze thousands of DNA sequences that are unique to each chromosome allowing for the accurate identification of extra or missing chromosomes (iGLS, 2022).

PacGenomics has developed several PGT's for aneuploidies (PGT-A), structural chromosomal rearrangements (PGT-SR) with a subsequent PGT-SR Plus® which is used to differentiate between normal and balanced translocation carrier embryos in translocation cases, and for monogenic/single gene disorders (PGT-M). PGT-A for aneuploidies is performed on embryos created through IVF to screen for chromosomal abnormalities. PGT-SR is a genetic test performed on embryos created through IVF to screen for chromosomal structural rearrangements caused by balanced translocations and inversions. PGT-SR-Plus® can be added to PGT-SR which helps identify translocation carriers in patients who are not initially suspected to be at significant risk. PGT-M is performed on embryos created through IVF that is designed for individuals who know they are at an increased risk of having a child with a specific genetic disorder (PacGenomics, 2022).

Reproductive Genetic Innovations (RGI) developed several PGT tests including PGT-A, PGT-SR, and PGT-M which has similar functions to the other proprietary tests. RGI provides an additional test, PGT-HLA, which identifies embryos that are HLA compatible with a child who needs a bone marrow or cord blood transplant to help treat blood disorders (RGI, 2022).

Clinical Utility and Validity

Dreesen et al. (2014) performed a study assessing the accuracy of diagnoses made based on PGD. A total of 940 cases covering 53 genetic disorders were re-evaluated

using a PCR-based test. Of the 940 embryos, 881 (93.7%) of these embryos had two agreeing diagnoses. The first evaluation breakdown was 234 unaffected embryos, 590 affected, and 116 aberrant whereas the re-evaluation's breakdown was 283 unaffected embryos, 578 affected, and 79 aberrant. The sensitivity of this method was 99.2%, and its specificity was 80.2%. Allelic drop-out, mosaicism, and human error were the three most common causes of error (Dreesen et al., 2014).

Ghiossi et al. (2018) performed a study focusing on couples' decisions based on expanded carrier screening. Forty-five couples took a survey of their reproductive decision making after receiving their results, and of those 45, 28 said they would plan IVF with PGD or a prenatal diagnosis in future pregnancies. Of the 19 pregnant respondents, eight chose a prenatal diagnosis route, two planned amniocenteses but miscarried, and nine considered the condition insufficiently severe to warrant invasive testing. Three of the eight that chose the prenatal diagnosis route were affected by a condition, and two pregnancies were terminated. Disease severity was found to be a significant association with changes in decision making. Thirteen respondents did not plan to use the results from the carrier screening and four responses were unclear (Ghiossi et al., 2018).

Kamath et al. (2019) analyzed 207,697 data sets from women undergoing single-embryo transfer after PGT or IVF without PGT between the year 2000 and 2016. Results showed a significantly higher incidence of zygotic splitting following PGT (2.4%) compared to following non-PGT IVF (1.5%); this shows "a likely increased risk of monozygotic splitting following embryo biopsy" (Kamath et al., 2019) and highlights a potential risk with PGT embryonic biopsies.

A new study was published which compared the live birth rates of embryos fertilized without chromosome analysis compared to those analyzed via PGT-A and comprehensive chromosome screening of the first and second polar body (Verpoest et al., 2018). All mothers were of advanced maternal age between 36 and 40 years old. A total of 396 women enrolled in this multicenter, randomized clinical trial. Two hundred and five women had chromosomal screening, and 50 (24%) had a live birth within a year; in the group without intervention, which was comprised of 191 women, 45 (24%) had a live birth within a year (Verpoest et al., 2018). It is important to note that the groups had a slightly different number of participants. This study shows that PGT-A allows for similar birth rates when compared to embryos fertilized without chromosome analysis via intracytoplasmic sperm injection (ICSI). However, the authors warn that "Whether these benefits outweigh drawbacks such as the cost for the patient, the higher workload for the IVF lab and the potential effect on the children born after prolonged culture and/or cryopreservation remains to be shown" (Verpoest et al., 2018).

A meta-analysis focusing on evaluating the effectiveness and safety of PGT-A in women undergoing an IVF treatment was conducted in 2020. Thirteen randomized controlled trials—involving a total of 2794 women—reporting data on clinical outcomes were included. The meta-analysis concluded that there existed insufficient evidence for preimplantation genetic testing for abnormal chromosomes numbers to provide a difference in cumulative live birth rate, live birth rate after the first embryo transfer, or miscarriage rate between IVF with and IVF without PGT-A as currently performed, and therefore "the effect of PGT-A on clinical pregnancy rate is uncertain." The evidence evinced that though the observed cumulative live birth rate (cLBR) was 24% in the control group, the chance of live birth following the results of one IVF cycle with PGT-A is between 17% and 34%. Similarly, trials focusing on IVF with addition of PGT-A boasted an average cLBR of 29% in the control group, but the chance of live birth following the results of one IVF cycle with PGT-A was between 12% and 29%. When PGT-A is performed with FISH, the chance of live births after the first transfer in the control group (31%) fell to between 16% and 29% for those tested. Thus, the authors caution that "Women need to be aware that it is uncertain whether PGT-A with the use of genome-wide analyses is an effective addition to IVF, especially in view of the invasiveness and costs involved in PGT-A", going so far as to state that "PGT-A using FISH for the genetic analysis is probably harmful" (Cornelisse et al., 2020).

Next generation sequencing can be used for PGS to screen for aneuploidies in IVF scenarios. A study by Yap et al. (2019) analyzed results from a total of 391 IVF pregnancies whose embryos were cultured to the blastocyst stage; a total of 1361 blastocysts were analyzed (Yap et al., 2019). Of the 1361 blastocysts, 423 were identified as aneuploid, 723 as euploid and 216 as mosaic (contained varying cell lines) (Yap et al., 2019). These results show that next generation sequencing can be used to identify mosaic and aneuploid blastocysts and is an effective PGS tool.

Zeevi et al. (2021) studied the clinical validity of Haploseek, a method for preimplantation genetic testing and compared the results to polymerase chain reaction (PCR)-based PGT case results. A total of 151 embryo biopsies from 27 PGT cases were obtained and sequenced using Haploseek to predict the chromosome copy-number variants (CNVs) and relevant variant-flanking haplotypes in each embryo. For each of the 151 embryo biopsies, all Haploseek-derived haplotypes and CNVs were concordant with clinical PGT results. The authors conclude that "Haploseek is clinically accurate and fit for all standard clinical PGT applications" (Zeevi et al., 2021).

Kumar et al. (2022) noted the lack of comprehensive embryo genetic assessment in PGT. The authors used parental genome sequencing and embryo genotyping to create a whole-genome reconstruction. Using a combination of molecular and statistic techniques, the authors could infer inherited genome sequences and model

susceptibility to common conditions. The study included 110 embryos from ten couples and investigated 12 common conditions including cancer and autoimmune diseases. The method resulted in "genotype accuracy of 99.0–99.4% at sites relevant to polygenic risk scoring in cases from day-5 embryo biopsies and 97.2–99.1% in cases from day-3 embryo biopsies." The authors conclude that these results can "inform the discussion of utility and implementation of genome-based PGT in clinical practice" (Kumar et al., 2022).

VI. Guidelines and Recommendations

American College of Obstetricians and Gynecologists (ACOG)

In 2017, the ACOG noted that if a carrier couple (carriers for the same condition) is identified, genetic counselling is encouraged so that options such as preimplantation genetic diagnosis or prenatal diagnosis may be discussed. This guideline was reaffirmed in 2023 (ACOG, 2017).

In 2020, the ACOG published a series of recommendations in their "ACOG Committee Opinion" Number 799. The committee opinion was reaffirmed in 2023. These recommendations are shortened for brevity and reported below:

- "Preimplantation genetic testing-monogenic uses only a few cells from the early embryo, usually at the blastocyst stage, and misdiagnosis is possible but rare with modern techniques. Confirmation of preimplantation genetic testing-monogenic results with chorionic villus sampling (CVS) or amniocentesis should be offered."
- "To detect structural chromosomal abnormalities such as translocations, preimplantation genetic testing-structural rearrangements (known as PGT-SR) is used. Confirmation of preimplantation genetic testing-structural rearrangements results with CVS or amniocentesis should be offered."
- "The main purpose of preimplantation genetic testing-aneuploidy (known as PGT-A) is to screen embryos for whole chromosome abnormalities. Traditional diagnostic testing or screening for aneuploidy should be offered to all patients who have had preimplantation genetic testing-aneuploidy, in accordance with recommendations for all pregnant patients" (ACOG et al., 2020).

European Society for Human Reproduction and Embryology (ESHRE) PGD Consortium

In 2010, the ESHRE issued detailed guidelines related to technical aspects of PGD, specifically for the use of amplification techniques and for FISH. The ESHRE recommends that "misdiagnosis rates should be calculated for each type of assay and for all assays from a particular Centre." Additionally, they note that "Follow-up of

pregnancies (including multiple pregnancy rate and outcome), deliveries, and the health of children at birth and beyond should be attempted and maintained along with the cycle data" (Harton et al., 2010).

In 2020, the ESHRE expanded upon their practice recommendations for preimplantation genetic testing. For the organization of PGT, the ESHRE provided patient inclusion/exclusion criteria. In general, "It is recommended that PGT is only applied when genetic diagnosis is technically feasible, and the reliability of the diagnosis is high. Current procedures in most IVF/PGT centres allow for overall error rates (resulting in misdiagnosis) as low as 1 to 3%. Each centre should be aware of their error rates and include this information in their informed consents and reports in an open communication with the patient.

When considering PGT, safety issues, female age, impossibility to retrieve male or female gametes, body mass index (BMI) and other contraindications for IVF should be considered as possible exclusion criteria.

Furthermore, exclusion from PGT should be considered if the woman has serious signs and symptoms of an autosomal dominant or X-linked disorder (for which PGT is requested), which could introduce medical complications during ovarian stimulation, oocyte retrieval or pregnancy or medical risks at birth. PGT should be carefully considered if one of the partners has serious physical or psychological problems, either linked to the tested disease or due to other conditions."

Different preimplantation genetic testing for specific defects and disorders carried their own caveats and recommendations in terms of inclusion and exclusion of patients:

For *PGT-M, mitochondrial disorders and HLA*: "Cases of genetic variants of unknown significance that are not predictive of a phenotype should be excluded from PGT. PGT testing is inappropriate in case of uncertain genetic diagnosis (for example genetic/molecular heterogeneity), or in case of uncertain mode of inheritance.

For autosomal recessive disorders, where a single pathogenic variant has been diagnosed in the proband and only one parent, it is acceptable to offer PGT if the pathogenic genotype is attributed to a single gene and sufficient evidence from the family pedigree allows identification of the disease-associated haplotypes. Similarly, it is acceptable to offer PGT for known X-linked recessive single gene disorders with a clear unequivocal clinical diagnosis where no pathogenic variant was found in the proband but low- and high-risk haplotypes can be identified based on the family history.

Exclusion or non-disclosure testing can be indicated for late-onset disorders, such as Huntington's disease, to avoid pre-symptomatic testing of the partner with a family history of the disease. Exclusion testing is preferred over PGT with non-disclosure of the direct test results to the couple."

For *PGT for mitochondrial disorders*: "PGT is not indicated in case of homoplasmy. In cases where the causative pathogenic variant of the mitochondrial disease is encoded by nuclear DNA, testing is the same as for other monogenic disorders."

For *HLA Typing*: "When all other clinical options have been exhausted, selection of HLA-matched embryos via PGT is acceptable for couples who already have a child affected with a malignant, acquired disorder or a genetic disorder where the affected child is likely to be cured or life expectancy is substantially prolonged by transplantation with stem cells from an HLA-matched sibling. Testing can be performed for HLA typing alone, if the recurrence risk of the disease is low, or in combination with autosomal dominant/recessive or X-linked disorders."

For *PGT-SR*: "Depending on the technology used (FISH, quantitative real-time PCR (qPCR), comprehensive testing methods [array-based comparative genomic hybridisation (aCGH), single nucleotide polymorphism (SNP) array or next generation sequencing (NGS)]), different inclusion/exclusion criteria may apply. In general, PGT-SR is only recommended if the technique applied is able to detect all expected unbalanced forms of the chromosomal rearrangement. When comprehensive testing strategies are applied, it is acceptable to use information on copy number of nonindication chromosomes to refine embryo transfer strategies."

For *PGT-A*: "For all, but in particular for RIF, RM and SMF couples, a previous karyotype of both partners is recommended since there is a higher chance of structural rearrangements for these indications. If an abnormal karyotype is identified, the technology for the detection of unbalanced abnormalities can differ from the regular PGT-A" (ESHRE PGT Consortium Steering Committee et al., 2020).

Ethics Committee of the American Society for Reproductive Medicine (ASRM)

The ASRM ethics committee has published several opinion guidelines over the years.

In 2018, the ASRM published a committee opinion on the use of preimplantation genetic testing for monogenic defects (PGT-M) for adult-onset conditions. These guidelines stated the following:

- "Preimplantation genetic testing for monogenic disease (PGT-M) for adult-onset conditions is ethically justified when the condition is serious and no safe, effective interventions are available."

- "Reproductive liberty arguments ethically allow for PGT-M for adult-onset conditions of lesser severity or penetrance. In the latter cases, the application of the technology hinges on the evidence that PGT-M is a relatively low-risk procedure; this evidence may change."
- "The Committee to strongly recommend that an experienced genetic counselor with knowledge about PGT-M play a major role in counseling patients considering such procedures" (ASRM, 2018).

The ASRM also published an updated committee opinion in July 2023 detailing indications and management of preimplantation genetic testing for monogenic conditions, not limited to adult onset. These recommendations state the following:

- "Suggested categorization of PGT-M indications (derived from table):
  - Childhood-onset, lethal or severe condition
  - Serious adult-onset conditions
  - Mild conditions or indications of limited or questionable risk reduction:
    - Low penetrance or susceptibility genes
    - Mild genetic variants
    - Carrier status for autosomal recessive conditions with carrier manifestations
    - Mild, common, and/or treatable conditions
    - Variants appearing to be de novo in an affected child
    - Variants of uncertain significance
- Indications for which PGT-M is not recommended:
  - Autosomal recessive carrier status without manifestations of symptoms
  - Combination of variants not associated with disease
  - Pseudodeficiency alleles
  - Somatic-only variants"
- "Preimplantation genetic testing for monogenic conditions should be offered if a significant reproductive risk is identified. Acceptance of PGT-M should be optional"

However, with these categorizations, the committee reiterates some key considerations surrounding the application of PGT-M and the ethical arguments in certain situations: "The initial application of PGT-M was primarily to prevent the transmission of severe, untreatable, or life-threatening childhood-onset conditions. Today, however, the use of the technology extends to a much broader range of genetic conditions for which support of PGT-M is less clear or even controversial, including those with mild to moderate phenotypes and later age of onset and of much greater clinical variability and/or reduced penetrance. A survey of laboratory genetic counselors in 2021 found that all participants believed that PGT-M should be

allowed for conditions of lower penetrance, citing patient autonomy as a primary consideration. At this time, no guidelines exist to offer direction to those involved in determining when to offer or decline to perform PGT-M, although some ethical arguments exist. As such, physicians, genetic counselors, and PGT laboratories may develop internal policies regarding PGT-M availability within their institution. If certain types of conditions or clinical scenarios are deemed not appropriate for PGT-M, the patient should be informed of the policy as early in the process as possible." (Practice Committee and Genetic Counseling Professional Group of the American Society for Reproductive Medicine, 2023).

Similar guidelines were also published in 2013 by the ASRM regarding the use of PGD for serious adult-onset conditions:

- "Preimplantation genetic diagnosis (PGD) for adult-onset conditions is ethically justifiable when the conditions are serious and when there are no known interventions for the conditions or the available interventions are either inadequately effective or significantly burdensome.
- For conditions that are less serious or of lower penetrance, PGD for adult onset conditions is ethically acceptable as a matter of reproductive liberty. It should be discouraged, however, if the risks of PGD are found to be more than merely speculative.
- Physicians and patients should be aware that much remains unknown about the long-term effects of embryo biopsy on any developing fetus. Though thought to be without serious side effects, PGD for adult onset diseases of variable penetrance should only be considered after patients are carefully and thoroughly counseled to weigh the risks of what is unknown about the technology and the biopsy itself against the expected benefit of its use.
- It is important to involve the participation of a genetic counselor experienced in such conditions before patients undertake PGD. Counseling should also address the patient specific prognosis for achieving pregnancy and birth through in vitro fertilization (IVF) with PGD" (Ethics Committee of the American Society for Reproductive Medicine ASRM, 2013).

Additional guidelines were published in 2008, which stated the following:

- "Before PGD is performed, genetic counseling must be provided to ensure that patients fully understand the risk for having an affected child, the impact of the disease on an affected child, and the limitations of available options that may help to avoid the birth of an affected child.

- Prenatal diagnostic testing to confirm the results of PGD is encouraged strongly because the methods used for PGD have technical limitations that include the possibility for a false negative result.
- Available evidence does not support the use of PGS as currently performed to improve live-birth rates in patients with advanced maternal age.
- Available evidence does not support the use of PGS as currently performed to improve live-birth rates in patients with previous implantation failure.
- Available evidence does not support the use of PGS as currently performed to reduce miscarriage rates in patients with recurrent pregnancy loss related to aneuploidy" (ASRM, 2008).

Preimplantation Genetic Diagnosis International Society (PGDIS)

In the report from the 2021 PGDIS Expert Consultation on Mosaic Embryo Transfer, the PGDIS published a position statement on the transfer of mosaic embryos, including the following recommendations for clinicians:

- "Patients should continue to be advised that any genetic test based on sampling one or small number of cells biopsied from preimplantation embryos cannot be 100% accurate because of a combination of technical and biological factors, including cell mosaicism;
- Patient information and consent forms for aneuploidy testing should be modified to include the possibility of mosaic results; and
- In general, transfer of blastocysts with a normal euploid result should be prioritized over those with mosaic results unless other indications, such as patient preference, are raised" (Leigh et al., 2022).

American College of Medical Genetics and Genomics (ACMG)

The ACMG has released guidelines on prenatal/preconception carrier screening, primarily when to test:

- "Disorders should be of a nature that most at-risk patients and their partners identified in the screening program would consider having a prenatal diagnosis to facilitate making decisions surrounding reproduction.
- For each disorder, the causative gene(s), mutations, and mutation frequencies should be known in the population being tested, so that meaningful residual risk in individuals who test negative can be assessed."
- There must be validated clinical association between the mutation(s) detected and the severity of the disorder" (Grody et al., 2013).

In 2021 the ACMG published recommendations on screening for autosomal recessive and X-linked conditions during pregnancy and preconception. They reference an overlapping, tiered approach to testing defined as:

- Tier 1: Cystic fibrosis, spinal muscular atrophy, and risk based screening.
- Tier 2: "Conditions that have a severe or moderate phenotype and a carrier frequency of at least 1/100." (Includes Tier 1).
- Tier 3: "Conditions with a carrier frequency ≥ 1/200." (Includes Tier 2). Includes X-linked conditions.
- Tier 4: "Genes less common than those in Tier 3 and can identify additional at-risk couples." Conditions with a carrier frequency <1/200. (Includes Tier 3).

In terms of what screening approaches should be offered, the ACMG recommends that "all pregnant patients and those planning a pregnancy should be offered Tier 3 carrier screening. Tier 4 screening should be considered: when a pregnancy stems from a known or possible consanguineous relationship (second cousins or closer); [or] when a family or personal medical history warrants." The ACMG does NOT recommend "Offering Tier 1 and/or Tier 2 screening, because these do not provide equitable evaluation of all racial/ethnic groups [or] routine offering of Tier 4 panels."

In terms of what autosomal recessive conditions are appropriate for carrier screening, the ACMG recommends that "All pregnant patients and those planning a pregnancy should be offered Tier 3 carrier screening for autosomal recessive and X-linked conditions. Reproductive partners of pregnant patients and those planning a pregnancy may be offered Tier 3 carrier screening for autosomal recessive conditions when carrier screening is performed simultaneously with their partner."

In terms of which X-linked conditions are appropriate for carrier screening, they note that "All XX patients should be offered screening for only those X-linked genes [listed here] as part of Tier 3 screening." The X-linked genes are: *ABCD1, AFF2, ARX, DMD, F8, F9, FMR1, GLA, L1CAM, MID1, NR0B1, OTC, PLP1, PRGR, RS1, SLC6A8*.

Lastly, the ACMG notes the "critical" importance of education and counseling in carrier screening and recommends that "carrier screening counseling should be provided by knowledgeable and appropriately trained health-care professionals and should be performed pre- and post-test" (Gregg et al., 2021).

British Fertility Society (BFS) Policy and Practice Guidelines

The BFS have published guidelines regarding PGS. These guidelines state that "It remains possible that PGS may be of benefit under certain circumstances. However at present patients should be informed that there is no robust evidence that PGS for

advanced maternal age improves live birth rate per cycle started, and PGS should preferably be offered within the context of robustly designed randomised trials performed in suitably experienced centres" (Anderson & Pickering, 2008).

Indian Society for Assisted Reproduction (ISAR)

The Indian Society for Assisted Reproduction released consensus guidelines about preimplantation genetic testing in *In vitro* fertilization clinics in India. The recommendations for PGT-A are: "PGT-A is recommended for: advanced maternal age (36–40 years), or repeated pregnancy loss – known etiologies. PGT-A is not recommended for: young, good prognosis patients (<35 years), or unexplained RPL, or low AMH – limited eggs; multiple IVF cycles may be necessary in order to obtain one euploid blastocyst."

The clinical recommendations for genetic testing for monogenic indications are:

- "Can be offered to all patients with single or multiple gene disorders with a positive mutation report
- Cannot be offered for diseases which as multifactorial and nongenetic based diseases
- In case PGT-A is desired, it should be performed on PGT-M screened embryos" (Malhotra et al., 2021).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81228	Cytogenomic (genome-wide) analysis for constitution chromosomal abnormalities; interrogation of genomic regions for copy number variants, comparative genomic hybridization [CGH] microarray analysis
81229	Cytogenomic (genome-wide) analysis for consititutional chromosomal abnormalties; interrogation og genomic regions for copy number and single nucleotide polymorphism (SNP) variants, comparative genomic hybridization (CGH) microarray analysis
81349	Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of genomic regions for copy number and loss-of-heterozygosity variants, low-pass sequencing analysis
81479	Unlisted molecular pathology procedure
0254U	Reproductive medicine (preimplantation genetic assessment), analysis of 24 chromosomes using embryonic DNA genomic sequence analysis for aneuploidy, and a mitochondrial DNA score in euploid embryos, results reported as normal (euploidy), monosomy, trisomy, or partial deletion/duplications, mosaicism, and segmental aneuploidy, per embryo tested Proprietary test: SMART PGT-A (Pre-implantation Genetic Testing - Aneuploidy) Lab/Manufacturer: Igenomix®
0396U	Obstetrics (pre-implantation genetic testing), evaluation of 300000 DNA single-nucleotide polymorphisms (SNPs) by microarray, embryonic tissue, algorithm reported as a probability for single-gene germline conditions Proprietary test: Spectrum PGT-M Lab/Manufacturer: Strata Oncology, Inc

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

ACOG. (2017). Carrier Screening in the Age of Genomic Medicine. https://www.acog.org/clinical/clinical-guidance/committee-opinion/articles/2017/03/carrier-screening-in-the-age-of-genomic-medicine

ACOG, Klugman, S., & Rollene, N. (2020). Preimplantation Genetic Testing. *Obstetrics & Gynecology*, *135*(3). https://www.acog.org/-

/media/project/acog/acogorg/clinical/files/committee-opinion/articles/2020/03/preimplantation-genetic-testing.pdf
Alteri, A., Corti, L., Sanchez, A. M., Rabellotti, E., Papaleo, E., & Vigano, P. (2019). Assessment of pre-implantation genetic testing for embryo aneuploidies: A SWOT analysis. *Clin Genet*, *95*(4), 479-487. https://doi.org/10.1111/cge.13510
Anderson, R. A., & Pickering, S. (2008). The current status of preimplantation genetic screening: British Fertility Society Policy and Practice Guidelines. *Hum Fertil (Camb)*, *11*(2), 71-75. https://doi.org/10.1080/14647270802041607
ASRM. (2008). Preimplantation genetic testing: a Practice Committee opinion. *Fertil Steril*, *90*(5 Suppl), S136-143. https://doi.org/10.1016/j.fertnstert.2008.08.062
ASRM. (2013). Use of preimplantation genetic diagnosis for serious adult onset conditions: a committee opinion. *Fertil Steril*, *100*(1), 54-57. https://doi.org/10.1016/j.fertnstert.2013.02.043
ASRM. (2015). Use of reproductive technology for sex selection for nonmedical reasons. *Fertil Steril*, *103*(6), 1418-1422. https://doi.org/10.1016/j.fertnstert.2015.03.035
ASRM. (2018). *Use of preimplantation genetic testing for monogenic defects (PGT-M) for adult-onset conditions: an Ethics Committee opinion*. https://www.asrm.org/globalassets/asrm/asrm-content/news-and-publications/ethics-committee-opinions/use-of-pgt-for-monogenic-defects-foradult-onset-conditions.pdf
Brezina, P. R., Anchan, R., & Kearns, W. G. (2016). Preimplantation genetic testing for aneuploidy: what technology should you use and what are the differences? *J Assist Reprod Genet*, *33*(7), 823-832. https://doi.org/10.1007/s10815-016-0740-2
Cornelisse, S., Zagers, M., Kostova, E., Fleischer, K., Wely, M., & Mastenbroek, S. (2020). Preimplantation genetic testing for aneuploidies (abnormal number of chromosomes) in in vitro fertilisation. *Cochrane Database of Systematic Reviews*(9). https://doi.org/10.1002/14651858.CD005291.pub3
Dreesen, J., Destouni, A., Kourlaba, G., Degn, B., Mette, W. C., Carvalho, F., Moutou, C., Sengupta, S., Dhanjal, S., Renwick, P., Davies, S., Kanavakis, E., Harton, G., & Traeger-Synodinos, J. (2014). Evaluation of PCR-based preimplantation genetic diagnosis applied to monogenic diseases: a collaborative ESHRE PGD consortium study. *Eur J Hum Genet*, *22*(8), 1012-1018. https://doi.org/10.1038/ejhg.2013.277
ESHRE PGT Consortium Steering Committee, Carvalho, F., Coonen, E., Goossens, V., Kokkali, G., Rubio, C., Meijer-Hoogeveen, M., Moutou, C., Vermeulen, N., & De Rycke, M. (2020). ESHRE PGT Consortium good practice recommendations for the organisation of PGT†. *Human Reproduction Open*, *2020*(3). https://doi.org/10.1093/hropen/hoaa021
Ethics Committee of the American Society for Reproductive Medicine ASRM. (2013). Use of preimplantation genetic diagnosis for serious adult onset conditions: a committee opinion. *Fertil Steril*, *100*(1), 54-57. https://doi.org/10.1016/j.fertnstert.2013.02.043

Farra, C., Choucair, F., & Awwad, J. (2018). Non-invasive pre-implantation genetic testing of human embryos: an emerging concept. *Hum Reprod*, *33*(12), 2162-2167. https://doi.org/10.1093/humrep/dey314

Feldman, B., Aizer, A., Brengauz, M., Dotan, K., Levron, J., Schiff, E., & Orvieto, R. (2017). Pre-implantation genetic diagnosis-should we use ICSI for all? *J Assist Reprod Genet*, *34*(9), 1179-1183. https://doi.org/10.1007/s10815-017-0966-7

García-Herrero, S., Martínez-Fernández, A., Marin, L., Nieto, J., Campos-Gallindo, I., Peinado, V., García-Pascual, C., Rodrigo, L., Rubio, C., & Simón, C. (2019). New high-throughput semiautomated Next Generation Sequencing (NGS) platform for Pre-implantation Genetic Testing for aneuploidies (PGT-A). *Reprod Biomed Online*, *38*. https://www.sciencedirect.com/science/article/abs/pii/S1472648319301543

Ghiossi, C. E., Goldberg, J. D., Haque, I. S., Lazarin, G. A., & Wong, K. K. (2018). Clinical Utility of Expanded Carrier Screening: Reproductive Behaviors of At-Risk Couples. *J Genet Couns*, *27*(3), 616-625. https://doi.org/10.1007/s10897-017-0160-1

Gregg, A. R., Aarabi, M., Klugman, S., Leach, N. T., Bashford, M. T., Goldwaser, T., Chen, E., Sparks, T. N., Reddi, H. V., Rajkovic, A., & Dungan, J. S. (2021). Screening for autosomal recessive and X-linked conditions during pregnancy and preconception: a practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *23*(10), 1793-1806. https://doi.org/10.1038/s41436-021-01203-z

Grody, W. W., Thompson, B. H., Gregg, A. R., Bean, L. H., Monaghan, K. G., Schneider, A., & Lebo, R. V. (2013). ACMG position statement on prenatal/preconception expanded carrier screening. *Genet Med*, *15*(6), 482-483. https://doi.org/10.1038/gim.2013.47

Harton, G. L., De Rycke, M., Fiorentino, F., Moutou, C., SenGupta, S., Traeger-Synodinos, J., & Harper, J. C. (2010). ESHRE PGD consortium best practice guidelines for amplification-based PGD†. *Human Reproduction*, *26*(1), 33-40. https://doi.org/10.1093/humrep/deq231

iGLS. (2022). Preimplantation Genetic Testing for Aneuploidy. https://www.igls.net/services/pgt-a/

Kamath, M. S., Antonisamy, B., & Sunkara, S. K. (2019). Zygotic splitting following embryo biopsy: a cohort study of 207 697 single-embryo transfers following IVF treatment. *Bjog*. https://doi.org/10.1111/1471-0528.16045

Kumar, A., Im, K., Banjevic, M., Ng, P. C., Tunstall, T., Garcia, G., Galhardo, L., Sun, J., Schaedel, O. N., Levy, B., Hongo, D., Kijacic, D., Kiehl, M., Tran, N. D., Klatsky, P. C., & Rabinowitz, M. (2022). Whole-genome risk prediction of common diseases in human preimplantation embryos. *Nat Med*, *28*(3), 513-516. https://doi.org/10.1038/s41591-022-01735-0

Ledger, W. (2019). Preimplantation genetic screening should be used in all in vitro fertilisation cycles in women over the age of 35 years: AGAINST: Pre-implantation genetic screening should not be used in all IVF cycles in women over the age of 35 years. *Bjog*, *126*(13), 1555. https://doi.org/10.1111/1471-0528.15942

Leigh, D., Cram, D. S., Rechitsky, S., Handyside, A., Wells, D., Munne, S., Kahraman, S., Grifo, J., Katz-Jaffe, M., Rubio, C., Viotti, M., Forman, E., Xu, K., Gordon, T., Madjunkova, S., Qiao, J., Chen, Z. J., Harton, G., Gianaroli, L., . . . Kuliev, A. (2022). PGDIS position statement on the transfer of mosaic embryos 2021. *Reprod Biomed Online*, *45*(1), 19-25. https://doi.org/10.1016/j.rbmo.2022.03.013

Malhotra, J., Malhotra, K., Majumdar, G., Hari, R., Chelur, V., Kandari, S., Sharma, D., Chimote, N., Mehta, M. S., Singh, S., Sethi, F., Mangoli, V. S., Gopinath, P., Chaitanya, K., & Selvaraj, P. (2021). Indian Society for Assisted Reproduction Consensus Guidelines on Preimplantation Genetic Testing in In vitro Fertilization Clinics. *J Hum Reprod Sci*, *14*(Suppl 1), S31-s47. https://doi.org/10.4103/0974-1208.330503

Montazeri, F., Foroughmand, A. M., Kalantar, S. M., Aflatoonian, A., & Khalilli, M. A. (2018). Tips and Tricks in Fluorescence In-situ Hybridization (FISH)-based Preimplantation Genetic Diagnosis/Screening (PGD/PGS). *International Journal of Medical Laboratory*, *5*, 84-98. https://pdfs.semanticscholar.org/961d/7648113976ca31c7655e29b471078bd1026b.pdf

Natera. (2022). *Spectrum®*. https://www.natera.com/spectrum-pgt

PacGenomics. (2022). Preimplantation Genetic Testing (PGT). https://pacgenomics.com/pgt/

PGDIS. (2008). Guidelines for good practice in PGD: programme requirements and laboratory quality assurance. *Reprod Biomed Online*, *16*(1), 134-147. https://www.rbmojournal.com/article/S1472-6483(10)60567-6/pdf

Practice Committee and Genetic Counseling Professional Group of the American Society for Reproductive Medicine. (2023). Indications and management of preimplantation genetic testing for monogenic conditions: a committee opinion. *Fertil Steril*, *120*(1), 61-71. https://doi.org/10.1016/j.fertnstert.2023.03.003

RGI. (2022). What is PGT. https://rgiscience.com/

Schattman, G. L., & Xu, K. (2024, January 10, 2024). *Preimplantation genetic testing*. https://www.uptodate.com/contents/preimplantation-genetic-testing

Scott, Upham, Forman, Zhao, & Treff, N. R. (2013). Cleavage-stage biopsy significantly impairs human embryonic implantation potential while blastocyst biopsy does not: a randomized and paired clinical trial. *Fertil Steril*, *100*(3), 624-630. https://doi.org/10.1016/j.fertnstert.2013.04.039

Scott, K. L., Hong, K. H., & Scott, R. T., Jr. (2013). Selecting the optimal time to perform biopsy for preimplantation genetic testing. *Fertil Steril*, *100*(3), 608-614. https://doi.org/10.1016/j.fertnstert.2013.07.004

Simon, A. L., Kiehl, M., Fischer, E., Proctor, J. G., Bush, M. R., Givens, C., Rabinowitz, M., & Demko, Z. P. (2018). Pregnancy outcomes from more than 1,800 in vitro fertilization cycles with the use of 24-chromosome single-nucleotide polymorphism-based

preimplantation genetic testing for aneuploidy. *Fertil Steril*, *110*(1), 113-121. https://doi.org/10.1016/j.fertnstert.2018.03.026

Stern, H. J. (2014). Preimplantation Genetic Diagnosis: Prenatal Testing for Embryos Finally Achieving Its Potential. *J Clin Med*, *3*(1), 280-309. https://doi.org/10.3390/jcm3010280

Sullivan-Pyke, C., & Dokras, A. (2018). Preimplantation Genetic Screening and Preimplantation Genetic Diagnosis. *Obstet Gynecol Clin North Am*, *45*(1), 113-125. https://doi.org/10.1016/j.ogc.2017.10.009

Vaiarelli, A., Cimadomo, D., Capalbo, A., Orlando, G., Sapienza, F., Colamaria, S., Palagiano, A., Bulletti, C., Rienzi, L., & Ubaldi, F. M. (2016). Pre-implantation genetic testing in ART: who will benefit and what is the evidence? *J Assist Reprod Genet*, *33*(10), 1273-1278. https://doi.org/10.1007/s10815-016-0785-2

Verpoest, W., Staessen, C., Bossuyt, P. M., Goossens, V., Altarescu, G., Bonduelle, M., Devesa, M., Eldar-Geva, T., Gianaroli, L., Griesinger, G., Kakourou, G., Kokkali, G., Liebenthron, J., Magli, M. C., Parriego, M., Schmutzler, A. G., Tobler, M., van der Ven, K., Geraedts, J., & Sermon, K. (2018). Preimplantation genetic testing for aneuploidy by microarray analysis of polar bodies in advanced maternal age: a randomized clinical trial. *Hum Reprod*, *33*(9), 1767-1776. https://doi.org/10.1093/humrep/dey262

Yap, W., Lee, C., Chan, W., & Lim, Y. (2019). Detection of Mosaicism in Blastocyst using High Resolution Next Generation Sequencing Preimplantation Genetic Screening (hr-NGS). *Reprod Biomed Online*, *38*. https://www.sciencedirect.com/science/article/abs/pii/S1472648319301671

Zeevi, D. A., Backenroth, D., Hakam-Spector, E., Renbaum, P., Mann, T., Zahdeh, F., Segel, R., Zeligson, S., Eldar-Geva, T., Ben-Ami, I., Ben-Yehuda, A., Carmi, S., & Altarescu, G. (2021). Expanded clinical validation of Haploseek for comprehensive preimplantation genetic testing. *Genetics in Medicine*, *23*(7), 1334-1340. https://doi.org/10.1038/s41436-021-01145-6

Prenatal Screening (Genetic)

Policy Number: AHS – M2179– Prenatal Screening (Genetic)	Prior Policy Name and Number, as applicable:
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Prenatal screening encompasses any testing done to determine the health status of the pregnant individual and/or fetus. Genetic prenatal screening encompasses screening to determine risk of fetal abnormalities, including genetic and developmental abnormalities. Any individual undergoing screening tests, especially genetic carrier screenings, must realize the limitations of screening tests and the difference between screening and diagnostic testing. Screening refers to testing of asymptomatic or healthy individuals to search for a condition that may affect the pregnancy or individual, whereas diagnostic testing is used to either confirm or refute true abnormalities in an individual (Grant & Mohide, 1982; Lockwood & Magriples, 2023).

This policy addresses broad prenatal genetic screening, as well as screening for conditions not addressed in condition-specific policies. For situations in which prenatal and preconception screening may be discussed in further detail, please see the "Related Policies" section of this policy document.

Terms such as male and female are used when necessary to refer to sex assigned at birth.

II. Related Policies

Policy Number	Policy Title
AHS-G2035	Prenatal Screening (Nongenetic)
AHS-G2055	Prenatal Screening for Fetal Aneuploidy
AHS-G2148	Genetic Testing for Hereditary Hearing Loss
AHS-M2017	Genetic Testing for Cystic Fibrosis
AHS-M2024	Genetic Testing for Polyposis Syndromes
AHS-M2028	Genetic Testing for *FMR1* Mutations

AHS-M2033	Chromosomal Microarray and Low-pass Whole Genome
AHS-M2039	Pre-Implantation Genetic Testing
AHS-M2077	Genetic Testing for Fanconi Anemia
AHS-M2167	Genetic Testing for Neurodegenerative Disorders
AHS-M2170	Red Blood Cell Molecular Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals who are pregnant **or** who are capable of becoming pregnant and seeking pre-conception care, single gene or multi-gene panel screening of the individual for conditions classified through ACMG as a Tier 1, Tier 2, or Tier 3 condition (see Note 1) **MEETS COVERAGE CRITERIA**.

2) For pregnant individuals and those capable of becoming pregnant who come from a family with a genetic disorder for which a properly validated test is available, the following testing **MEETS COVERAGE CRITERIA**:

   a) Testing restricted to the known mutation.

   b) Comprehensive genetic testing, including multi-gene panel testing specific to the familial genetic disorder, when the specific familial mutation is unknown.

3) For individuals planning a pregnancy with a reproductive partner who is known or found to be a carrier of a recessively inherited disorder, genetic testing specific to the genes for which the reproductive partner is a carrier **MEETS COVERAGE CRITERIA**.

4) For RHD negative pregnant individuals, fetal RHD genotyping using maternal plasma **MEETS COVERAGE CRITERIA**.

5) For fetuses with a high risk for a genetic disorder, prenatal genetic testing using cells obtained for diagnostic cytogenetic testing (i.e., amniocentesis or chorionic villus sampling [CVS]) **MEETS COVERAGE CRITERIA**.

6) Carrier screening for the same gene more than once per lifetime **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

7) To screen for single-gene mutations (i.e., autosomal recessive, autosomal dominant, X-linked) in the fetus, the use of non-invasive prenatal screening (NIPS) **DOES NOT MEET COVERAGE CRITERIA.**

8) For all other inherited medical disorders not meeting the above criteria, pre-conceptional or prenatal genetic testing **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Please see the "Guidelines and Recommendations" section of this policy for ACMG's tiered system based on carrier frequency (Tables 1-6).

Note 2: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
ACMG	American College of Medical Genetics and Genomics
ACOG	American College of Obstetricians and Gynecologists
ADA	American Diabetes Association
CAP	College of American Pathologists
CAVD	Congenital absence of the vas deferens
cfDNA	Cell-free deoxyribonucleic acid
CFTR	*CF transmembrane conductance regulator*
CMA	Chromosomal microarray
CNVs	Copy number variants
CVS	Chorionic villus sampling
DMD	Dystrophin
DNA	Deoxyribonucleic acid
FRAXE	Fragile site, folic acid type, rare, Fra(X)(Q28) E
GJB6	Gap junction protein
HBB	Human beta-globin gene
HDFN	Hemolytic disease of the fetus and newborn
ISPD	International Society for Prenatal Diagnosis
MMS	Microdeletion/microduplication syndromes
NGS	Next generation sequencing
NIPS-	Expanded non-invasive prenatal screening
NIPT	Non-invasive prenatal testing
NT	Nuchal translucency
PPVs	Positive predictive values
PQF	Perinatal Quality Foundation
RBC	Red blood cells

RHD	Rh blood group D antigen
sgNIPS	Single-gene noninvasive prenatal screening
SMA	Spinal muscular atrophy
SMFM	Society for Maternal Fetal Medicine
SMN1	*Survival of motor neuron 1*
SNP	Single nucleotide polymorphism
TMRC	Transfusion Medicine Resource Committee

V. Scientific Background

Prenatal screening is a part of overall prenatal care to promote optimal care of both mother and baby. Prenatal screening allows for assessment and monitoring of the fetus for the presence of congenital defects or disease. Various professional medical organizations provide guidelines for prenatal screening. "Screening is an offer on the initiative of the health system or society, rather than a medical intervention in answer to a patient's complaint or health problem. Screening aims at obtaining population health gains through early detection that enables prevention or treatment" (de Jong et al., 2015).

Genetic screening tests, including carrier screening for genetic mutations and fetal testing for chromosomal aneuploidy, can be a part of prenatal screening. Aneuploidy screening may be performed on cell-free DNA in maternal circulation or by examining maternal serum levels of specific biochemical markers for trisomy (Lockwood & Magriples, 2023). These non-invasive prenatal testing (NIPT) can possibly decrease the number of more invasive procedures and the risks of unwanted side effects. A chromosomal microarray (CMA) can screen all chromosomes in a single test and "can detect many very small variants that cannot be detected by traditional karyotyping" (de Jong et al., 2015). The American College of Obstetricians and Gynecologists (ACOG) recommends CMA for instances where the ultrasound of a fetus shows a major structural abnormality (ACOG, 2016a). CMA in this situation should be performed on DNA from amniotic fluid, chorionic villus cells, or cord blood, rather than on maternal serum cell-free DNA since the process does not include an amplification step and the maternal DNA signal would be many times higher than the fetal DNA (Miller, 2023).

Several companies, such as LabCorp, have developed panels to test for potential genetic mutations in pregnant individuals, or in individuals planning to become pregnant. This includes the Inheritest® Carrier Screening which encompasses six different panels to identify potential genetic mutations. These six panels include the Inheritest® 500 PLUS Panel (which screens 525 genes for several clinically relevant genetic disorders), the Inheritest® Comprehensive Panel (which screens for more than 110 disorders), the Inheritest® Ashkenazi Jewish Panel (which screens for more than 40 Ashkenazi Jewish related disorders), the Inheritest® Society-Guided Panel (which

screens for more than 13 disorders highlighted in the American College of Medical Genetics and Genomics and the American Congress of Obstetricians and Gynecologists guidelines), the Inheritest® Core Panel (which screens for cystic fibrosis, fragile X syndrome, and spinal muscular atrophy), and the Inheritest® CF/SMA (spinal muscular atrophy) Panel (which screens only for cystic fibrosis and spinal muscular atrophy) (LabCorp, 2023).

Additionally, the company BillionToOne has created a noninvasive prenatal screening test. UNITY Complete® uses cell-free DNA from a maternal blood draw and assesses for seven aneuploidies (trisomy 21, trisomy 18, trisomy 13, monosomy X, XXX, XXY, and XYY), and five recessive conditions (cystic fibrosis, spinal muscular atrophy, sickle cell disease, alpha thalassemia, and beta thalassemia). This screen functions in a sequential manner. First, the screen uses NGS of genomic DNA to assesses maternal carrier status for genes associated with the most common single-gene recessive disorders. If the pregnant individual is identified as a carrier for a pathogenic variant in one or more of these genes, the sample is then reflexed to single-gene noninvasive prenatal screening (sgNIPS). In sgNIPS, NGS is performed on cfDNA extracted from the original blood sample, from which fetal risk is calculated. Fetal risk assessment is summarized as low risk (fetal risk 1/500), high risk (fetal risk >1/4), increased risk or decreased risk (fetal risk between 1/500 and 1/4), or no result (BillionToOne, 2023; Hoskovec et al., 2023).

Red blood cell antigen discrepancy between a mother and fetus may also occur during pregnancy. This is known as hemolytic disease of the fetus and newborn (HDFN), and causes maternal antibodies to destroy the red blood cells of the neonate or fetus (Calhoun, 2023). Alloimmunization is the immune response which occurs in the mother due to foreign antigens after exposure to genetically foreign cells, occurring almost exclusively in mothers with type O blood. However, while ABO blood type incompatibility is identified in almost 15% of pregnancies, HDFN is only identified in approximately 4% of pregnancies (Calhoun, 2023). Another important inherited antigen sometimes found on the surface of red blood cells is known as the Rhesus (Rh)D antigen. During pregnancy and delivery, individuals who are RhD negative may be exposed to RhD positive fetal cells, which can lead to the development of anti-RhD antibodies. This exposure typically happens during delivery and affects subsequent pregnancies; infants with RhD incompatibility tend to experience a more severe form of HDFN than those with ABO incompatibility. The clinical presentation of HDFN may be mild (such as hyperbilirubinemia with mild to moderate anemia) to severe and life-threatening anemia (such as hydrops fetalis). Less severely affected infants may develop hyperbilirubinemia within the first day of life; infants with RhD HDFN may also present with symptomatic anemia requiring a blood transfusion. In more severe cases, infants with severe life-threatening anemia, such as hydrops fetalis, may exhibit shock at delivery requiring an emergent blood transfusion (Calhoun, 2023).

The administration of anti-D immune globulin has been able to dramatically reduce, but not eliminate, the number of RhD alloimmunization cases. "Anti-D immune globulin is manufactured from pooled plasma selected for high titers of IgG antibodies to D-positive erythrocytes" (Moise, 2024). Before the development of this anti-D immune globulin, it has been reported that 16% of pregnant RhD-negative individuals with two deliveries of RhD-positive ABO-compatible infants became alloimmunized. However, this rate falls to 1-2% with routine postpartum administration of a single dose of anti-D immune globulin. An additional administration in the third trimester of pregnancy further reduces the incidents of alloimmunization to 0.1-0.3% (Moise, 2024).

Fetal RhD genotyping using cell-free fetal DNA from maternal plasma can be performed to identify fetal blood type most accurately after 11 weeks of gestation. While the United States has not implemented fetal RhD genotyping for routine prophylaxis and fetal monitoring protocols, several European countries, such as Denmark, the Netherlands, England, Sweden, France and Finland, do utilize fetal RhD determination so that the administration of anti-D immune globulin can be avoided when an RhD-negative fetus is identified (Moise, 2024). Daniels et al. (2007) report that approximately 40% of RhD-negative pregnant individuals are carrying a RhD-negative fetus; genotypic screening would, therefore, be very valuable in preventing these individuals from receiving unnecessary anti-D immune globulin. Kent et al. (2014) suggest that the administration of anti-D immune globulin to the one third of pregnant individuals who do not require this administration is unethical, and that the availability of RhD genotyping to all RhD-negative pregnant individuals would assist in more informed choices being made regarding anti-D immune globulin administration. Finning et al. (2008) agree with the previous statements, declaring that "high throughput RHD genotyping of fetuses in all RhD negative [individuals] is feasible and would substantially reduce unnecessary administration of anti-RhD immunoglobulin to RhD negative pregnant [individuals] with an RhD negative fetus."

Analytical Validity

A prospective cohort study by de Haas et al. (2016) completed a nationwide program in the Netherlands to determine the sensitivity of fetal RhD screening for the safe guidance of targeted anti-immune globulin prophylaxis. A total of 25,789 RhD-negative pregnant individuals participated in this study. Fetal testing for the *RHD* gene was assessed in the 27th week of pregnancy. Fetal *RHD* test results were compared to serological cord blood results after birth. "Sensitivity for detection of fetal *RHD* was 99.94% (95% confidence interval 99.89% to 99.97%) and specificity was 97.74% (97.43% to 98.02%). Nine false-negative results for fetal RHD testing were registered (0.03%, 95% confidence interval 0.01% to 0.06%)" (de Haas et al., 2016). They conclude that fetal RhD testing is a highly reliable testing method.

Manfroi et al. (2018) completed fetal *RhD* genotyping with real-time polymerase chain reaction (qPCR) using cell-free fetal DNA extracted from maternal plasma. A commercial multiple-exon assay was used to determine fetal *RHD* genotypic accuracy. A total of 367 plasma samples obtained between the 24th and 28th weeks of pregnancy were used for this study. Neonatal results were available for 284 of the pregnancies. The sensitivity was reported at 100% and specificity at 97.5%. The diagnostic accuracy was 96.1% with the inclusion of 9/284 inconclusive results (Manfroi et al., 2018). The authors conclude that this is therefore an accurate and reliable tool for targeted prenatal immunoprophylaxis.

Clinical Utility and Validity

Education and counseling are a key factor in prenatal screening and diagnostic tests. Yesilcinar and Guvenc (2021) found that a proactive intervention approach decreased anxiety and decisional conflict in the pregnant individual and increased attitudes towards the tests, having a positive effect on the pregnant individual's knowledge level and decision satisfaction. This allowed the individual to make more informed decisions, such as opting to have screening and diagnostic testing performed. Decreasing anxiety during pregnancy is beneficial to the fetus and individuals receiving educational intervention showed decreased anxiety when receiving genetic screening results as compared to individuals not receiving the same intervention (Yesilcinar & Guvenc, 2021). Migliorini et al. (2020) have also reported that the use of cell free DNA (cfDNA) screening, combined with a detailed ultrasound examination, as a first-trimester risk assessment is associated with improved maternal reassurance and satisfaction and decreased anxiety, as compared to individuals who received standard first-trimester combined screening with nuchal translucency (NT) and biochemistry (Migliorini et al., 2020).

Biro et al. (2020) report on a noninvasive prenatal testing method for congenital heart disease, utilizing the measurement of cell-free nucleic acid and protein biomarkers in maternal blood. Congenital heart disease is considered the most common fetal malformation.. While prenatal ultrasonography is currently used to diagnose congenital heart disease, it is not the most accurate method. After a large review completed with PubMed and Web of Sciences databases, the authors conclude that most fetal congenital heart disease related disorders can be diagnosed by noninvasive prenatal testing (NIPT) techniques. Further, cell-free RNAs and circulating proteins are potential biomarkers for fetal congenital heart disease and may be able to improve the detection rate in early pregnancies (Biro et al., 2020).

A study by Persico et al. (2016) investigated the clinical implication of cfDNA testing in high-risk pregnancies. In their cohort of 259 singleton pregnancies, cfDNA testing

provided results in 249 (96.1%). Further, cfDNA testing identified 97.2% (35/36) of trisomy 21, 100% (13/13) of trisomy 18, 100% of trisomy 13 (5/5), and 75% of sex chromosome aneuploidies (3/4). The authors conclude that "a policy of performing an invasive test in [individuals] with a combined risk of ≥1 in 10 or NT ≥4 mm and offering cfDNA testing to the remaining cases would detect all cases of trisomy 21, 18 or 13, 80% of sex aneuploidies and 62.5% of other defects and would avoid an invasive procedure in 82.4% of euploid fetuses" (Persico et al., 2016). These data support the earlier meta-analysis that reported NIPT sensitivity of trisomy 21, trisomy 18, and trisomy 13 of 99%, 96.8%, and 92.1%, respectively and specificities of 99.92%, 99.85%, and 99.80%, respectively, for trisomies 21, 18, and 13 (Dondorp et al., 2015; Gil et al., 2014).

A multi-year study of more than 5000 patients in public hospitals in Spain examined the effect of NIPT on the number of invasive procedures performed, showing that the introduction of NIPT drastically reduced the incidences of invasive procedures. The data shows that despite a 60.5% reduction occurred in invasive procedures, the chromosomopathy detection rate was unaffected; moreover, the ratio of positive invasive procedures was improved to 50%, indicating that unwarranted invasive procedures had been avoided (Martinez-Payo et al., 2018). The authors of the study concluded, "NIPT introduction has caused a significant reduction of 60.5% of IP [invasive procedures] in high chromosomopathy risk patients after combined screening without modifying detection rate" (Martinez-Payo et al., 2018).

A meta-analysis was completed by Mackie et al. (2017), researching the accuracy of cell-free fetal DNA NIPT testing in singleton pregnancies. A total of 117 studies were included, analyzing 18 different conditions. For RHD testing, a sensitivity of 0.993 and specificity of 0.984 was identified and for fetal sex identification, a sensitivity of 0.989 and a specificity of 0.996 was calculated (Mackie et al., 2017). With such high sensitivity and specificity calculations, NIPT testing for fetal sex and RHD status may be considered accurate diagnostic tools.

Clausen et al. (2014) completed a two-year evaluation of nationwide prenatal RhD screening in Denmark. A total of 12,668 pregnancies were analyzed, with blood samples drawn in week 25 of pregnancy. DNA was extracted from these blood samples and was analyzed for the *RHD* gene. Results were later compared to the serological typing of the newborns after birth. "The sensitivity for the detection of fetal *RHD* was 99.9% (95% CI: 99.7-99.9%). Unnecessary recommendation of prenatal RhD prophylaxis was avoided in 97.3% of the [individuals] carrying an RhD-negative fetus. Fetuses that were seropositive for RhD were not detected in 11 pregnancies (0.087%)" (Clausen et al., 2014). This study shows high sensitivity of fetal *RHD* genotyping, results which were recently supported by another large-scale meta-analysis completed by Yang et al.

(2019), focusing on NIPT testing for fetal RhD status. A total of 3921 results confirmed that "High-throughput NIPT is sufficiently accurate to detect fetal RhD status in RhD-negative [individuals] and would considerably reduce unnecessary treatment with routine anti-D immunoglobulin" (Yang et al., 2019).

Darlington et al. (2018) completed an analysis of 11 French Obstetric Departments with a total of 949 patients to determine the effectiveness of RhD genotyping. The patients were separated into two groups (genotyping group: n=515, and control group: n=335). The authors concluded that "Early knowledge of the RHD status of the fetus using non-invasive fetal *RHD* genotyping significantly improved the management of *RHD* negative pregnancies with a small increase in cost" (Darlington et al., 2018).

Runkel et al. (2020) completed a systematic review to determine the benefit of NIPT for fetal RhD status in RhD-negative pregnant individuals because "All non-sensitized Rhesus D (RhD)-negative pregnant [individuals] in Germany receive antenatal anti-D prophylaxis without knowledge of fetal RhD status." The meta-analysis included data from 60,000 participants, with the focus of the research on the impact of fetal and maternal morbidity. The researchers concluded that "NIPT for fetal RhD status is equivalent to conventional serologic testing using the newborn's blood. Studies investigating patient-relevant outcomes are still lacking" (Runkel et al., 2020).

Hoskovec et al. (2023) evaluated the "clinical performance of carrier screening for cystic fibrosis, hemoglobinopathies, and spinal muscular atrophy with reflex single-gene noninvasive prenatal screening (sgNIPS)." In the study, 9151 pregnant individuals were screened for carrier status. As a result, 1669 (18.2%) of the sampled individuals were found to carry one or more harmful genetic variations and were subsequently tested using sgNIPS. The results of sgNIPS were then compared to the outcomes of 201 pregnancies, which were obtained from surveys completed by parents or reports from healthcare providers. In conclusion, carrier screening using sgNIPS during pregnancy presents an alternative approach that circumvents the need for a paternal sample. It offers accurate assessment of fetal risk promptly, facilitating prenatal counseling and pregnancy management.

Westin et al. (2022) conducted a retrospective study which aimed to "validate the sgNIPT in clinical samples and identify high-risk SCD fetuses in a cohort of at-risk pregnancies." This retrospective clinical investigation gathered 77 maternal blood samples from pregnant patients at either Baylor College of Medicine or the University of Alabama at Birmingham. These patients were identified as having at least one harmful HBB allele. The results of this study highlighted that sgNIPT screening promotes "efficient and accurate fetal risk assessment for SCD in pregnant patients" (Westin et al., 2022).

It is notable that the field continues to evolve, with potential shifts from one testing method to another in pursuit of optimality and comprehensiveness. A multicenter retrospective study of singleton high-risk pregnancies for chromosomal abnormalities was conducted by Zhu et al. (2020) to evaluate the utility of expanded noninvasive prenatal screening as compared with chromosomal microarray analysis (CMA). The analysis enrolled subjects who underwent expanded NIPS and CMA sequentially during pregnancy from 2015 through 2019. The study demonstrated that of the 943 high-risk pregnancies, 550 (58.3%) cases had positive NIPS results, while positive CMA results were detected in 308 (32.7%) cases, and the agreement rates between NIPS and CMA were 82.3%, 59.6% and 25.0% for trisomy 21, 18 and 13, respectively. Regarding rare aneuploidies and segmental imbalances, NIPS and CMA results were concordant in 7.5% and 33.3% of cases. However, copy number variants were better detected with CMA than with NIPS and additional genetic aberrations were detected by CMA in one of 17 high-risk pregnancies that were otherwise passed over when processed with NIPS. The researchers contend that CMA should be offered for high-risk pregnancies to provide comprehensive detection of chromosomal abnormalities in these pregnancies (Zhu et al., 2020).

This policy focuses on genetic testing performed during pre-conception and/or prenatal periods as part of a comprehensive prenatal care program.

VI. Guidelines and Recommendations

American College of Medical Genetics and Genomics (ACMG)

In 2021, ACMG released an updated guideline for screening for autosomal recessive and X-linked conditions during pregnancy and preconception. Their practice resource reviews aim to recommend "a consistent and equitable approach for offering carrier screening to all individuals during pregnancy and preconception" and replaces any earlier ACMG position statements on prenatal/preconception expanded carrier screening and provide the following recommendations:

- "Analytical validity of carrier screening is to be established by a laboratory in compliance with CLIA/CAP regulations and adhering to ACMG Laboratory Standards and Guidelines."
- "As evidence evolves, ClinVar and ClinGen continually update pathogenicity of variants and the association between genes and conditions, respectively."
- "Carrier screening enables those screened to consider their reproductive risks, reproductive options, and to make informed decisions."
- "Published evidence supports clinical utility for carrier screening of multiple conditions simultaneously."
- "The phrase "expanded carrier screening" be replaced by "carrier screening."

- "Adopting a more precise tiered system based on carrier frequency:
  - Tier 4: <1/200 carrier frequency (includes Tier 3) genes/condition will vary by lab
  - Tier 3: ≥ 1/200 carrier frequency (includes Tier 2) includes X-linked conditions
  - Tier 2: ≥1/100 carrier frequency (includes Tier 1)
  - Tier 1: *CF* [Cystic Fibrosis] + *SMA* [spinal muscular atrophy] + Risk Based Screening
    - "Tier 1 screening conveys the recommendations previously adopted by ACMG and ACOG" and "adopts an ethnic and population neutral approach when screening for cystic fibrosis and spinal muscular atrophy. Beyond these two conditions, additional carrier screening is determined after risk assessment, which incorporates personal medical and family history as well as laboratory and imaging information where appropriate."
    - "Tier 2 carrier screening stems from an ACOG recommendation for conditions that have a severe or moderate phenotype and a carrier frequency of at least 1/100." However, "data demonstrate that carrier screening for two common conditions using a carrier frequency threshold of 1/100 may not be equitable across diverse populations. Others have shown that limiting the carrier frequency to ≥1/100 creates missed opportunities to identify couples at risk for serious conditions."
    - "We define Tier 3 screening as carrier screening for conditions with a carrier frequency ≥1/200 . . . Tier 2 and Tier 3 screening prioritize carrier frequency as a way to think about conditions most appropriate for screening in the general population. However, when ACOG proposed this level, they did not specify whether it was thinking about carrier frequency in terms of the global population or subpopulations. We use "carrier frequency" to mean in any ethnic group with reasonable representation in the United States."
    - "Tier 4 includes genes less common than those in Tier 3 and can identify additional at-risk couples. Tier 4 has no lower limit carrier screening frequency and can greatly extend the number of conditions screened . . . the clinical validity at this level of carrier screening may be less compelling, therefore we suggest reserving this level of screening for consanguineous pregnancies (second cousins or closer) and in couples where family or medical history suggests Tier 4 screening might be beneficial . . . Importantly, patients should understand that their chance of being a carrier for one or more conditions increases as the number of conditions screened is increased."
- "All pregnant patients and those planning a pregnancy should be offered Tier 3 carrier screening.

- Tier 4 screening should be considered:
  - When a pregnancy stems from a known or possible consanguineous relationship (second cousins or closer);
  - When a family or personal medical history warrants.
- ACMG does NOT recommend:
  - Offering Tier 1 and/or Tier 2 screening, because these do not provide equitable evaluation of all racial/ethnic groups.
  - Routine offering of Tier 4 panels.
- "Carrier screening paradigms should be ethnic and population neutral and more inclusive of diverse populations to promote equity and inclusion."
- "All pregnant patients and those planning a pregnancy should be offered Tier 3 carrier screening for autosomal recessive (Tables 1–5) and X-linked (Table 6) conditions."
- "Reproductive partners of pregnant patients and those planning a pregnancy may be offered Tier 3 carrier screening for autosomal recessive conditions (Tables 1–5) when carrier screening is performed simultaneously with their partner."
- "All XX patients should be offered screening for only those X-linked genes listed in Table 6 as part of Tier 3 screening."
- "When Tier 1 or Tier 2 carrier screening was performed in a prior pregnancy, Tier 3 screening should be offered" (Gregg et al., 2021).

Table 1. Autosomal recessive genes for screening with carrier frequency ≥1/50.

OMIM gene	OMIM gene name	Maximum carrier frequency[a]	OMIM phenotype	Conditions
141900	*HBB*	0.119837	603903	Sickle cell anemia β-thalassemia
			613985	
613208	*XPC*	0.050885	278720	Xeroderma pigmentosum
606933	*TYR*	0.049337	203100	Oculocutaneous albinism type 1A and 1B
			606952	
613815	*CYP21A2*	0.048459	201910	Congenital adrenal hyperplasia due to 21-hydroxylase deficiency
612349	*PAH*	0.046068	261600	Phenylketonuria
602421	*CFTR*	0.040972	219700	Cystic fibrosis
600985	*TNXB*	0.035134	606408	Ehlers–Danlos-like syndrome due to tenascin-X deficiency
606869	*HEXA*	0.033146	272800	Tay–Sachs disease
121011	*GJB2*	0.026200	220290	Nonsyndromic hearing loss recessive 1A
			601544	Nonsyndromic hearing loss dominant 3A
602858	*DHCR7*	0.023709	270400	Smith–Lemli–Opitz syndrome
277900	*ATP7B*	0.021983	606882	Wilson disease
608034	*ASPA*	0.019856	271900	Canavan disease
607008	*ACADM*	0.016583	201450	Medium-chain acyl-coenzyme A dehydrogenase deficiency
602716	*NPHS1*	0.015994	256300	Finnish congenital nephrotic syndrome
601785	*PMM2*	0.015877	212065	Carbohydrate-deficient glycoprotein syndrome type Ia
607440	*FKTN*	0.015660	611615	Cardiomyopathy, dilated, 1X
			253800	Walker–Warburg congenital muscular dystrophy
605646	*SLC26A4*	0.015422	600791	Deafness autosomal recessive 4
			274600	Pendred syndrome
126340	*ERCC2*	0.015255	610756	Cerebrooculofacioskeletal syndrome 2
			601675	Trichothiodystrophy 1, photosensitive
603297	*DYNC2H1*	0.014817	613091	Short-rib thoracic dysplasia 3 with or without polydactyly

OMIM Online Mendelian Inheritance in Man.[55]
[a]Values round to ≥ 0.02 (two decimal places).

Table 2. Autosomal recessive genes for screening with carrier frequency <1/50 to ≥1/100.

OMIM gene	OMIM gene name	Maximum carrier frequency[a]	OMIM phenotype	Conditions
610142	*CEP290*	0.014422	610188	Joubert syndrome 5
			611755	Leber congenital amaurosis 10
607839	*GBE1*	0.013799	232500	Glycogen storage disease, type IV
			263570	GBE1-related disorders
606800	*GAA*	0.013565	232300	Glycogen storage disease, type II (Pompe disease)
100725	*CHRNE*	0.013526	100725	Myasthenic syndrome, congenital, 4A, slow-channel
				Myasthenic syndrome, congenital, 4B, fast-channel
613742	*G6PC*	0.013401	232200	Glycogen storage disease type IA
611409	*OCA2*	0.013113	203200	Oculocutaneous albinism brown and type II
120120	*COL7A1*	0.012995	226600	Recessive dystrophic epidermolysis bullosa
600509	*ABCC8*	0.012242	618857	Diabetes mellitus, permanent neonatal 3
612724	*ALDOB*	0.012119	229600	Hereditary fructosuria
613899	*FANCC*	0.011992	227645	Fanconi anemia, complementation group C
604597	*GRIP1*	0.011989	617667	Fraser syndrome
248611	*BCKDHB*	0.011760	245600	Maple syrup urine disease
613726	*ANO10*	0.010781	613728	Spinocerebellar ataxia 10
104170	*NAGA*	0.010637	609241	Schindler disease, type 1
				Schindler disease, type 3
607608	*SMPD1*	0.010259	257200	Niemann–Pick disease, type A
			607616	Niemann–Pick disease, type B
608400	*USH2A*	0.010203	276901	Usher syndrome, type 2A
609058	*MMUT*	0.009999	251000	Methylmalonic aciduria–methylmalonyl–CoA mutase deficiency
600650	*CPT2*	0.009742	600649	Carnitine palmitoyltransferase II deficiency, infantile
			608836	Carnitine palmitoyltransferase II deficiency, lethal neonatal
608894	*AHI1*	0.009740	608629	Joubert syndrome 3

OMIM Online Mendelian Inheritance in Man.[55]
[a]After rounding values are < 0.02 and ≥ 0.01 (two decimal places).

Table 3. Autosomal recessive genes for screening with carrier frequency <1/100 to ≥1/150.

OMIM gene	OMIM gene name	Maximum carrier frequency[a]	OMIM phenotype	Conditions
608172	*DHDDS*	0.009340	613861	Congenital disorder of glycosylation type 1
				Retinitis pigmentosa 59
606152	*SLC19A3*	0.009163	607483	Basal ganglia disease, biotin-responsive
606999	*GALT*	0.009132	230400	Galactosemia
118485	*CYP11A1*	0.008771	613743	Adrenal insufficiency, congenital, with 46, XY sex reversal, partial or complete
190000	*TF*	0.008615	209300	Atransferrinemia
609831	*MMACHC*	0.008610	277400	Methylmalonic aciduria with homocystinuria cblC type
601615	*ABCA3*	0.008587	610921	Surfactant metabolism dysfunction, pulmonary 3
606463	*GBA*	0.008572	230800	Gaucher disease, type I
			230900	Gaucher disease, type II
605248	*MCOLN1*	0.008531	252650	Mucolipidosis type IV
607840	*GNPTAB*	0.008454	252500	Mucolipidosis type II alpha/beta
			252600	Mucolipidosis type III alpha/beta
613228	*AGA*	0.008364	208400	Aspartylglucosaminuria
605514	*PCDH15*	0.008330	609533	Deafness, autosomal recessive 23
			602083	Usher syndrome, type 1F
613871	*FAH*	0.007716	276700	Tyrosinemia type I
607358	*AIRE*	0.007664	240300	Autoimmune polyendocrinopathy syndrome type I
606151	*BBS2*	0.007501	615981	Bardet–Biedl syndrome 2
			616562	Retinitis pigmentosa 74
606530	*CYP27A1*	0.007399	213700	Cerebrotendinous xanthomatosis
611204	*CCDC88C*	0.007282	236600	Congenital hydrocephalus 1
136132	*FMO3*	0.007190	602079	Trimethylaminuria
613277	*TMEM216*	0.007107	608091	Joubert syndrome 2
			603194	Meckel syndrome 2
605080	*CNGB3*	0.006849	262300	Achromatopsia 3
607117	*MCPH1*	0.006822	651200	Primary microcephaly 1, recessive
602671	*SLC37A4*	0.006748	232220	Glycogen storage disease Ib
			232240	Glycogen storage disease Ic
170280	*PRF1*	0.006734	603553	Hemophagocytic lymphohistiocytosis, familial, 2
604272	*SCO2*	0.006671	604377	Mitochondrial complex IV deficiency, nuclear type 2
604285	*AGXT*	0.006648	259900	Hyperoxaluria, primary type I

OMIM Online Mendelian Inheritance in Man.[55]
[a]After rounding values are < 0.01 and ≥ 0.007 (two decimal places).

Table 4. Autosomal recessive genes for screening with carrier frequency <1/150 to ≥1/200.

OMIM gene	OMIM gene name	Maximum carrier frequency[a]	OMIM phenotype	Conditions
609575	*ACADVL*	0.006419	201475	Very long chain acyl-CoA dehydrogenase deficiency
608310	*ASL*	0.006190	207900	Argininosuccinate aciduria
607261	*EVC2*	0.006083	225500	Chondroectodermal dysplasia
607574	*ARSA*	0.005986	250100	Metachromatic leukodystrophy
251170	*MVK*	0.005966	260920	Hyper-IgD syndrome
			610377	Mevalonic aciduria
606702	*PKHD1*	0.005960	263200	Autosomal recessive polycystic kidney disease
609019	*BTD*	0.005953	253260	Biotinidase deficiency
171760	*ALPL*	0.005719	146300	Hypophosphatasia, adult
			241510	Hypophosphatasia, childhood and infantile
209901	*BBS1*	0.005713	209900	Bardet–Biedl syndrome 1
118425	*CLCN1*	0.005688	255700	Congenital myotonia, autosomal recessive form
609506	*CYP27B1*	0.005512	264700	Vitamin D–dependent rickets, type 1
174763	*POLG*	0.005330	203700	Mitochondrial DNA depletion syndrome 4A
			613662	Mitochondrial DNA depletion syndrome 4B
609014	*MCCC2*	0.005184	210210	3-methylcrotonyl CoA carboxylase 2 deficiency
605908	*MLC1*	0.005058	604004	Megalencephalic leukoencephalopathy with subcortical cysts
607809	*ACAT1*	0.005000	203750	α-Methylacetoacetic aciduria
612013	*CC2D2A*	0.004969	612285	Joubert syndrome 9
			612284	Meckel syndrome 6
606718	*SLC26A2*	0.004715	226900	Epiphyseal dysplasia, multiple, 4
			600972	Achondrogenesis Ib
236200	*CBS*	0.004676	236200	Homocystinuria, B6 responsive and nonresponsive
600073	*LRP2*	0.004676	222448	Donnai–Barrow syndrome
252800	*IDUA*	0.004675	607014	Mucopolysaccharidosis, Ih (Hurler S)
			607015	Mucopolysaccharidosis, Ih/s (Hurler–Scheie S)
606596	*FKRP*	0.004668	613153	Muscular dystrophy–dystroglycanopathy, type A, 5
			606612	Muscular dystrophy–dystroglycanopathy, type B, 5
610326	*RNASEH2B*	0.004609	610181	Aicardi Goutieres syndrome 2
611524	*RARS2*	0.004592	611523	Pontocerebellar hypoplasia type 6

OMIM Online Mendelian Inheritance in Man.[55]
[a]After rounding values are < 0.007 and ≥ 0.005 (two decimal places).

Table 5. Genes that were ascertained for screening outside of the gnomAD criteria[a].

OMIM gene	OMIM gene name	Published carrier frequency[b]	Rationale for inclusion	Ethnic group	OMIM phenotype	Conditions
141800	*HBA1*	U[c]	Carrier frequency	SEA and others	604131	α-Thalassemia
141850	*HBA2*	U[c]	Carrier frequency	SEA and others	604131	α-Thalassemia
600354	*SMN1*	1/60[18]	ACOG/ACMG and carrier frequency	US panethnic	253300	
					253550	Spinal muscular
					253400	atrophy types: I, II, III, IV
					271150	
604982	*HPS1*	1/59[56–58]	Carrier frequency	PR	203300	Hermansky Pudlak S. 1
606118	*HPS3*	1/59[56]	Carrier frequency	PR	614072	Hermansky Pudlak S. 3
603722	*ELP1*	1/32[59]	ACOG/ACMG and carrier frequency	AJ	223900	Familial dysautonomia
606829	*FXN*	1/60–1/100[60]	Carrier frequency	Caucasians[d]	229300	Friedreich ataxia
238331	*DLD*	~1/100[59,61]	Carrier frequency	AJ	246900	Dihydrolipoamide dehydrogenase deficiency
161650	*NEB*	1/168[59]	Carrier frequency	AJ	256030	Nemaline myopathy 2
606397	*CLRN1*	1/120[59]	Carrier frequency	AJ	276902	Usher syndrome 3a
604610	*BLM*	1/100[59]	ACMG and carrier frequency	AJ	210900	Bloom syndrome

ACMG American College of Medical Genetics and Genomics, *ACOG* American College of Obstetricians and Gynecologists, *AJ* Ashkenazi Jewish (≥2% of the US population), *OMIM* Online Mendelian Inheritance in Man,[55] *PR* Puerto Rican, *SEA* South East Asian.
[a]Carrier frequency of a sequence variant is <1/200, if reported in gnomAD.[50]
[b]Diagnostic laboratory data was not used for carrier frequency data.
[c]Specific data for general US population not available; however, recognized as common among many US immigrant populations.[62]
[d]This term is no longer used by the journal but is used in the original article to which these studies refer. We have therefore not changed the term but recognize it does not accurately describe the ancestry of the populations originally studied.[46]

Table 6. X-linked genes recommended for carrier screening.

OMIM gene	OMIM gene name	OMIM phenotype	Phenotype
300371	*ABCD1*	300100	Adrenoleukodystrophy (ALD)
300806	*AFF2*	309548	Mental retardation, X-linked, associated with fragile site FRAXE
300382	*ARX*	308350	Developmental and epileptic encephalopathy 1 (DEE1)
300377	*DMD*	300376	Muscular dystrophy, Becker type (BMD)
		310200	Muscular dystrophy, Duchenne type (DMD)
306700	*F8*	300841	Hemophilia A (HEMA)
300746	*F9*	306900	Hemophilia B (HEMB)
309550	*FMR1*	300624	Fragile X syndrome (FXS)
300644	*GLA*	301500	Fabry disease
308840	*L1CAM*	307000	Hydrocephalus due to congenital stenosis of aqueduct of Sylvius (HSAS)
300552	*MID1*	300000	Opitz GBBB syndrome, type I (GBBB1)
300473	*NR0B1*	300200	Adrenal hypoplasia, congenital (AHC)
300461	*OTC*	311250	Ornithine transcarbamylase deficiency
300401	*PLP1*	312920	Spastic paraplegia 2, X-linked (SPG2)
312610	*RPGR*	300029	Retinitis pigmentosa 3 (RP3; RP)
		300455	Retinitis pigmentosa, X-linked, and sinorespiratory
		300834	Infections, with or without deafness
			Macular degeneration, X-linked atrophic
300839	*RS1*	312700	Retinoschisis 1, X-linked, juvenile (RS1)
300036	*SLC6A8*	300352	Cerebral creatine deficiency syndrome 1 (CCDS1)

OMIM Online Mendelian Inheritance in Man.[55]

Tables 1-6 from (Gregg et al., 2021)

CFTR Variant Testing

In 2020, the ACMG provided a technical standard for *CFTR* variant testing. These standards state the following as it pertains to pregnancy:

"During pregnancy, simultaneous testing may be desired depending on gestational age, family and personal history, ethnicity, or patient preferences. Carrier testing may be offered to individuals with a positive family history of CF, in partners of individuals with a positive family history, in partners of CAVD males, to reproductive age women, and to gamete donors. *CFTR* variant testing can also be performed for prenatal diagnosis using cells obtained for diagnostic cytogenetic testing (i.e., amniocentesis or chorionic villus sampling [CVS])" (Deignan et al., 2020).

"As a way to ensure that *CFTR* variant testing for carrier screening and diagnostic testing purposes remains inclusive, the ACMG recommends either a classification-based reporting approach or a classification-based (targeted) testing approach (which has historically been used for CFTR carrier screening). For those laboratories who wish to continue using a targeted testing approach, the ACMG-23 variant panel remains as the minimum list of CFTR variants that should be included. Laboratories may want to consider adding additional variants to their panel depending on the ethnic composition of their expected test population. However, the minimum list of CFTR variants recommended for pan-ethnic carrier screening has not been increased at this time" (Deignan et al., 2020).

In 2023, the ACMG provided updated recommendations for *CFTR* carrier screening which includes a new minimum *CFTR* variant set (increased from 23 to 100 variants). The updated ACMG position statement states the following:

"This new set now supersedes the previous set of 23 *CFTR* variants recommended by the ACMG. These revised recommendations apply only to carrier screening. They do not apply to *CFTR* variant testing for diagnosis or newborn screening. All other aspects of the updated 2020 ACMG *CFTR* technical standards still apply" (Deignan et al., 2020; Deignan et al., 2023).

American College of Obstetricians and Gynecologists (ACOG)

ACOG has several practice guidelines related to prenatal care as well as both pre-conception and prenatal testing. ACOG recommendations and guidelines include the following:

Genetic Testing and Genetic Counseling: Concerning genetic testing and genetic counseling, ACOG recommends:

- "The routine use of whole-genome or whole-exome sequencing for prenatal diagnosis is **not** recommended outside of the context of clinical trials until sufficient peer-reviewed data and validation studies are published" (ACOG, 2016a). This was reaffirmed in 2023.
- Chromosomal microarray analysis (CMA) is recommended for patients with a fetus with at least one major structure abnormality identified via ultrasound. CMA can be considered for all pregnant individuals who undergo prenatal diagnostic testing; however, "In a patient with a structurally normal fetus who is undergoing invasive prenatal diagnostic testing, either fetal karyotyping or a chromosomal microarray analysis can be performed. Chromosomal microarray analysis of fetal tissue (ie, amniotic fluid, placenta, or products of conception) is recommended in the evaluation of intrauterine fetal death or stillbirth when further cytogenetic analysis is desired because of the test's increased likelihood of obtaining results and improved detection of causative abnormalities" (ACOG, 2016a). This was reaffirmed in 2023.
- "All patients who are considering pregnancy or are already pregnant, regardless of screening strategy and ethnicity, should be offered carrier screening for cystic fibrosis and spinal muscular atrophy, as well as a complete blood count and screening for thalassemias and hemoglobinopathies. Fragile X premutation carrier screening is recommended for [individuals] with a family history of fragile X-related disorders or intellectual disability suggestive of fragile X syndrome, or [individuals] with a personal history of ovarian insufficiency. Additional screening also may be indicated based on family history or specific ethnicity" (ACOG, 2017a). This was reaffirmed in 2023.
- "The American College of Obstetricians and Gynecologists discourages direct-to-consumer genetic testing without appropriate counseling. . . Patients may present after direct-to-consumer testing already has been performed, and clinicians should be prepared to review these results or refer to a health care professional with the appropriate knowledge, training, and experience in interpreting test results. . . Given the insufficient data to support the use of single nucleotide polymorphisms (SNP) testing for medical purposes, SNP testing to provide individual risk assessment for a variety of diseases or to tailor drug therapy outside of an institutional review board-approved research protocol is not recommended. The American College of Obstetricians and Gynecologists recommends that the use of these technologies be viewed as investigational at this time" (ACOG, 2021).
- ACOG notes that "Cascade testing has been shown to be cost effective in part because testing for specific mutations (e.g., those identified in the affected relative) is less expensive than whole-gene sequencing" (ACOG, 2018). This was reaffirmed in 2022.

Prenatal Diagnostic Testing for Genetic Disorders: Concerning prenatal diagnostic testing for genetic disorders, ACOG has published the following recommendations:

- "An abnormal FISH result should not be considered diagnostic. Therefore, clinical decision making based on information from FISH should include at least one of the following additional results: confirmatory traditional metaphase chromosome analysis or chromosomal microarray, or consistent clinical information (such as abnormal ultrasonographic findings or a positive screening test result for Down syndrome or trisomy 18)."
- "All pregnant women should be offered prenatal assessment for aneuploidy by screening or diagnostic testing regardless of maternal age or other risk factors."
- "Prenatal genetic testing cannot identify all abnormalities or problems in a fetus, and any testing should be focused on the individual patient's risks, reproductive goals and preferences."
- "Genetic testing should be discussed as early as possible in pregnancy, ideally at the first obstetric visit, so that first-trimester options are available" (ACOG, 2016b).

Prevention of Rh D Alloimmunization: Concerning the prevention of Rh D alloimmunization, ACOG has published the guidelines supporting the administration of anti-D immune globulin to individuals in various scenarios. However, these guidelines do not mention the use of cell-free fetal DNA for fetal RHD testing to determine if anti-D immune globulin is needed (ACOG, 2017c).

Genetic Carrier Screening: Concerning genetic carrier screening, including testing for specific conditions, ACOG recommends [(ACOG, 2017a, 2017b) reaffirmed 2023]:

- "Carrier screening and counseling ideally should be performed before pregnancy.
- "If an individual is found to be a carrier for a specific condition, the individual's reproductive partner should be offered testing in order to receive informed genetic counseling about potential reproductive outcomes. Concurrent screening of the patient and her partner is suggested if there are time constraints for decisions about prenatal diagnostic evaluation."
- "Carrier screening for a particular condition generally should be performed only once in a person's lifetime, and the results should be documented in the patient's health record. Because of the rapid evolution of genetic testing, additional mutations may be included in newer screening panels. The decision to rescreen a patient should be undertaken only with the guidance of a genetics professional who can best assess the incremental benefit of repeat testing for additional mutations."

- "Prenatal carrier screening does not replace newborn screening, nor does newborn screening replace the potential value of prenatal carrier screening."
- "The cost of carrier screening for an individual condition may be higher than the cost of testing through commercially available expanded carrier screening panels. When selecting a carrier screening approach, the cost of each option to the patient and the health care system should be considered."
- "Screening for spinal muscular atrophy should be offered to all [individuals] who are considering pregnancy or are currently pregnant. In patients with a family history of spinal muscular atrophy, molecular testing reports of the affected individual and carrier testing of the related parent should be reviewed, if possible, before testing. If the reports are not available, *SMN1* deletion testing should be recommended for the low-risk partner."
- "Cystic fibrosis carrier screening should be offered to all [individuals] who are considering pregnancy or are currently pregnant. Complete analysis of the *CFTR* gene by DNA sequencing is not appropriate for routine carrier screening."
- "A complete blood count with red blood cell indices should be performed in all [individuals] who are currently pregnant to assess not only their risk of anemia but also to allow assessment for risk of a hemoglobinopathy. Ideally, this testing also should be offered to [individuals] before pregnancy. A hemoglobin electrophoresis should be performed in addition to a complete blood count if there is suspicion of hemoglobinopathy based on ethnicity (African, Mediterranean, Middle Eastern, Southeast Asian, or West Indian descent). If red blood cell indices indicate a low mean corpuscular hemoglobin or mean corpuscular volume, hemoglobin electrophoresis also should be performed."
- "Fragile X premutation carrier screening is recommended for [individuals] with a family history of fragile X-related disorders or intellectual disability suggestive of fragile X syndrome and who are considering pregnancy or are currently pregnant."
- "If a [individual] has unexplained ovarian insufficiency or failure or an elevated follicle-stimulating hormone level before age 40 years, fragile X carrier screening is recommended to determine whether she has an *FMR1* premutation."
- "All identified individuals with intermediate results and carriers of a fragile X premutation or full mutation should be provided follow-up genetic counseling to discuss the risk to their offspring of inheriting an expanded full-mutation fragile X allele and to discuss fragile X-associated disorders (premature ovarian insufficiency and fragile X tremor/ataxia syndrome)."
- "Prenatal diagnostic testing for fragile X syndrome should be offered to known carriers of the fragile X premutation or full mutation."

- "DNA-based molecular analysis (eg, Southern blot analysis and polymerase chain reaction) is the preferred method of diagnosis of fragile X syndrome and of determining *FMR1* triplet repeat number (e.g., premutations). In rare cases, the size of the triplet repeat and the methylation status do not correlate, which makes it difficult to predict the clinical phenotype. In cases of this discordance, the patient should be referred to a genetics professional."
- "When only one partner is of Ashkenazi Jewish descent, that individual should be offered screening first. If it is determined that this individual is a carrier, the other partner should be offered screening. However, the couple should be informed that the carrier frequency and the detection rate in non-Jewish individuals are unknown for most of these disorders, except for Tay–Sachs disease and cystic fibrosis. Therefore, it is difficult to accurately predict the couple's risk of having a child with the disorder."
- "Screening for Tay–Sachs disease should be offered when considering pregnancy or during pregnancy if either member of a couple is of Ashkenazi Jewish, French–Canadian, or Cajun descent. Those with a family history consistent with Tay–Sachs disease also should be offered screening. When one member of a couple is at high risk (i.e., of Ashkenazi Jewish, French–Canadian, or Cajun descent or has a family history consistent with Tay–Sachs disease) but the other partner is not, the high-risk partner should be offered screening. If the high-risk partner is found to be a carrier, the other partner also should be offered screening."
- "Enzyme testing in pregnant [individuals] and [individuals] taking oral contraceptives should be performed using leukocyte testing because serum testing is associated with an increased false-positive rate in these populations."
- "If Tay–Sachs disease screening is performed as part of pan-ethnic expanded carrier screening, it is important to recognize the limitations of the mutations screened in detecting carriers in the general population. In the presence of a family history of Tay–Sachs disease, expanded carrier screening panels are not the best approach to screening unless the familial mutation is included on the panel" (ACOG, 2017b).
- "Regarding expanded carrier screening panels, ACOG recommends that "the disorders selected for inclusion should meet several of the following consensus-determined criteria: have a carrier frequency of 1 in 100 or greater, have a well-defined phenotype, have a detrimental effect on quality of life, cause cognitive or physical impairment, require surgical or medical intervention, or have an onset early in life." ACOG further states that "screened conditions should be able to be diagnosed prenatally and may afford opportunities for antenatal intervention to improve perinatal outcomes, changes to delivery management to optimize newborn and infant outcomes, and education of the parents about special care needs after birth" (ACOG, 2017a).

Carrier Screening in the Age of Genomic Medicine: Concerning carrier screening in the age of genomic medicine, the ACOG has published the following guidelines (ACOG, 2017a):

- "Ethnic-specific, pan-ethnic and expanded carrier screening are acceptable strategies for prepregnancy and prenatal carrier screening.
- If a patient requests a screening strategy other than the one used by the obstetrician-gynecologist or other health care provider, the requested test should be made available to her after counseling on its limitations, benefits, and alternatives.
- All patients who are considering pregnancy or already pregnant, regardless of screening strategy and ethnicity, should be offered carrier screening for cystic fibrosis and spinal muscular atrophy, as well as a complete blood count and screening for thalassemias and hemoglobinopathies. Fragile X premutation carrier screening is also recommended for [individuals] with a family history of fragile x-related disorders or intellectual disability suggestive of fragile X syndrome, or [individuals] with a personal history of ovarian insufficiency. Additional screening also may be indicated based on family history or specific ethnicity.
- If a [individual] is found to be a carrier for a specific condition, her reproductive partner should be offered screening to provide accurate genetic counseling for the couple with regard to the risk of having an affected child. Additional genetic counseling should be provided to discuss the specific condition, residual risk, and options for prenatal testing.
- Individuals with a family history of a genetic disorder may benefit from the identification of the specific familial mutation or mutations rather than carrier screening. Knowledge of the specific familial mutation may allow for more specific and rapid prenatal diagnosis.
- Given the multitude of conditions that can be included in expanded carrier screening panels, the disorders selected for inclusion should meet several of the following consensus-determined criteria: have a carrier frequency of 1 in 100 or greater, have a well-defined phenotype, have a detrimental effect on quality of life, cause cognitive or physical impairment, require surgical or medical intervention, or have an onset early in life. Additionally, screened conditions should be able to be diagnosed prenatally and may afford opportunities for antenatal intervention to improve perinatal outcomes, changes to delivery management to optimize newborn and infant outcomes, and education of the parents about special care needs after birth.
- Carrier screening panels should not include conditions primarily associated with a disease of adult onset" (ACOG, 2017a). This guideline was reaffirmed in 2023.

International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM), and the Perinatal Quality Foundation (PQF)

The ISPD, SMFM and PQF published the following guidelines on the use of genome-wide sequencing for fetal diagnosis:

- The use of diagnostic sequencing is currently being introduced for evaluation of fetuses for whom standard diagnostic genetic testing, such as chromosomal microarray analysis (CMA), has already been performed and is uninformative, is offered concurrently according to accepted practice guidelines, or for whom expert genetic opinion determines that standard genetic testing is less optimal than sequencing for the presenting fetal phenotype.
- The routine use of prenatal sequencing as a diagnostic test cannot currently be supported due to insufficient validation data and knowledge about its benefits and pitfalls (ISPD, 2018).

In addition to the joint position statement released in 2018, the IPSD released a guideline in 2020 on the use of cfDNA screening for trisomies in multiple pregnancies:

- "The use of first trimester cfDNA screening for the common autosomal trisomies is appropriate for twin pregnancies due to sufficient evidence showing high detection and low false positive rates with high predictive values. **Moderate."**
- "It is preferable for laboratories performing cfDNA testing in multi-fetal pregnancies to take evidence of zygosity into consideration (eg, chorionicity, sex of the fetuses, embryo transfer history) for the interpretation of both test results and fetal fractions. **Moderate."**
- "Screening options for triplet pregnancies are lacking and cfDNA may be a potential option. However, diagnostic testing should always be offered and the limitations of screening tests stressed. **Low"** (Palomaki et al., 2021).

College of American Pathologists (CAP) Transfusion Medicine Resource Committee (TMRC) Work Group

The following recommendations were given by the CAP TMRC Work Group:

- The Work Group recommends that *RHD* genotyping be performed whenever a discordant RhD typing result and/or a serological weak D phenotype is detected in patients, including pregnant individuals, newborns, and potential transfusion recipients. It is anticipated that the immediate benefit will be fewer unnecessary injections of RhIG and increased availability of RhD-negative RBCs for transfusion.
- Other than RHD genotypes weak D type 1, 2, or 3, the Work Group recommends that individuals with a serological weak D phenotype receive conventional

prophylaxis with RhIG, including postpartum RhIG if the newborn is RhD-positive or has a serological weak D phenotype (Sandler et al., 2015).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website https://www.cms.gov/Medicare-Coverage-Database/search.aspx. For the most up-to-date Medicaid policies and coverage, please visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

The FDA has approved many tests for conditions that can be included in a prenatal screening, such as HSV, chlamydia, gonorrhea, syphilis, and diabetes. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	CPT Description
81171	AFF2 (ALF transcription elongation factor 2 [FMR2]) (eg, fragile X intellectual disability 2 [FRAXE]) gene analysis; evaluation to detect abnormal (eg, expanded) alleles
81172	AFF2 (ALF transcription elongation factor 2 [FMR2]) (eg, fragile X intellectual disability 2 [FRAXE]) gene analysis; characterization of alleles (eg, expanded size and methylation status)
81200	ASPA (aspartoacylase) (eg, Canavan disease) gene analysis, common variants (eg, E285A, Y231X)
81209	BLM (Bloom syndrome, RecQ helicase-like) (eg, Bloom syndrome) gene analysis, 2281del6ins7 variant
81241	F5 (coagulation factor V) (eg, hereditary hypercoagulability) gene analysis, Leiden variant

CPT	CPT Description
81242	FANCC (Fanconi anemia, complementation group C) (eg, Fanconi anemia, type C) gene analysis, common variant (eg, IVS4+4A>T)
81243	FMR1 (fragile X messenger ribonucleoprotein 1) (eg, fragile X syndrome, X-linked intellectual disability [XLID]) gene analysis; evaluation to detect abnormal (eg, expanded) alleles
81244	FMR1 (fragile X messenger ribonucleoprotein 1) (eg, fragile X syndrome, X-linked intellectual disability [XLID]) gene analysis; characterization of alleles (eg, expanded size and promoter methylation status)
81251	GBA (glucosidase, beta, acid) (eg, Gaucher disease) gene analysis, common variants (eg, N370S, 84GG, L444P, IVS2+1G>A)
81255	HEXA (hexosaminidase A [alpha polypeptide]) (eg, Tay-Sachs disease) gene analysis, common variants (eg, 1278insTATC, 1421+1G>C, G269S)
81257	HBA1/HBA2 (alpha globin 1 and alpha globin 2) (eg, alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis; common deletions or variant (eg, Southeast Asian, Thai, Filipino, Mediterranean, alpha3.7, alpha4.2, alpha20.5, Constant Spring)
81260	IKBKAP (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein) (eg, familial dysautonomia) gene analysis, common variants (eg, 2507+6T>C, R696P)
81290	MCOLN1 (mucolipin 1) (eg, Mucolipidosis, type IV) gene analysis, common variants (eg, IVS3-2A>G, del6.4kb)
81329	SMN1 (survival of motor neuron 1, telomeric) (eg, spinal muscular atrophy) gene analysis; dosage/deletion analysis (eg, carrier testing), includes SMN2 (survival of motor neuron 2, centromeric) analysis, if performed
81330	SMPD1(sphingomyelin phosphodiesterase 1, acid lysosomal) (eg, Niemann-Pick disease, Type A) gene analysis, common variants (eg, R496L, L302P, fsP330)
81400	Molecular pathology procedure, Level 1 (eg, identification of single germline variant [eg, SNP] by techniques such as restriction enzyme digestion or melt curve analysis)
81401	Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)
81403	Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)
81404	Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-

CPT	CPT Description
	10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons))
81412	Ashkenazi Jewish associated disorders (eg, Bloom syndrome, Canavan disease, cystic fibrosis, familial dysautonomia, Fanconi anemia group C, Gaucher disease, Tay-Sachs disease), genomic sequence analysis panel, must include sequencing of at least 9 genes, including ASPA, BLM, CFTR, FANCC, GBA, HEXA, IKBKAP, MCOLN1, and SMPD1
81443	Genetic testing for severe inherited conditions (eg, cystic fibrosis, Ashkenazi Jewish-associated disorders [eg, Bloom syndrome, Canavan disease, Fanconi anemia type C, mucolipidosis type VI, Gaucher disease, Tay-Sachs disease], beta hemoglobinopathies, phenylketonuria, galactosemia), genomic sequence analysis panel, must include sequencing of at least 15 genes (eg, ACADM, ARSA, ASPA, ATP7B, BCKDHA, BCKDHB, BLM, CFTR, DHCR7, FANCC, G6PC, GAA, GALT, GBA, GBE1, HBB, HEXA, IKBKAP, MCOLN1, PAH)
81479	Unlisted molecular pathology procedure
81599	Unlisted multianalyte assay with algorithmic analysis
S3845	Genetic testing for alpha-thalassemia
S3846	Genetic testing for hemoglobin E beta-thalassemia
S3849	Genetic testing for niemann-pick disease
0400U	Obstetrics (expanded carrier screening), 145 genes by next-generation sequencing, fragment analysis and multiplex ligation-dependent probe amplification, DNA, reported as carrier positive or negative Proprietary test: Genesys Carrier Panel Lab/Manufacturer: Genesys Diagnostics, Inc
0449U	Carrier screening for severe inherited conditions (eg, cystic fibrosis, spinal muscular atrophy, beta hemoglobinopathies [including sickle cell disease], alpha thalassemia), regardless of race or self-identified ancestry, genomic sequence analysis panel, must include analysis of 5 genes (CFTR, SMN1, HBB, HBA1, HBA2 Proprietary test: UNITY Carrier Screen™ Lab/Manufacturer: BillionToOne Laboratory, BillionToOne, Inc

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

ACOG. (2016a). Committee Opinion No.682: Microarrays and Next-Generation Sequencing Technology: The Use of Advanced Genetic Diagnostic Tools in Obstetrics and Gynecology. *Obstet Gynecol*, *128*(6), e262-e268. https://doi.org/10.1097/aog.0000000000001817

ACOG. (2016b). Prenatal Diagnostic Testing for Genetic Disorders. https://s3.amazonaws.com/cdn.smfm.org/publications/223/download-f5260f3bc6686c15e4780f8100c74448.pdf

ACOG. (2017a). Committee Opinion No. 690: Carrier Screening in the Age of Genomic Medicine. *Obstet Gynecol*, *129*(3), e35-e40. https://doi.org/10.1097/aog.0000000000001951

ACOG. (2017b). Committee Opinion No. 691: Carrier Screening for Genetic Conditions. *Obstet Gynecol*, *129*(3), e41-e55. https://doi.org/10.1097/aog.0000000000001952

ACOG. (2017c). Practice Bulletin No. 181: Prevention of Rh D Alloimmunization. https://journals.lww.com/greenjournal/fulltext/2017/08000/Practice_Bulletin_No__181__Prevention_of_Rh_D.54.aspx

ACOG. (2018). ACOG Committee Opinion No. 727: Cascade Testing: Testing Women for Known Hereditary Genetic Mutations Associated With Cancer. *Obstet Gynecol*, *131*(1), e31-e34. https://doi.org/10.1097/aog.0000000000002457

ACOG. (2021). Consumer Testing for Disease Risk: ACOG Committee Opinion, Number 816. *Obstet Gynecol*, *137*(1), e1-e6. https://doi.org/10.1097/AOG.0000000000004200

BillionToOne. (2023). *UNITY Complete: The New Standard In Prenatal Care*. https://www.unityscreen.com/

Biro, O., Rigo, J., Jr., & Nagy, B. (2020). Noninvasive prenatal testing for congenital heart disease - cell-free nucleic acid and protein biomarkers in maternal blood. *J Matern Fetal Neonatal Med*, *33*(6), 1044-1050. https://doi.org/10.1080/14767058.2018.1508437

Calhoun, D. (2023, 03/03/2023). *Postnatal diagnosis and management of hemolytic disease of the fetus and newborn*. Retrieved 03/14/24 from https://www.uptodate.com/contents/postnatal-diagnosis-and-management-of-hemolytic-disease-of-the-fetus-and-newborn?topicRef=6773&source=see_link

Clausen, F. B., Steffensen, R., Christiansen, M., Rudby, M., Jakobsen, M. A., Jakobsen, T. R., Krog, G. R., Madsen, R. D., Nielsen, K. R., Rieneck, K., Sprogoe, U., Homburg, K. M., Baech, J., Dziegiel, M. H., & Grunnet, N. (2014). Routine noninvasive prenatal screening for fetal RHD in plasma of RhD-negative pregnant women-2 years of

screening experience from Denmark. *Prenat Diagn*, *34*(10), 1000-1005. https://doi.org/10.1002/pd.4419

Daniels, G., Finning, K., Martin, P., & Summers, J. (2007). Fetal RhD genotyping: a more efficient use of anti-D immunoglobulin. *Transfus Clin Biol*, *14*(6), 568-571. https://doi.org/10.1016/j.tracli.2008.03.007

Darlington, M., Carbonne, B., Mailloux, A., Brossard, Y., Levy-Mozziconacci, A., Cortey, A., Maoulida, H., Simon, T., Rousseau, A., & Durand-Zaleski, I. (2018). Effectiveness and costs of non-invasive foetal RHD genotyping in rhesus-D negative mothers: a French multicentric two-arm study of 850 women. *BMC Pregnancy Childbirth*, *18*(1), 496. https://doi.org/10.1186/s12884-018-2114-5

de Haas, M., Thurik, F. F., van der Ploeg, C. P., Veldhuisen, B., Hirschberg, H., Soussan, A. A., Woortmeijer, H., Abbink, F., Page-Christiaens, G. C., Scheffer, P. G., & Ellen van der Schoot, C. (2016). Sensitivity of fetal RHD screening for safe guidance of targeted anti-D immunoglobulin prophylaxis: prospective cohort study of a nationwide programme in the Netherlands. *Bmj*, *355*, i5789. https://doi.org/10.1136/bmj.i5789

de Jong, A., Maya, I., & van Lith, J. M. (2015). Prenatal screening: current practice, new developments, ethical challenges. *Bioethics*, *29*(1), 1-8. https://doi.org/10.1111/bioe.12123

Deignan, J. L., Astbury, C., Cutting, G. R., del Gaudio, D., Gregg, A. R., Grody, W. W., Monaghan, K. G., & Richards, S. (2020). CFTR variant testing: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genetics in Medicine*, *22*(8), 1288-1295. https://doi.org/10.1038/s41436-020-0822-5

Deignan, J. L., Gregg, A. R., Grody, W. W., Guo, M. H., Kearney, H., Monaghan, K. G., Raraigh, K. S., Taylor, J., Zepeda-Mendoza, C. J., & Ziats, C. (2023). Updated recommendations for CFTR carrier screening: A position statement of the American College of Medical Genetics and Genomics (ACMG). *Genetics in Medicine*, *25*(8), 100867. https://doi.org/10.1016/j.gim.2023.100867

Dondorp, W., de Wert, G., Bombard, Y., Bianchi, D. W., Bergmann, C., Borry, P., Chitty, L. S., Fellmann, F., Forzano, F., Hall, A., Henneman, L., Howard, H. C., Lucassen, A., Ormond, K., Peterlin, B., Radojkovic, D., Rogowski, W., Soller, M., Tibben, A., . . . American Society of Human, G. (2015). Non-invasive prenatal testing for aneuploidy and beyond: challenges of responsible innovation in prenatal screening. *European journal of human genetics : EJHG*, *23*(11), 1438-1450. https://doi.org/10.1038/ejhg.2015.57

Finning, K., Martin, P., Summers, J., Massey, E., Poole, G., & Daniels, G. (2008). Effect of high throughput RHD typing of fetal DNA in maternal plasma on use of anti-RhD immunoglobulin in RhD negative pregnant women: prospective feasibility study. *Bmj*, *336*(7648), 816-818. https://doi.org/10.1136/bmj.39518.463206.25

Gil, M. M., Akolekar, R., Quezada, M. S., Bregant, B., & Nicolaides, K. H. (2014). Analysis of cell-free DNA in maternal blood in screening for aneuploidies: meta-analysis. *Fetal Diagn Ther*, *35*(3), 156-173. https://doi.org/10.1159/000358326

Grant, A., & Mohide, P. (1982). Screening and diagnostic tests in antenatal care. *Effectiveness and satisfaction in antenatal care*, 22-59. https://books.google.com/books?hl=en&lr=&id=fVH-JYbe2isC&oi=fnd&pg=PA22&dq=screening+versus+diagnostic+tests&ots=WXVxt6ALwT&sig=DUy8K33sGYU72yPEjPHIyTT3ppA#v=onepage&q=screening%20versus%20diagnostic%20tests&f=false

Gregg, A. R., Aarabi, M., Klugman, S., Leach, N. T., Bashford, M. T., Goldwaser, T., Chen, E., Sparks, T. N., Reddi, H. V., Rajkovic, A., Dungan, J. S., Practice, A. P., & Guidelines, C. (2021). Screening for autosomal recessive and X-linked conditions during pregnancy and preconception: a practice resource of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *23*(10), 1793-1806. https://doi.org/10.1038/s41436-021-01203-z

Hoskovec, J., Hardisty, E. E., Talati, A. N., Carozza, J. A., Wynn, J., Riku, S., Ten Bosch, J. R., & Vora, N. L. (2023). Maternal carrier screening with single-gene NIPS provides accurate fetal risk assessments for recessive conditions. *Genetics in Medicine*, *25*(2), 100334. https://doi.org/10.1016/j.gim.2022.10.014

ISPD. (2018). Joint Position Statement from the International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM), and the Perinatal Quality Foundation (PQF) on the use of genome-wide sequencing for fetal diagnosis. *Prenat Diagn*, *38*(1), 6-9. https://doi.org/10.1002/pd.5195

Kent, J., Farrell, A. M., & Soothill, P. (2014). Routine administration of Anti-D: the ethical case for offering pregnant women fetal RHD genotyping and a review of policy and practice. *BMC Pregnancy Childbirth*, *14*, 87. https://doi.org/10.1186/1471-2393-14-87

LabCorp. (2023). *Inheritest*. https://www.integratedgenetics.com/patients/pre-pregnancy/inheritest

Lockwood, C. J., & Magriples, U. (2023). *Prenatal care: Initial assessment*. Wolters Kluwer. Retrieved 05/03/2023 from https://www.uptodate.com/contents/prenatal-care-initial-assessment

Mackie, F. L., Hemming, K., Allen, S., Morris, R. K., & Kilby, M. D. (2017). The accuracy of cell-free fetal DNA-based non-invasive prenatal testing in singleton pregnancies: a systematic review and bivariate meta-analysis. *Bjog*, *124*(1), 32-46. https://doi.org/10.1111/1471-0528.14050

Manfroi, S., Calisesi, C., Fagiani, P., Gabriele, A., Lodi, G., Nucci, S., Pelliconi, S., Righini, L., & Randi, V. (2018). Prenatal non-invasive foetal RHD genotyping: diagnostic accuracy of a test as a guide for appropriate administration of antenatal anti-D immunoprophylaxis. *Blood Transfus*, *16*(6), 514-524. https://doi.org/10.2450/2018.0270-17

Martinez-Payo, C., Bada-Bosch, I., Martinez-Moya, M., & Perez-Medina, T. (2018). Clinical results after the implementation of cell-free fetal DNA detection in maternal plasma. *J Obstet Gynaecol Res*, *44*(8), 1369-1376. https://doi.org/10.1111/jog.13672

Migliorini, S., Saccone, G., Silvestro, F., Massaro, G., Arduino, B., D'Alessandro, P., Petti, M. T., Paino, J. A. C., Guida, M., Locci, M., & Zullo, F. (2020). First-trimester screening based on cell-free DNA vs combined screening: A randomized clinical trial on women's experience. *Prenat Diagn*, *40*(11), 1482-1488. https://doi.org/10.1002/pd.5800

Miller, D. (2023). *Use of chromosomal microarray in obstetrics*. Wolters Kluwer. Retrieved 05/03/2023 from https://www.uptodate.com/contents/use-of-chromosomal-microarray-in-obstetrics

Moise, K. (2024, 03/07/2024). *Prevention of RhD alloimmunization in pregnancy*. Retrieved 03/20/2024 from https://www.uptodate.com/contents/prevention-of-rhd-alloimmunization-in-pregnancy

Palomaki, G. E., Chiu, R. W. K., Pertile, M. D., Sistermans, E. A., Yaron, Y., Vermeesch, J. R., Vora, N. L., Best, R. G., & Wilkins-Haug, L. (2021). International Society for Prenatal Diagnosis Position Statement: cell free (cf)DNA screening for Down syndrome in multiple pregnancies. *Prenat Diagn*, *41*(10), 1222-1232. https://doi.org/10.1002/pd.5832

Persico, N., Boito, S., Ischia, B., Cordisco, A., De Robertis, V., Fabietti, I., Periti, E., Volpe, P., Fedele, L., & Rembouskos, G. J. P. D. V. (2016). Cell-free DNA testing in the maternal blood in high-risk pregnancies after first-trimester combined screening. *Prenatal Diagnosis*, *36*(3), 232-236.

Runkel, B., Bein, G., Sieben, W., Sow, D., Polus, S., & Fleer, D. (2020). Targeted antenatal anti-D prophylaxis for RhD-negative pregnant women: a systematic review. *BMC Pregnancy Childbirth*, *20*(1), 83. https://doi.org/10.1186/s12884-020-2742-4

Sandler, S. G., Flegel, W. A., Westhoff, C. M., Denomme, G. A., Delaney, M., Keller, M. A., Johnson, S. T., Katz, L., Queenan, J. T., Vassallo, R. R., & Simon, C. D. (2015). It's time to phase in RHD genotyping for patients with a serologic weak D phenotype. College of American Pathologists Transfusion Medicine Resource Committee Work Group. *Transfusion*, *55*(3), 680-689. https://doi.org/10.1111/trf.12941

Westin, E. R., Tsao, D. S., Atay, O., Landry, B. P., Ye, P. P., Chandler-Brown, D., Alford, B., Hoskovec, J., Subramaniam, A., Pawlik, K. M., Kuper, S. G., Goldman, F. D., Townes, T. M., & Sheehan, V. A. (2022). Validation of single-gene noninvasive prenatal testing for sickle cell disease. *American Journal of Hematology*, *97*(7). https://doi.org/10.1002/ajh.26570

Yang, H., Llewellyn, A., Walker, R., Harden, M., Saramago, P., Griffin, S., & Simmonds, M. (2019). High-throughput, non-invasive prenatal testing for fetal rhesus D status in RhD-negative women: a systematic review and meta-analysis. *BMC Med*, *17*(1), 37. https://doi.org/10.1186/s12916-019-1254-4

Yesilcinar, I., & Guvenc, G. (2021). Counselling and education for prenatal screening and diagnostic tests for pregnant women: Randomized controlled trial. *Int J Nurs Pract*, *27*(5), e13000. https://doi.org/10.1111/ijn.13000

Zhu, X., Chen, M., Wang, H., Guo, Y., Chau, M. H. K., Yan, H., Cao, Y., Kwok, Y. K. Y., Chen, J., Hui, A. S. Y., Zhang, R., Meng, Z., Zhu, Y., Leung, T. Y., Xiong, L., Kong, X., & Choy, K. W. (2020). Clinical utility of expanded noninvasive prenatal screening and chromosomal microarray analysis in high risk pregnancies. *Ultrasound Obstet Gynecol*. https://doi.org/10.1002/uog.22021

Prenatal Testing for Fetal Aneuploidy

Policy Number: AHS – G2055 – Prenatal Testing for Fetal Aneuploidy	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Aneuploidy is defined as an abnormal number of chromosomes present in the cell. Fetal aneuploidy is a condition where the fetus has one or more extra or missing chromosomes leading to either a nonviable pregnancy, offspring that may not survive after birth, or surviving newborn with congenital birth defects and functional abnormalities. The most common fetal aneuploidies associated with an additional chromosome are Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), and Patau syndrome (trisomy 13). Prenatal screening for fetal aneuploidy is an assessment of the pregnant individual's risk of carrying a fetus with fetal aneuploidy using markers found in maternal serum (ACOG, 2016). Noninvasive-invasive prenatal screening is a method for screening for chromosomal abnormalities using a maternal blood sample where cell-free fetal DNA (cffDNA) is extracted and screened for aneuploidies (McKanna et al., 2018).

For guidance on the use of chromosomal microarray in the confirmation of fetal aneuploidy, please see AHS-M2033-Chromosomal Microarray and Low-pass Whole Genome Sequencing.

II. Related Policies

Policy Number	Policy Title
AHS-G2035	Prenatal Screening
AHS-M2033	Chromosomal Microarray and Low-pass Whole Genome

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For pregnant individuals who desire information on the risk of having a child with fetal aneuploidy, the following screening tests to detect fetal aneuploidy of chromosomes 13, 18, and 21 **MEET COVERAGE CRITERIA**:
    a) First-trimester (defined as 11-14 weeks) screening incorporating maternal serum markers (hCG, PAPP-A with NT).
    b) Second-trimester (15-22 weeks) screening incorporating triple maternal serum markers (hCG, AFP, uE3 with NT) and quad maternal serum markers (hCG, AFP, uE3, DIA with NT).
    c) First (11-14 weeks) and second (15-22 weeks) trimester integrated screening incorporating maternal serum markers (PAPP-A with NT) and quad maternal serum markers (hCG, AFP, uE3, DIA with NT).
    d) First (11-14 weeks) and second (15-22 weeks) trimester sequential screening incorporating maternal serum markers (PAPP-A, hCG with NT) and quad maternal serum markers (hCG, AFP, uE3, DIA with NT).
    e) First (11-14 weeks) and second (15-22 weeks) trimester contingent screening incorporating maternal serum markers (PAPP-A, hCG with NT); if positive, quad maternal serum markers (hCG, AFP, uE3, DIA with NT).
2) For pregnant individuals who desire information on the risk of having a child with fetal aneuploidy, non-invasive prenatal screening (NIPS) to detect fetal aneuploidy of chromosomes 13, 18, 21, X, and Y (singleton or twin pregnancies of at least 10 weeks gestation) **MEETS COVERAGE CRITERIA**.
3) For pregnant individuals who desire information on the risk of having a child with fetal aneuploidy or to pursue additional confirmatory testing of equivocal or positive results from the above testing, karyotyping to confirm fetal aneuploidy **MEETS COVERAGE CRITERIA**.
4) To detect fetal aneuploidy, the use of the "penta" screen (hCG, AFP, uE3, DIA with NT, and hyperglycosylated hCG) **DOES NOT MEET COVERAGE CRITERIA**.
5) Screening for the detection of fetal aneuploidies **DOES NOT MEET COVERAGE CRITERIA** in any of the following situations:
    a) Parallel or simultaneous testing with multiple screening methodologies for fetal aneuploidy.
    b) For the screening of pregnant individuals with higher order multiple gestation pregnancies.
    c) Repeat screening for pregnant individuals with negative screening results.

d) For the detection of other chromosomal abnormalities (e.g., microdeletion syndromes, unbalanced translocations, deletions, duplications) not addressed above.

e) For the determination of fetal sex.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

6) For the diagnosis of fetal aneuploidy, the use of single cell genotyping in trophoblasts isolated from maternal serum (e.g., Luna Prenatal Test) **DOES NOT MEET COVERAGE CRITERIA**.

IV. Table of Terminology

Term	Definition
ACOG	The American College of Obstetrics and Gynecologists
ACMG	The American College of Medical Genetics and Genomics
AFP	Alpha-fetoprotein
ASRM	American Society for Reproductive Medicine
BMI	Body mass index
cfDNA	Cell-free deoxyribose nucleic acid
CffDNA	Cell-free fetal deoxyribose nucleic acid
CGH	Comparative genomic hybridization
CLIA '88	The Clinical Laboratory Improvement Amendments of 1988
CMA	Chromosomal microarray
CMS	Centers for Medicare and Medicaid
CPM	Confined placental mosaicism
CVS	Chorionic villa sampling
DIA	Dimeric inhibin A
DNA	Deoxyribose nucleic acid
dPCR-NIPT	Droplet digital polymerase chain reaction-non-invasive prenatal testing
E3	Estriol
FASTER	First and second trimester evaluation of risk
FDA	Food and Drug Administration
FPR	False-positive rate
GCPG	Practice Committee and Genetic Counseling Professional Group
β-hCG	β-human chorionic gonadotropin

hCG	Human chorionic gonadotropin
h-hCG	Hyperglycosylated human chorionic gonadotropin
Ifs	Incidental findings
ISPD	The International Society for Prenatal Diagnosis
ITA	Invasive trophoblast antigen
LDT	Laboratory-developed test
MPS	Massively parallel sequencing
MSS	Maternal serum screening
NEXT	Noninvasive examination of trisomy
NGS	Next generation sequencing
NIPD	Noninvasive prenatal diagnosis
NIPS	Noninvasive prenatal screening
NIPT	Noninvasive prenatal testing
NSGC	The National Society of Genetic Counselors
NT	Nuchal translucency
PAPP-A	Pregnancy-associated plasma protein-A
PPV	Positive predictive value
RATs	Rare autosomal trisomies
SCAs	Sex chromosome aneuploidies
SMFM	Society for Maternal-Fetal Medicine
SNPs	Single nucleotide polymorphisms
SURUSS	Serum, Urine and Ultrasound Screening Study
uE3	Unconjugated estriol

V. Scientific Background

Pregnant individuals are routinely offered blood-based screening or invasive diagnostic testing for identification of the most common fetal aneuploidies: trisomy 13 (Patau syndrome), trisomy 18 (Edwards syndrome) and trisomy 21 (Down syndrome). Approximately 90% of chromosomal abnormalities are due to an incorrect number of chromosomes, especially in these three triploid conditions as well as monosomy X (Turner syndrome). Approximately 15-20% of clinically recognized pregnancies result in first trimester spontaneous abortions, with 50% of these spontaneous abortions due to chromosomal abnormalities (Witters et al., 2011).

Historically, noninvasive blood-based aneuploidy screening has taken the form of first- and/or second-trimester analysis of biomarkers in maternal circulation, sometimes along with ultrasound measurement of fetal nuchal translucency (NT). Although both the sensitivity (detection rate) and specificity (true positive rate) of maternal serum screening tests for aneuploidy have improved significantly over time, the false positive

rate (two to five percent) remains higher than desirable. The detection rate of Down syndrome in the first trimester using a combination of NT and biochemical markers is typically 79% - 90% (Dey et al., 2013). Positive maternal serum screen results are usually followed by an invasive diagnostic test, such as karyotyping of a chorionic villus sample (in first trimester) or karyotyping of an amniotic fluid sample (second trimester).

Additionally, detection rates of maternal serum screens are typically below 99%, resulting in the inability of a normal result to confer complete confidence that the fetus is unaffected with aneuploidy (Dey et al., 2013). Thus, many pregnant individuals who are in a high-risk category due to age or other factors may opt for the more definitive, diagnostic, invasive testing, which has its own risks and relatively high costs. The availability of noninvasive testing may improve both the sensitivity and specificity of aneuploidy detection while resulting in fewer invasive procedures, less risk, and less overall cost.

Screening Tests

Chromosomal anomalies are a leading cause of perinatal mortality and developmental abnormality. The goal of prenatal testing is to screen for chromosomal anomalies and to provide genetic counseling for parents. The American College of Obstetrics and Gynecology (ACOG) recommends that prenatal testing is offered to all pregnant individuals (ACOG, 2016, 2020). Invasive testing, including chorionic villi sampling or amniocentesis, should be limited to high-risk patients owing to the potential risks for procedure-related pregnancy loss.

Genome-wide sequencing tests for fetal diagnostics have also been employed and are expected to increase in popularity as the cost decreases and as new tools are developed. These tests include DNA sequencing methods, such as whole exome-sequencing and targeted clinical panels, which can further evaluate fetal structural anomalies first detected in an ultrasound (ISPD, 2018). This diagnostic sequencing method has been used for various fetal diagnostic measures including standard genetic testing and chromosomal microarray analysis. While cfDNA could theoretically be analyzed to screen for other genetic disorders beyond common aneuploidies, no professional guideline currently recommend expanded screening for additional genetic disorders (Glenn E Palomaki, 2024). Screening for these additional genetic conditions (other aneuploidies, microdeletions/microduplications, and single gene disorders) while increasingly commercially available, has not been recommended for routine use by leading medical societies and is considered “investigational” (Glenn E Palomaki, 2024).

Chromosomal microarray (CMA) testing refers to the use of comparative genomic hybridization (CGH) arrays to compare the DNA of a patient with a normal control (Aradhya et al., 2007). CMA is significantly more sensitive (10 to 100 kb) than traditional karyotyping (5 to 10 Mb) and has a turnaround time of five days quicker than karyotyping (Robson et al., 2017), while providing an alternative to karyotyping when dividing cells are not available for analysis. This technique may be used for several different purposes, such as identifying a cause of pregnancy loss or identifying other aneuploid conditions, such as Down syndrome (Reddy et al., 2012). This method of diagnostic prenatal sequencing is currently investigational because of limited data and is utilized most prominently in research settings or clinically on a case-by-case basis (ISPD, 2018).

Biochemical Markers in Maternal Serum

Many studies revealed that maternal age, fetal NT, maternal serum free β-human chorionic gonadotropin (hCG) and pregnancy-associated plasma protein-A (PAPP-A) have been associated with aneuploidy. The "Quad screen", comprising alpha-fetoprotein (AFP), hCG, unconjugated estriol (E3), and inhibin A, is the most efficient multiple-marker screening test in the second trimester. In addition, there are more options such as integrated, sequential testing, and cell-free DNA screening. Many studies are ongoing to reveal the most sensitive, specific and effective screening tools for use during the first trimester (Park et al., 2016).

To improve the accuracy of serum markers, ultrasound markers are used. NT refers to the fluid filled space measured on the dorsal aspect of the fetal neck. An enlarged NT (>3.0 mm/99th percentile of the crown-rump length) is independently associated with fetal aneuploidy and structural malformations (ACOG, 2016, 2020).

Screening studies of pregnant individuals reported an association between increased NT in the first trimester of pregnancy (10-13 weeks of gestation) and chromosomal defects, most commonly Down syndrome (trisomy 21) but also trisomy 18 and 13. NT could be done alone as a first-trimester screen or in combination with maternal serum markers, free beta subunit of human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A). All three trisomies (chromosomes 13, 18, and 21) "are associated with increased maternal age, increased fetal NT and decreased PAPP-A, but in trisomy 21 serum free β-hCG is increased whereas in trisomies 18 and 13 free β-hCG is decreased" (Shiefa et al., 2013). Low β-hCG in the first trimester has also been associated with an increased risk of significant copy number variants on chromosomal microarray analyses (Bornstein et al., 2018).

Analytical Validity of Biochemical Markers

Screening for chromosomal abnormalities using biochemical markers include the first trimester combined test, triple test, quadruple test, sequential test, and integrated test. Except for the first trimester combined test, all others can provide screening results in the second trimester. In the first trimester combined test, the risk is calculated based on the ultrasonographic findings of NT and maternal serum levels of free β-hCG and PAPP-A. First-trimester screening not only allows early reassurance or early diagnosis of aneuploidy, but it also provides an option of earlier and safer termination of pregnancy in affected cases. Consequently, the first trimester combined test has become one of the most popular and useful screening strategies. Lee et al. (2013) conducted a 13-year study of 25,104 pregnant individuals using the first trimester Down syndrome screening. "The detection rates for trisomy 21, trisomy 18, Turner syndrome, and other chromosome anomalies were 87.5% (21/24), 69.2% (9/13), 81.8% (9/11), and 60% (18/30), respectively, with a false positive rate (FPR) of 5.4% (1353/25,026). Further evaluation of the detection rates for trisomy 21, by gestational age at 11, 12, and 13 weeks, were 92.3%, 87.5%, and 66.7%, respectively" (Lee et al., 2013).

For second-trimester screening for Down syndrome, the sensitivity and specificity of the triple test—co-testing AFP, unconjugated E3, and free β-hCG—are higher than screening with AFP alone. However, when the false-positive rate is fixed at five percent, to compare the screening performance between the screening tools, the detection rate was found to be 66.8% to 77% with the triple test and 75.9% to 92% with the first trimester combined test. The sensitivity of the triple test was lower than the combined test (Baer et al., 2015).

The quadruple test, which uses the fourth marker, inhibin A, in addition to the other three markers, has seven percent higher sensitivity when applying a fixed five percent false-positive rate. A study conducted by Wald et al. (2003) revealed that when inhibin A was added to the traditional triple marker test, a detection rate of 83% was achieved, which was six percent higher than the 77% detection rate found with the triple test. This result was similar to that produced with the first trimester combined test (Park et al., 2016).

Many studies, including the Serum, Urine and Ultrasound Screening Study (SURUSS) (Wald et al., 2003) and the First and Second-Trimester Evaluation of Risk (FASTER) study (Malone et al., 2005), have offered evidence suggesting that first-trimester screening for Down syndrome with measurement of fetal NT and maternal serum markers is at least as accurate as alternative tests and may allow for earlier confirmation or exclusion of Down syndrome. These studies evaluated several tests in parallel, including first trimester testing with NT and maternal markers, the triple test, second-semester quadruple test and a combined first- and second-trimester test (both

with and without NT), stepwise sequential testing (results given after first-trimester testing, move on to second-trimester testing), and integrated screening (results given only after first and second-trimester testing). In a direct comparison of the first-trimester test to the triple test, the SURUSS study has shown that setting the false-positive rate at 5% resulted in an 83% detection rate, which was superior to what was historically expected of the triple test (Wald et al., 2003). SURUSS results were based on data from 47,053 pregnancies (101 with Down syndrome). The FASTER trial was conducted in the United States and was sponsored by the National Institutes of Health. The study enrolled 38,167 pregnant individuals and provided further evidence that first-trimester combined screening was effective, but it did not provide NT measurement alone; results showed that integrated first- and second-trimester screening provided higher detection rates. The SURUSS and FASTER studies also found that overall, first-trimester screening with NT alone is inferior to either first- or second-trimester combined screening. Additional testing may not be necessary in those few cases when NT is at least 4.0 mm due to the high likelihood of Down syndrome in these cases (Malone et al., 2005; Wald et al., 2009; Wald et al., 2003).

Studies have found a high rate of successful imaging of the fetal nasal bone and an association between absent nasal bone and the presence of Down syndrome in high-risk populations. However, there is insufficient evidence on the performance of fetal nasal bone assessment in average-risk populations. Of concern is the low performance of fetal nasal bone assessment in a subsample of the FASTER study conducted in a general population sample. Two studies conducted outside of the United States have found that, when added to a first-trimester screening program evaluating maternal serum markers and NT, fetal nasal bone assessment can result in a modest decrease in the false-positive rate. Several experts in the field are proposing that fetal nasal bone assessment be used as a second stage of screening to screen pregnant individuals found to be of borderline risk using maternal serum markers and NT. Considering the uncertainty of test performance in average-risk populations and the lack of standardization in the approach to incorporating this test into a first-trimester screening program, detection of fetal nasal bone is considered investigational (Wald et al., 2009).

Palomaki et al. (2004) demonstrate that hyperglycosylated hCG (h-hCG), also known as invasive trophoblast antigen (ITA), may be a promising screening marker for Down syndrome detection in the second trimester. In the study, serum samples of 45 Down syndrome cases and 238 unaffected control pregnancies between 14 to 20 weeks of gestation were collected and measured for h-hCG, along with other screening markers (Palomaki et al., 2004). As seen in the figure below, h-hCG, in combination with four other screening markers, increased the detection rate to 83% at a five percent false-positive rate from the 72% detection rate by the tripe test (Palomaki et al., 2004;

QuestDiagnostics, 2022). In addition, "The median [h-hCG] in Down syndrome pregnancies was >3.00 multiples of the median, higher than that found for human chorionic gonadotropin (hCG)." The author recommends that "the highest screening performance for Down syndrome can be obtained by integrating first- and second-trimester serum and ultrasound markers into a single interpretation in the second trimester. This integrated test approach can detect 90% of Down syndrome pregnancies at a 3% false-positive rate" (Palomaki et al., 2004).

Cell-Free Fetal DNA from Maternal Serum

In 1997, researchers reported the identification of cell-free fetal DNA (cffDNA) in the circulation of pregnant individuals (Lo et al., 1997). The fetal fraction of cffdna increases throughout gestation. cffDNA is usually detectable within six to seven weeks of gestation, with the earliest detection reported at 4.5 weeks. Therefore, it allows for noninvasive procedures to be performed much earlier in the pregnancy and eschews the need for standard biochemical and invasive screens. Moreover, given that cffDNA is cleared from maternal circulation within hours following birth and removal of the placenta, cffDNA is specific to the ongoing pregnancy at the time of sampling (Shaw et al., 2020). At 10 weeks of gestation, the fetal fraction comprises at least three to four percent whereas it can account for up to 50% of the total cell-free DNA at term (Palomaki et al., 2024). Since then, laboratories have validated several different techniques for the use of cell-free DNA as a screening test for fetal aneuploidy, and these methods have been termed noninvasive prenatal screening (NIPS) or noninvasive prenatal testing (NIPT).

Noninvasive prenatal screening is a testing method which utilizes cell-free DNA from the plasma of pregnant individuals to screen for fetal aneuploidy. It is important to note that cell-free DNA screening does not assess risk of fetal anomalies, including neural tube defects or ventral wall defects (ACOG, 2015). NIPS methods only provide an estimate of whether the risk of aneuploidy is increased or decreased; NIPS does not provide a definitive diagnosis of aneuploidy. As with other aneuploidy screening tests, it is recommended that positive results of NIPS be followed by diagnostic testing such as traditional karyotyping of fetal cells obtained via chorionic villus sampling or amniocentesis (Gregg et al., 2016).

One cell-free fetal DNA detection method for NIPS, known as massively parallel sequencing (MPS), is a technique in which millions of pieces of maternal and fetal chromosomal material are sequenced and quantified. The MPS method is able to detect many types of aneuploidies, including those which are less commonly seen (Devers et al., 2013). MPS can detect common aneuploidies with both high sensitivity and high specificity for trisomies 13, 18, and 21. Bianchi et al. (2012) found the

detection rate sensitivity for trisomy 21 to be 100%, the detection rate sensitivity for trisomy 18 to be 97.2%, and the detection rate sensitivity for trisomy 13 to be 78.6%; specificity was 100% for all three of the aforementioned trisomies.

Detection of aneuploidy using circulating cell-free fetal DNA can also be performed using selective analysis of specific loci only from the chromosomes of interest, as opposed to sequencing of all chromosomes performed in MPS. This directed analysis of cell-free fetal DNA has also been shown to have high sensitivity and high specificity for the common trisomies. Lee et al. (2019) utilized plasma from 1,055 pregnant individuals and found that NIPT with cell-free fetal DNA "showed 100% sensitivity and 99.9% specificity for trisomy 21, and 92.9% sensitivity and 100% specificity for trisomy 18, and 100% sensitivity and 99.9% specificity for trisomy 13" (Lee et al., 2019).

The third approach to detect aneuploidy from cffdna is based on the amplification of single nucleotide polymorphisms (SNPs) on the chromosome of interest. In a study by Eiben et al. (2015), 2,942 patients underwent SNP-based noninvasive prenatal screening (NIPS) in which the source for cffdna was derived from placental cells. Sixty-five patients (2.2%) had positive noninvasive prenatal screening results for aneuploidy and further invasive testing confirmed aneuploidy in fifty-nine of those patients (90.8%). The remaining six patients were false positives due to a discrepancy between the genetic status of the fetus and placenta, a condition known as confined placental mosaicism (CPM). The fetal fraction was abnormally low (less than eight percent) and indicative of fetal-placental discrepancies. Although a reliable screening method, the author suggests that SNP-based NIPS "cannot be used as a standalone test without ultrasound examination or invasive confirmation" (Eiben et al., 2015).

Despite the apparent advantages of NIPS over standard maternal serum screening in screening for common aneuploidies, there are limitations. "Reported Ifs [incidental findings] range from fetal or maternal deletions and duplications or mosaic sex chromosome aneuploidy in the mother or fetus, presenting as aneuploidy risk on NIPS, to mosaicism and uniparental disomy to abnormal results because of the presence of cell-free DNA originating from an undiagnosed maternal tumor" (Westerfield et al., 2014). When ultrasound evaluation reveals fetal anomalies that may be consistent with one of those scenarios, invasive diagnostic testing with karyotyping or microarray may be more appropriate. NIPS also cannot distinguish the cause of aneuploidy, nor can it differentiate among the presence of an extra chromosome, a Robertsonian translocation, or high-level mosaicism. The determination of the type of aneuploidy is important for accurate counseling and future risk assessment (Neufeld-Kaiser et al., 2015; Strom et al., 2017; Westerfield et al., 2014). Also, some samples contain insufficient amounts of cell-free DNA, which is unknown until the test procedure has commenced. Early gestational age (<10 weeks) and high body mass index have been

shown to be associated with reduced amounts of circulating cell-free fetal DNA. Additionally, NIPS for aneuploidy does not detect the presence of neural tube defects, which is included in traditional second trimester maternal serum screening. It has been suggested that the testing of maternal serum AFP in the second trimester should be offered to pregnant individuals who underwent first-trimester aneuploidy screenings (Palomaki et al., 2024).

And so, while promising on the screening front, research has yet to support NIPT's diagnostic prowess. NIPT platforms typically screen for common trisomies with or without sex chromosome anomalies, and therefore overlook most other chromosomal rearrangements (Al Toukhi et al., 2019; Shaw et al., 2020). Furthermore, the power of NIPT is limited by discordant—e.g., false-positive and false-negative—results, due to issues including vanishing twin syndrome, where a spontaneous early miscarriage may still release cffDNA and interfere with early NIPT results. Abnormal maternal cells mixing with normal fetal cells, producing mosaicism as aforementioned, has been reportedly repeatedly and therefore is an incidental cause of discordant results, suggesting that pregnant individuals with known malignancies should be dissuaded from NIPT (Bianchi et al., 2015; Shaw et al., 2020).

Extension of NIPT to sex chromosome aneuploidies and rare autosomal trisomies has also been explored, though its utility remains controversial. The increased variability in its use here is due in part to the sensitivity of NIPT to detect sex chromosome aneuploidies—e.g., Turner syndrome (45, X) and Klinefelter syndrome (45, XXY)—being lower than that of common trisomies. Moreover, as NIPT screens were originally limited in scope to identify trisomies 13, 18, and 21, the utility of NGS-based NIPT to also detect rare autosomal trisomies (RATs) has yet to be informed by the clinical community, and offers inspiration for future directions (Shaw et al., 2020).

Analytical Validity of Cell-free Fetal DNA Testing

A study by Palomaki et al. (2012) of 4664 pregnancies at high-risk for Down syndrome using the MPS method had a detection rate of 98.6% with a false-positive rate of only 0.20% (3/1471) (Palomaki et al., 2011). Results also identified MPS as a successful detection method for both trisomy 18 and trisomy 13. The authors state, "Among the 99.1% of samples interpreted (1,971/1,988), observed trisomy 18 and 13 detection rates were 100% (59/59) and 91.7% (11/12) at false-positive rates of 0.28% and 0.97%, respectively… Among high-risk pregnancies, sequencing circulating cell-free DNA detects nearly all cases of Down syndrome, trisomy 18, and trisomy 13, at a low false-positive rate. This can potentially reduce invasive diagnostic procedures and related fetal losses by 95%. Evidence supports clinical testing for these aneuploidies" (Palomaki et al., 2012).

Norton et al. (2015) reported near-perfect accuracy of detection for trisomy 21 (Down syndrome) with the use of cell-free DNA (cfDNA) (sensitivity, 100% [38 of 38 cases of trisomy 21]; false-positive rate, 0.06% [9 false positives among 15,841 pregnant individuals]) in the Noninvasive Examination of Trisomy (NEXT) study. Norton and colleagues found that cfDNA testing for trisomy 21, as compared with standard screening, had a better global performance during the first trimester of pregnancy. However, they did not provide information about the 14 fetal chromosomal abnormalities in the 15,841 screened pregnancies, other than for trisomies 13, 18, and 21 (Norton et al., 2015).

In 2017, the Dutch Ministry of Health introduced a nationwide implementation study on NIPT as a first-tier strategy offered to all pregnant individuals in the TRIDENT-2 study. TRIDENT-2 was specific in its scope, as it excluded pregnancies with a vanishing or dischorionic twin, fetal ultrasound including a nuchal translucency greater than or equal to 3.5mm, or gestational age less than 11 weeks. Moreover, pregnant individuals with a history of being high-risk for the common trisomies and who have had an organ transplant were excluded as well, as were pregnant individuals with malignant neoplasia. Of all pregnancies that year, 73,239 (42%) opted for NIPT, it was found that though the number of common trisomies 13, 18, and 21 detected by NIPT was comparable to those of earlier studies, PPVs were higher than expected (53% PPV, 98%, 96%, respectively) with high sensitivities (100%, 91%, 98%, respectively), as confirmed by invasive prenatal testing or by postnatal bloodwork (van der Meij et al., 2019). However, the researchers do acknowledge potential limitations, namely not having presented data on sex chromosome aneuploidies and using different sequencing methods (e.g., NextSeq vs. HiSeq) and fetal fraction benchmarks for rejection across their three testing centers. However, despite issues to external validity, the authors conclude that "this study has confirmed that genome-wide NIPT is a reliable and robust screening test for the detection of fetal trisomies 21, 18, and 13" as they urge further research on screening for fetal pathology and adverse pregnancy outcomes (van der Meij et al., 2019).

As a secondary finding of the TRIDENT-2 study, NIPT may play a future role in raising suspicion of rare maternal malignancies. The TRIDENT-2 study enrolled 231,896 pregnant patients with NIPT results and showed incidental findings of malignancy-suspicious-NIPT in a small proportion of patients (Heesterbeek et al., 2022). The most common malignancies in individuals capable of becoming pregnant who are also of reproductive age include breast, cervical, ovarian, and colorectal cancers; leukemia; Hodgkin and non-Hodgkin lymphoma; thyroid cancer; and melanoma. When tumor cfDNA is incidentally found in maternal blood, there are no current professional guidelines that address the clinical management of cfDNA results. While only (0.02%) of NIPT results in the Heesterbeek et al. (2022)study showed indications of a maternal

malignancy, researchers pointed out this information has clinical significance and can prompt a diagnostic workup through magnetic resonance imaging and computed tomography to enable counseling and confirmative diagnosis in these rare instances (Heesterbeek et al., 2022).

Luo et al. (2021) aimed to explore the efficacy of using NIPT to predict sex chromosome aneuploidies (SCAs) in a 34,717-patient sample study in China. Of the clinical pregnancies examined, 229 (0.66%) were associated with sex chromosome aneuploidies, with 78 of the cases reporting positive for 45,X and 151 sex chromosome trisomies (47,XXX, 47,XXY, 47XYY). In total, 193 of the 229 NIPT positive results acquiesced to confirmatory invasive prenatal diagnosis via karyotyping analysis of amniotic fluid and fluorescent *in situ* hybridization, and it was found that only 67 (34.7%) were true positives. The authors reported similarly low PPVs, with 23.07% for 45,X and 36%, 50%, and 27.27% for 47,XXX, 47,XXY, 47XYY, respectively. Given this performance of the NIPT, the authors concluded that "Confirmatory testing of abnormal results is recommended prenatally or after birth," insinuating the current impotency of NIPT (Luo et al., 2021).

A two-year longitudinal study which utilized 11,414 material blood samples for NIPT. Among the 11,213 cases with confirmatory results, "94 T21, 39 T18, 8 T13, 15 XO, 6 XXX, 3 XYY, 5 XXY and 11,042 euploid cases were detected." Overall, the sensitivity of NIPT was 98.90% (T21), 100.00% (T18), 100.00% (T13), 90.91% (XO), 100.00% (XXX), 100.00% (XYY), and 100.00% (XXY), while the specificity was 99.96% (T21), 99.97% (T18), 99.99% (T13), 99.96% (XO), 99.98% (XXX), 100.00% (XYY), and 99.99% (XXY). The authors concluded that "NIPT showed excellent performance as screening test for the detection of fetal T21, T18, T13 and SCA [sex chromosome aneuploidies] in mixed-risk pregnancies in Iran" (Garshasbi et al., 2019).

A study out of the Illumina laboratory (formerly Verinata) compared NIPS to standard maternal serum screening in pregnant individuals at average-risk for fetal aneuploidy. Their report included data of 5974 samples tested for trisomies 13, 18, and 21 as well as monosomy X. Aneuploidy was detected in 4.8% of samples with only 0.2% putative false-positives and 0.08% false-negatives; however, 2.8% of cases had indefinite results for a single chromosome (Futch et al., 2013). Illumina more recently reported a more extensive study consisting of 85,298 clinical cases. "Aneuploidy was detected or suspected in 2142 (2.5%) samples. For aneuploidy detected cases with known clinical outcomes, the overall positive predictive value (PPV) was 83.5% (608/728); observed PPVs for trisomies 21, 18, and 13 ranged from 50.0 to 92.8%" (Taneja et al., 2016).

Liang et al. (2019) used cell-free plasma DNA to assess the clinical utility of using an expanded noninvasive prenatal screening ("NIPS-Plus") to detect aneuploidy and

genome-wide microdeletion/microduplication syndromes (MMS). Of the 94,085 individuals with singleton pregnancies enrolled in the study, 1128 were suspected of having clinically significant fetal chromosomal abnormalities. Follow-up testing in the study reported the positive predictive values (PPVs) of 95%, 82%, 46%, 29%, and 47% for T21, T18, T13, rare trisomies, and sex chromosome aneuploidies, respectively. For known MMS (n=32), PPVs were 93% (DiGeorge), 68% (22q11.22 microduplication), 75% (Prader-Willi/Angelman), and 50% (Cri-du-Chat). Thus, the researchers conclude that "the data have potential significance in demonstrating the usefulness of cfDNA profiling" and that "NIPS-Plus can be used as a first-tier pregnancy screening method to improve detection rates of clinically significant fetal chromosome abnormalities" (Liang et al., 2019).

A retrospective study by Wu et al. (2020) compared positive noninvasive prenatal screening (NIPS) results for aneuploidy to standard diagnostic tests such as traditional karyotyping and chromosomal microarray analysis (CMA). The study enrolled 551 pregnant individuals who screened positive for trisomy 13, trisomy 18, trisomy 21, and other sex chromosomal aneuploidies. Samples were obtained from either amniotic fluid or fetal cord blood and subsequent karyotyping or CMA confirmed a total of 256 out of 551 cases (46.4%) to possess chromosomal abnormalities concordant or partially concordant with NIPT results. Placental biopsies were obtained to assess the etiology of NIPS false positives and confined placental mosaicism (CPM) was found in 60% of the biopsies. The authors also reported that pregnant individuals with advanced maternal age (>35 years) had the highest positive predictive value (PPV) for trisomy 21 (87.8%), trisomy 18 (59.3%), and trisomy 13 (37.5%), while the PPV was significantly lower for pregnant individuals with young maternal age (<34 years) for trisomy 21 (71.9%), trisomy 18 (0%), and trisomy 13 (16.7%). This suggests that NIPS performs better in predicting aneuploidies for pregnancies with advanced maternal age than for pregnancies with young maternal age. However, the author notes that the PPVs showed "no significant upward trend when compared based on specific age categories (an interval of 5 years), which suggested that NIPT-positive result deserves equal attention from both providers and patients regardless of maternal age" (Wu et al., 2020).

Table 1. Down Syndrome Detection Rates (DRs) Obtained from Various Screening Tests[12]

Test Name	Alias	Markers Included	DR, %[a]
Triple Screen	Maternal Serum Screen 3	Age, AFP, hCG, uE3	72
Quad Screen	Maternal Serum Screen 4	Age, AFP, hCG, uE3, DIA	79
Penta Screen	Maternal Serum Screen 5	Age, AFP, hCG, uE3, DIA, h-hCG	83

[a] Detection rate at a 5% false-positive rate.

Muzzey et al. (2020) studied and compared the sensitivities of both traditional aneuploidy screening and customized whole genome sequencing NIPS for pregnant individuals with high body mass index (BMI). Generally, pregnant individuals with high BMI have reduced fetal fraction during cf-DNA based NIPS, causing up to 24.3% of tests to fail. The study included 58105 patients, and the authors analyzed fetal fraction, aneuploidy, and BMI. Overall, across all obesity classes, and assuming no fetal fraction related rest failures, "analytical sensitivity for the investigated NIPS exceeded that of traditional aneuploidy screening for trisomies 13, 18, and 21." The authors conclude that customized NIPS with "high accuracy at low FF and a low test-failure rate" is preferable over traditional aneuploidy screening for individuals with high BMI (Muzzey et al., 2020).

Dar et al. (2022) investigated the performance of cell-free DNA screening against a genetic confirmation of results with the goal of analyzing test performance and test failure (no-call rates). A total of 20,194 pregnant individuals were enrolled with a median gestational pregnancy of 12.6 weeks. The results of the genetic test were confirmed for 17,851 cases (88.4%). Among these were 13,043 low-risk and 4808 high-risk cases for aneuploidy. A total of 133 trisomies were diagnosed. These were composed of 100 (trisomy 21), 18 (trisomy 18) and 15 (trisomy 13). The positive rate of cfDNA screens was lower in the low-risk group as compared to the high-risk cohort (0.27% versus 2.2% P<.0001). Sensitivity and specificity were comparable between the two risk groups. The PPV for the low- and high-risk groups was 85.7% versus 97.5%, respectively. There were also 602 individuals who had a "no-call" outcome post-draw and 287 (1.61%) after a second draw. The authors concluded that in pregnant individuals "at a low risk for aneuploidy, single-nucleotide polymorphism-based cell-free DNA has high sensitivity and specificity, positive predictive value of 85.7% for trisomy 21 and 74.3% for the three common trisomies." They also noted that "patients who receive a no-call result are at an increased risk of aneuploidy and require additional investigation" (Dar et al., 2022).

Dai et al. (2022) studied the sensitivity and specificity of a multiplex droplet digital PCR NIPT (dPCR-NIPT) assay. The authors propose that this test has a higher sensitivity than traditional serum biochemical tests and has a lower cost than NGS-NIPTs. The clinical performance of the dPCR-NIPT assay was tested with 238 maternal plasma samples, 36 of which were trisomy positive. "The dPCR-NIPT assay demonstrated a detection sensitivity of 100% and a specificity of 95.12%." The authors noted that this is the first study done on the dPCR-NIPT assay but concluded that it is "superior" to traditional serum biochemical tests and NGS-NIPTs (Dai et al., 2022).

Proprietary Testing

Several methods for detection of fetal aneuploidy by analysis of circulating cell-free fetal DNA are commercially available. All have been validated in pregnancies deemed to be at high-risk for aneuploidy. Evaluation of this technology for use in low- or average-risk pregnancies is ongoing.

Current commercially available laboratory-developed noninvasive prenatal tests for aneuploidy include: the MaterniT21™ Plus Test(Integrated Genetics/LabCorp) (LabCorp, 2023a), the verifi™ Prenatal Test and verifi™ Plus Prenatal Test (Illumina) (Illumina, 2023), the Harmony Prenatal Test™ (Roche) (Roche, 2023), the Panorama test (Natera, available from several reference laboratories) (Natera, 2023), QNatal® Advanced test (QuestDiagnostics, 2023), Prequel test (Myriad, 2023), CentoNIPT® test (Centogene, 2023), ClariTest™ Core (GenPath, 2023), IONA® test and Sage™ prenatal screen (YourgeneHealth, 2023), Invitae NIPS test (Invitae, 2023), and Clarigo test (AgilentTechnologies, 2022). Other examples include, but are not limited to, the VERACITY® test out of NIPD Genetics (NIPD, 2023), the Vanadis® NIPT system (PerkinElmer, 2023), the NIFTY® Test and NIFTY® Test Pro (BGI, 2023), and the informaSeq® Prenatal Test (Genetics, 2016).

Regarding serum screening options for common birth defects, Integrated Genetics, a LabCorp Specialty Testing Group, names the Afp4®, which screens for Down syndrome, trisomy 18, and open neural tube defects in the second trimester, and SerumIntegratedScreen®, which screens for the same, but combines results from both the first and second trimesters. Other tests mentioned include the FirstScreen®, IntegratedScreen®, and SequentialScreen®, the latter of which boasts that "Part 1 [between 10th and 14th weeks of pregnancy] leads to the detection of approximately 70% of Down syndrome cases and 80% of trisomy 18 cases, and Part two [between 15th and 22nd weeks of pregnancy] leads to detection of approximately 90% of Down syndrome cases, 90% of trisomy 18 cases and 80% of open neural tube defects" (LabCorp, 2023b).

The Luna Prenatal Test is a prenatal genetic test that uses a cell-based approach to extract fetal DNA from fetal trophoblast cells present in maternal blood (Luna, 2022a). A key advantage of this test, highlighted in a white paper, is its ability to accurately analyze a single fetal cell, ensuring access to pure fetal DNA.

In an analytical validation overview of the Luna Prenatal Test, two groups of pregnant individuals (one group composed of 59 high-risk individuals who received CVS or amniocentesis) and a second group (158 low-risk individuals who had no known diagnostic testing) were recruited. A third set of test samples was analyzed for comparison composed of "seven Coriell cell lines harboring known chromosome abnormalities" (Luna, 2022b). Single cells (enriched from maternal whole blood) were

processed individually to access pure fetal or cell line DNA. Subsequently, the DNA from each individual cell was "sequenced by low-pass massively parallel sequencing technology to generate a genome-wide copy number profile, which was evaluated for genome-wide copy number changes larger than 1.5 Mb"(Luna, 2022b). The first group of 59 samples had 11 samples excluded. Of the remaining 48 pregnancies (49 fetuses), results indicated an accuracy of 100% (92.75-100% confidence interval), specificity 100% (54.07-100% confidence interval), sensitivity of 100% (91.78-100% confidence interval), PPV of 100%, and NPV of 100% in detecting chromosomal abnormalities (complete agreement between CVS/amniocentesis and Luna); they also noted a plan to increase the sample size to 200 for a larger data set as this was a limited study.

For the second test group, a report was issued for 91.1% of cases, with 8.9% receiving a result of "no scorable cells." In the second group validation study, they found "complete intra-case concordance for 404 cells from 118 cases" (Luna, 2022b). The third set used human cultured cells (lymphoblast or fibroblast) with known aneuploidy or deletion. From the results: "out of a total of 148 scorable cells, the known abnormality was detected and called by the NxC software in every cell except one. Thus, the analytic sensitivity and specificity were 100% with the expected finding being reported in every cell" (Luna, 2022b).

The test makers also note: "We do not claim that the Luna Prenatal Test is diagnostic for aneuploidy, because there are recommendations from professional organizations that a final diagnosis should not rest on testing of trophoblasts alone" (Luna, 2022b).

Bellair et al. (2024) evaluated the noninvasive prenatal genetic "Luna test" using sequence-based copy number analysis of single trophoblasts from maternal blood. Blood samples were collected from 401 pregnant individuals (8-22 weeks), processed to isolate and sequence trophoblast cells. Of the 243 pregnancies undergoing chorionic villus sampling (CVS) or amniocentesis, 160 showed normal results, while 15 had abnormalities, including 14 aneuploidy cases and one Williams syndrome deletion. Placental mosaicism occurred in 7 of 236 Luna test cases and 3 of 188 CVS cases, with no usable trophoblasts recovered in 32 samples. In 158 low-risk pregnancies, 133 were normal, seven showed aneuploidy, and three had likely pathogenic deletions/duplications. Although the sample size was small, the Luna test identified aneuploidies and genetic deletions/duplications, showing consistency with CVS/amniocentesis results, though further data is needed for precise test accuracy (Bellair et al., 2024).

VI. Guidelines and Recommendations

The American College of Obstetrics and Gynecologists (ACOG) and the Society for Maternal-Fetal Medicine (SMFM)

The American College of Obstetrics and Gynecologists and the Society for Maternal-Fetal Medicine offered Recommendations for Clinical Management Guidelines for Obstetricians and Gynecologists on Screening for Aneuploidy.

The following recommendations and conclusions are based on good and consistent scientific evidence (Level A):

- "Prenatal genetic screening (serum screening with or without nuchal translucency [NT] ultrasound or cell-free DNA screening) and diagnostic testing (chorionic villus sampling [CVS] or amniocentesis) options should be discussed and offered to all pregnant [individuals] regardless of maternal age or risk of chromosomal abnormality. After review and discussion, every patient has the right to pursue or decline prenatal genetic screening and diagnostic testing.
- If screening is accepted, patients should have one prenatal screening approach, and should not have multiple screening tests performed simultaneously.
- Cell-free DNA is the most sensitive and specific screening test for the common fetal aneuploidies. Nevertheless, it has the potential for false-positive and false-negative results. Furthermore, cell-free DNA testing is not equivalent to diagnostic testing.
- All patients should be offered a second-trimester ultrasound for fetal structural defects, since these may occur with or without fetal aneuploidy; ideally this is performed between 18 and 22 weeks of gestation (with or without second-trimester maternal serum alpha-fetoprotein).
- Patients with a positive screening test result for fetal aneuploidy should undergo genetic counseling and a comprehensive ultrasound evaluation with an opportunity for diagnostic testing to confirm results.
- Patients with a negative screening test result should be made aware that this substantially decreases their risk of the targeted aneuploidy but does not ensure that the fetus is unaffected. The potential for a fetus to be affected by genetic disorders that are not evaluated by the screening or diagnostic test should also be reviewed. Even if patients have a negative screening test result, they may choose diagnostic testing later in pregnancy, particularly if additional findings such as fetal anomalies identified on ultrasound examination become evident.
- Patients whose cell-free DNA screening test results are not reported by the laboratory or are uninterpretable (a no-call test result) should be informed that test failure is associated with an increased risk of aneuploidy, receive further genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing.
- If an enlarged nuchal translucency or an anomaly is identified on ultrasound examination, the patient should be offered genetic counseling and diagnostic testing for genetic conditions as well as a comprehensive ultrasound evaluation

including detailed ultrasonography at 18–22 weeks of gestation to assess for structural abnormalities.
- [Individuals] with a positive screening test result for fetal aneuploidy should be offered further detailed counseling and testing" (ACOG, 2020).

The following recommendations and conclusions are based on limited or inconsistent scientific evidence (Level B):

- "The use of cell-free DNA screening as follow-up for patients with a screen positive serum analyte screening test result is an option for patients who want to avoid a diagnostic test. However, patients should be informed that this approach may delay definitive diagnosis and will fail to identify some fetuses with chromosomal abnormalities.
- In clinical situations of an isolated soft ultrasonographic marker (such as echogenic cardiac focus, choroid plexus cyst, pyelectasis, short humerus or femur length) where aneuploidy screening has not been performed, the patient should be counseled regarding the risk of aneuploidy associated with the finding and cell-free DNA, quad screen testing, or amniocentesis should be offered. If aneuploidy testing is performed and is low-risk, then no further risk assessment is needed. If more than one marker is identified, then genetic counseling, maternal–fetal medicine consultation, or both are recommended.
- No method of aneuploidy screening that includes a serum sample is as accurate in twin gestations as it is in singleton pregnancies; this information should be incorporated into pretest counseling for patients with multiple gestations.
- Cell-free DNA screening can be performed in twin pregnancies. Overall, performance of screening for trisomy 21 by cell-free DNA in twin pregnancies is encouraging, but the total number of reported affected cases is small. Given the small number of affected cases it is difficult to determine an accurate detection rate for trisomy 18 and 13" (ACOG, 2020).

The following recommendations and conclusions are based primarily on consensus and expert opinion (Level C):

- "The use of multiple serum screening approaches performed independently (eg, a first-trimester screening test followed by a quad screen as an unlinked test) is not recommended because it will result in an unacceptably high positive screening rate and could deliver contradictory risk estimates.
- In multifetal gestations, if a fetal demise, vanishing twin, or anomaly is identified in one fetus, there is a significant risk of an inaccurate test result if serum-based

aneuploidy screening or cell-free DNA is used. This information should be reviewed with the patient and diagnostic testing should be offered.

- Patients with unusual or multiple aneuploidies detected by cell-free DNA should be referred for genetic counseling and maternal–fetal medicine consultation" (ACOG, 2020).

The American College of Obstetrics and Gynecologists also comments on the specific types of screening, which include triple, quadruple (quad) and "penta" screens. These screens may be performed in the first trimester (10-14 weeks gestation) or second trimester (15-22 weeks). Triple screens measure serum hCG [human chorionic gonadotropin], AFP [alphafetoprotein], and uE3 [unconjugated estriol], while the quad screen includes DIA [dimeric inhibin A] with the three previously mentioned markers. Some laboratories have been noted to offer a "penta" screen, which includes hyperglycosylated hCG along with the four analytes of the quad screen, but ACOG states that "its performance has not been evaluated rigorously in prospective studies". ACOG discusses several testing algorithms in the include, which are summarized in the table below:

Screening Approach	Approximate Gestational Age Range for Screening (Weeks)	Detection Rate (DR) for Trisomy 21 (%)	Screen Positive Rate* (%)	Advantages	Disadvantages	Method
Cell-free DNA†	9–10 to term	99	2–4% Includes inability to obtain results, which is associated with increased risk†	1. Highest DR 2. Can be performed at any gestational age after 9–10 weeks 3. Lowest false-positive rate	Results may reflect underlying maternal aneuploidy or maternal disease	Several molecular methods
First trimester‡	10–13 6/7§	82–87‖	5	1. Early screening 2. Single time point test	Lower DR than tests with first and second trimester component NT required	NT+PAPP-A, free beta hCG, +/− AFP¶
Quad screen‡	15–22	81	5	1. Single time point test 2. No specialized US required	Lower DR than first trimester and first and second trimester combined tests	hCG, AFP, uE3, DIA
Integrated‡	10–13 6/7§, then 15–22	96	5	High DR	Two samples needed No first-trimester results NT required	NT+PAPP-A, then quad screen
Serum integrated‡	10–13 6/7§, then 15–22	88	5	1. DR compares favorably with first-trimester screening 2. No specialized US required	Two samples needed No first-trimester results	PAPP-A + quad screen
Sequential#: stepwise	10–13 6/7§, then 15–22	95	5	1. First-trimester results provided 2. Comparable performance to integrated, but FTS results provided	Two samples needed NT required	NT+ free beta hCG + PAPP-A, +/− AFP¶, then quad screen
Contingent screening**		88–94	5	First-trimester test result: Positive: diagnostic test or cell-free DNA offered Negative: no further testing Intermediate: second-trimester test offered Final: risk assessment incorporates first- and second-trimester results	Possibly two samples needed NT required	NT+hCG+ PAPP-A, +/− AFP¶, then quad screen

Finally, ACOG notes that other trisomies, such as trisomies 16 or 22, can be tested for. However, ACOG recommends against screening for these two aneuploidies due to lack of validated data (ACOG, 2020).

The National Society of Genetic Counselors (NSGC)

The NSGC issued a position statement that supports noninvasive prenatal screening as an option for pregnancies considered high-risk for trisomy 13, 18 or 21. "The National

Society of Genetic Counselors currently supports Noninvasive Prenatal Testing/Noninvasive Prenatal Diagnosis (NIPT/NIPD) as an option for patients whose pregnancies are considered to be at an increased risk for certain chromosome abnormalities. NSGC urges that NIPT/NIPD only be offered in the context of informed consent, education, and counseling by a qualified provider, such as a certified genetic counselor. Patients whose NIPT/NIPD results are abnormal, or who have other factors suggestive of a chromosome abnormality, should receive genetic counseling and be given the option of standard confirmatory diagnostic testing" (Devers et al., 2013).

The NSGC expounded upon their recommendations for prenatal screening and diagnostic testing for chromosomal aneuploidy in a set of practice guidelines. For all patients, it is recommended that "Providers should offer the options of maternal serum screening (MSS) and diagnostic testing for chromosome aneuploidy to every patient", provided that the providers themselves are made aware of factors that may impact their patients' options and that the patients are made aware of the costs and benefits of such options. However, "An ultrasound to assess the fetal anatomy is suggested at approximately 18w0d-20w0d gestation for all patients regardless of whether or not they choose to have screening or diagnostic testing" (Wilson et al., 2013).

The NSGC also presented the following recommendations for low-risk patients less than 14 weeks of gestation:

- "For patients who may consider CVS [chorionic villi screening] or amniocentesis, *stepwise sequential screening or combined first trimester screening* should be considered."
- "If CVS is not an option, *integrated screening* may be considered in order to maximize detection rates."
- "If a patient completes combined first trimester screening, a separate second trimester MSS for chromosome aneuploidy is *NOT* indicated. Screening for chromosome aneuploidy in the second trimester in patients who present prior to 14 weeks should *ONLY* be performed as a part of integrated, serum integrated, stepwise sequential, or contingency screening."
- "Patients who have an increased NT [nuchal translucency] (≥ 95th % or ≥ 3.0mm) should be offered diagnostic testing by either CVS or amniocentesis. A referral for a fetal echocardiogram should also be considered if the NT ≥3.5mm."
- "Early amniocentesis (prior to 15 weeks of gestation) is *not* recommended due to the increased risks for pregnancy loss, clubfoot, and fluid leakage. CVS should be offered as the diagnostic testing option for chromosome aneuploidy in the first trimester."

For low-risk patients after 14 weeks of gestation, they recommend the following:

- "Patients who desire MSS but did not have MSS in the first trimester should be offered a *quad* or *penta* screen rather than a triple screen due to the increased detection rates.
- Amniocentesis should be offered as the diagnostic testing option for chromosome aneuploidy for patients after 15 weeks of gestation."

The NSGC also recommend for those patients at increased risk for chromosome aneuploidy that if the patient presents prior to 14 weeks gestation, "CVS and amniocentesis should both be offered as diagnostic testing options for chromosome aneuploidy", whereas if the patient presents after 14 weeks gestation, "amniocentesis should be offered as the diagnostic testing option for chromosome aneuploidy." Lastly, the NSGC reiterated that patients may be offered NIPT (noninvasive prenatal testing) should they desire screening information (Wilson et al., 2013).

The International Society for Prenatal Diagnosis (ISPD)

The ISPD recognizes that massively parallel sequencing for detection of Down syndrome can be helpful for individuals with high-risk pregnancies, when "suitable genetic counseling" is provided (Benn et al., 2013).

The International Society for Prenatal Diagnosis released a statement regarding cell-free DNA screening for Down syndrome in multifetal pregnancies in 2020. The following recommendations were made:

- "The use of first trimester cfDNA screening for the common autosomal trisomies is appropriate for twin pregnancies due to sufficient evidence showing high detection and low false positive rates with high predictive values.
- The finding of an increased risk on a cfDNA screening test in multiple pregnancies should be followed by counseling and an offer of diagnostic testing to confirm results…CVS and amniocentesis procedures in multiple pregnancies are reliable and safe when performed by a provider experienced in these situations; subsequent diagnostic tests are highly reliable… Maternal age and nuchal translucency (with or without biochemistry) detects up to 80% of Down syndrome at a 5% false positive rate in twin pregnancies.
- It is preferable for laboratories performing cfDNA testing in multifetal pregnancies to take evidence of zygosity (e.g., chorionicity, sex of the fetuses, embryo transfer history) for the interpretation of both test results and fetal fractions…Interpretation of the cfDNA test results could differ depending on test methodology, fetal fraction and chorionicity/zygosity…Fetal fractions are higher in

twin pregnancies, but lower for individual fetuses when compared to singletons. Fetal fractions are correlated between dizygous twins, but can still vary two-fold.
- cfDNA based screening for common trisomies in twins provides higher positive predictive values among twin pregnancies compared with traditional serum and nuchal translucency-based screening in twins, but are associated with test failures…When a cfDNA test failure occurs consider ultrasound and diagnostic testing. If there is sufficient time, a second sample draw may also be considered.
- Screening options for triplet pregnancies are lacking and cfDNA may be a potential option. However, diagnostic testing should always be offered and the limitations of screening tests stressed" (Palomaki et al., 2020).

The American College of Medical Genetics and Genomics (ACMG)

The ACMG notes that "Pretest information should be provided … to ensure patients make informed decisions. Aneuploidy screening is not a routine prenatal test; it is acceptable for patients to decline screening." The ACMG also cautions that "All reports should clearly state that NIPS is a screening test and not diagnostic," and that results be presented in a clear and easily understandable fashion. ACMG guidelines state that "in pregnancies with multiple gestations and/or donor oocytes, testing laboratories should be contacted regarding the validity of NIPS before it is offered to the patient as a screening option" (Gregg et al., 2016).

The American College of Medical Genetics and Genomics published an evidence-based clinical guideline in 2023 on noninvasive prenatal screening (NIPS) for fetal chromosome abnormalities in a general-risk population. Their recommendations are reported below.

- "Recommendation: ACMG Recommends NIPS Over Traditional Screening Methods for All Pregnant Patients With Singleton Gestation for Fetal Trisomies 21, 18, and 13 (Strong Recommendation Based on High Certainty of Evidence)"
- "Recommendation: ACMG Recommends NIPS Over Traditional Methods for Trisomy Screening in Twin Gestations (Strong Recommendation, Based on High Certainty of Evidence)"
- "Recommendation: ACMG Recommends That NIPS Be Offered to Patients with a singleton gestation to Screen for Fetal SCA [sex chromosome aneuploidies] (Strong Recommendation, Based on High Certainty of Evidence)"
- "Recommendation: ACMG Suggests That NIPS for 22q11.2 Deletion Syndrome Be Offered to All Patients (Conditional Recommendation, Based on Moderate Certainty of the Evidence)"
    - "A conditional recommendation should be interpreted as follows: most patients would request this and most clinicians would offer NIPS for this

purpose, after a discussion about the benefits and limitations of screening and in the context of shared decision-making."

- "Recommendation: At This Time, There Is Insufficient Evidence to Recommend Routine Screening for CNVs Other Than 22q11.2 Deletions (No Recommendation, Owing to Lack of Clinically Relevant Evidence and Validation)"

"Recommendation: At This Time, There Is Insufficient Evidence to Recommend or Not Recommend NIPS for the Identification of RATs [rare autosomal trisomies] (No Recommendation, Owing to Lack of Clinically Relevant Evidence)" (Dungan et al., 2023)

Practice Committee and Genetic Counseling Professional Group (GCPG) of the American Society for Reproductive Medicine)

The American Society of Reproductive Medicine notes that screening tests (maternal serum biochemical screening, nuchal translucency and fetal anatomy scan, and noninvasive prenatal screening with cell-free DNA) cannot diagnose chromosomal aneuploidy. "In some cases, ultrasound and biochemical analytes may help identify congenital anomalies that may be associated with an aneuploid pregnancy; however, many aneuploidies (and mosaic aneuploidies in particular) do not result in visible ultrasound anomalies or skewed biochemical analytes and may be easily missed." Cell-free DNA testing "may test for a select number of full and partial aneuploidies, or all aneuploidies within a specified chromosomal resolution, depending on the specific test used by the laboratory. If the chromosome or chromosomal segment of interest is in fact able to be assessed by the assay used, an aneuploidy may be detected. However, it is important to recognize that NIPT is not designed for the detection of mosaicism and may result in false-negative results. False-positive results may also occur because NIPT analyzes placental (and not fetal) DNA" (ASRM, 2020).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration

In April 2021, the FDA released a statement titled, "Genetic Non-Invasive Prenatal Screening Tests May Have False Results: FDA Safety Communication," that detailed safety concerns regarding the use of NIPS tests outside of standard screening purposes. Specifically, they reinforced that NIPS tests are laboratory-developed tests not regulated by the FDA and that the use of NIPS should be confined to screenings, rather than diagnoses. "The accuracy and performance of NIPS tests have not been evaluated by the FDA and these tests can give false results, such as reporting a genetic abnormality when the fetus does not actually have one. NIPS tests are screening tests, which means the NIPS test may only tell you the risk of the fetus having certain genetic abnormalities. They are not diagnostic tests, which are generally used to more definitively confirm or rule out a suspected genetic abnormality." Additionally, "The FDA recommends that patients discuss the benefits and risks of NIPS tests with a genetic counselor or other health care provider before deciding to get these tests. Patients should also discuss the results of NIPS tests with a genetic counselor or other health care provider before making any decisions about their pregnancy. Health care providers should be aware of the risks and limitations of using these screening tests and should not use the results from these tests alone to diagnose chromosomal (genetic) abnormalities or disorders" (FDA, 2022).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81420	Fetal chromosomal aneuploidy (eg, trisomy 21, monosomy X) genomic sequence analysis panel, circulating cell-free fetal DNA in maternal blood, must include analysis of chromosomes 13, 18, and 21
81422	Fetal chromosomal microdeletion(s) genomic sequence analysis (eg, DiGeorge syndrome, Cri-du-chat syndrome), circulating cell-free fetal DNA in maternal blood
81479	Unlisted molecular pathology procedure
81507	Fetal aneuploidy (trisomy 21, 18, and 13) DNA sequence analysis of selected regions using maternal plasma, algorithm reported as a risk score for each trisomy Proprietary test: Harmony™ Prenatal Test Lab/Manufacturer: Ariosa Diagnostics

81508	Fetal congenital abnormalities, biochemical assays of two proteins (PAPP-A, hCG [any form]), utilizing maternal serum, algorithm reported as a risk score
81509	Fetal congenital abnormalities, biochemical assays of three proteins (PAPP-A, hCG [any form], DIA), utilizing maternal serum, algorithm reported as a risk score
81510	Fetal congenital abnormalities, biochemical assays of three analytes (AFP, uE3, hCG [any form]), utilizing maternal serum, algorithm reported as a risk score
81511	Fetal congenital abnormalities, biochemical assays of four analytes (AFP, uE3, hCG [any form], DIA) utilizing maternal serum, algorithm reported as a risk score (may include additional results from previous biochemical testing)
81512	Fetal congenital abnormalities, biochemical assays of five analytes (AFP, uE3, total hCG, hyperglycosylated hCG, DIA) utilizing maternal serum, algorithm reported as a risk score
81599	Unlisted multianalyte assay with algorithmic analysis
82105	Alpha-fetoprotein (AFP); serum
82106	Alpha-fetoprotein (AFP); amniotic fluid
82677	Estriol
84163	Pregnancy-associated plasma protein-A (PAPP-A)
84702	Gonadotropin, chorionic (hCG); quantitative
84703	Gonadotropin, chorionic (hCG); qualitative
84704	Gonadotropin, chorionic (hCG); free beta chain
86336	Inhibin A
88235	Tissue culture for non-neoplastic disorders; amniotic fluid or chorionic villus cells
88267	Chromosome analysis, amniotic fluid or chorionic villus, count 15 cells, 1 karyotype, with banding
88269	Chromosome analysis, in situ for amniotic fluid cells, count cells from 6-12 colonies, 1 karyotype, with banding
88271	Molecular cytogenetics; DNA probe, each (eg, FISH)
88280	Chromosome analysis; additional karyotypes, each study
88285	Chromosome analysis; additional cells counted, each study
0327U	Fetal aneuploidy (trisomy 13, 18, and 21), DNA sequence analysis of selected regions using maternal plasma, algorithm reported as a risk score for each trisomy, includes sex reporting, if performed Proprietary test: Vasistera™ Lab/Manufacturer: Natera, Inc
0341U	Fetal aneuploidy DNA sequencing comparative analysis, fetal DNA from products of conception, reported as normal (euploidy), monosomy, trisomy,

	or partial deletion/duplication, mosaicism, and segmental aneuploid Proprietary test: Single Cell Prenatal Diagnosis (SCPD) Test Lab/Manufacturer: Luna Genetics, Inc

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

ACOG. (2015). Committee Opinion No. 640: Cell-Free DNA Screening For Fetal Aneuploidy. *Obstet Gynecol*, *126*(3), e31-37. https://doi.org/10.1097/aog.0000000000001051

ACOG. (2016). Practice Bulletin No. 163: Screening for Fetal Aneuploidy. *Obstet Gynecol*, *127*(5), e123-137. https://doi.org/10.1097/aog.0000000000001406

ACOG. (2020). ACOG Practice Bulletin Number 226: Screening for Fetal Chromosomal Abnormalities. *American College of Obstetricians and Gynecologists'Committee on Practice Bulletins*. https://doi.org/10.1097/AOG.0000000000004084

AgilentTechnologies. (2022). Clarigo https://www.agilent.com/cs/library/brochures/Clarigo%20patient%20brochure%20v1708-EN.pdf

Al Toukhi, S., Chitayat, D., Keunen, J., Roifman, M., Seaward, G., Windrim, R., Ryan, G., & Van Mieghem, T. (2019). Impact of introduction of noninvasive prenatal testing on uptake of genetic testing in fetuses with central nervous system anomalies. *Prenatal Diagnosis*, *39*, 544-548. https://doi.org/10.1002/pd.5466

Aradhya, S., Manning, M. A., Splendore, A., & Cherry, A. M. (2007). Whole-genome array-CGH identifies novel contiguous gene deletions and duplications associated with developmental delay, mental retardation, and dysmorphic features. *American Journal of Medical Genetics Part A*, *143A*(13), 1431-1441. https://doi.org/10.1002/ajmg.a.31773

ASRM. (2020). Clinical management of mosaic results from preimplantation genetic testing for aneuploidy (PGT-A) of blastocysts: a committee opinion. *Fertility and Sterility*, *114*(2), 246-254. https://doi.org/10.1016/j.fertnstert.2020.05.014

Baer, R. J., Flessel, M. C., Jelliffe-Pawlowski, L. L., Goldman, S., Hudgins, L., Hull, A. D., Norton, M. E., & Currier, R. J. (2015). Detection Rates for Aneuploidy by First-Trimester and Sequential Screening. *126*(4), 753-759. https://doi.org/10.1097/aog.0000000000001040

Bellair, M., Amaral, E., Ouren, M., Roark, C., Kim, J., O'Connor, A., Soriano, A., Schindler, M. L., Wapner, R. J., Stone, J. L., Tavella, N., Merriam, A., Perley, L., Breman, A. M., & Beaudet, A. L. (2024). Noninvasive single-cell-based prenatal genetic testing: A proof

of concept clinical study. *Prenatal Diagnosis*, *44*(3), 304-316. https://doi.org/10.1002/pd.6529

Benn, P., Borell, A., Chiu, R., Cuckle, H., Dugoff, L., Faas, B., Gross, S., Johnson, J., Maymon, R., Norton, M., Odibo, A., Schielen, P., Spencer, K., Huang, T., Wright, D., & Yaron, Y. (2013). Position statement from the Aneuploidy Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. *Prenat Diagn*, *33*(7), 622-629. https://doi.org/10.1002/pd.4139

BGI. (2023). *The NIFTY® Test*. https://www.bgi.com/global/molecular-genetics/nifty-non-invasive-prenatal-testing/the-nifty-test-non-invasive-prenatal-testing/

Bianchi, D., Chudova, D., Sehnert, A., Bhatt, S., Murray, K., Prosen, T., Garber, J., Wilkins-Haug, L., Vora, N., & Warsof, S. (2015). Noninvasive prenatal testing and incidental detection of occult maternal malignancies. *JAMA*, *314*, 162-169. https://doi.org/10.1001/jama.2015.7120

Bianchi, D. W., Platt, L. D., Goldberg, J. D., Abuhamad, A. Z., Sehnert, A. J., & Rava, R. P. (2012). Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. *Obstet Gynecol*, *119*(5), 890-901. https://doi.org/10.1097/AOG.0b013e31824fb482

Bornstein, E., Gulersen, M., Krantz, D., Cheung, S. W., Maliszewski, K., & Divon, M. Y. (2018). Microarray analysis: First-trimester maternal serum free beta-hCG and the risk of significant copy number variants. *Prenat Diagn*, *38*(12), 971-978. https://doi.org/10.1002/pd.5350

Centogene. (2023). CentoNIPT®. https://www.centogene.com/diagnostics/our-tests/non-invasive-prenatal-testing/

Dai, P., Yang, Y., Zhao, G., Gu, Z., Ren, H., Hu, S., Liu, N., Jiao, W., Li, J., & Kong, X. (2022). A dPCR-NIPT assay for detections of trisomies 21, 18 and 13 in a single-tube reaction-could it replace serum biochemical tests as a primary maternal plasma screening tool? *J Transl Med*, *20*(1), 269. https://doi.org/10.1186/s12967-022-03455-y

Dar, P., Jacobsson, B., MacPherson, C., Egbert, M., Malone, F., Wapner, R. J., Roman, A. S., Khalil, A., Faro, R., Madankumar, R., Edwards, L., Haeri, S., Silver, R., Vohra, N., Hyett, J., Clunie, G., Demko, Z., Martin, K., Rabinowitz, M., . . . Norton, M. E. (2022). Cell-free DNA screening for trisomies 21, 18, and 13 in pregnancies at low and high risk for aneuploidy with genetic confirmation. *Am J Obstet Gynecol*, *227*(2), 259.e251-259.e214. https://doi.org/10.1016/j.ajog.2022.01.019

Devers, P. L., Cronister, A., Ormond, K. E., Facio, F., Brasington, C. K., & Flodman, P. (2013). Noninvasive prenatal testing/noninvasive prenatal diagnosis: the position of the National Society of Genetic Counselors. *J Genet Couns*, *22*(3), 291-295. https://doi.org/10.1007/s10897-012-9564-0

Dey, M., Sharma, S., & Aggarwal, S. (2013). Prenatal screening methods for aneuploidies. *N Am J Med Sci*, *5*(3), 182-190. https://pubmed.ncbi.nlm.nih.gov/23626953/

Dungan, J. S., Klugman, S., Darilek, S., Malinowski, J., Akkari, Y. M. N., Monaghan, K. G., Erwin, A., & Best, R. G. (2023). Noninvasive prenatal screening (NIPS) for fetal chromosome abnormalities in a general-risk population: An evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *25*(2), 100336. https://doi.org/10.1016/j.gim.2022.11.004

Eiben, B., Krapp, M., Borth, H., Kutur, N., Kreiselmaier, P., Glaubitz, R., Deutinger, J., & Merz, E. (2015). Single Nucleotide Polymorphism-Based Analysis of Cell-Free Fetal DNA in 3000 Cases from Germany and Austria. *Ultrasound Int Open*, *1*(1), E8-e11. https://doi.org/10.1055/s-0035-1555765

FDA. (2022). *Genetic Non-Invasive Prenatal Screening Tests May Have False Results: FDA Safety Communication*. https://www.fda.gov/medical-devices/safety-communications/genetic-non-invasive-prenatal-screening-tests-may-have-false-results-fda-safety-communication?

Futch, T., Spinosa, J., Bhatt, S., de Feo, E., Rava, R. P., & Sehnert, A. J. (2013). Initial clinical laboratory experience in noninvasive prenatal testing for fetal aneuploidy from maternal plasma DNA samples. *Prenat Diagn*, *33*(6), 569-574. https://doi.org/10.1002/pd.4123

Garshasbi, M., Wang, Y., Hantoosh Zadeh, S., Giti, S., Piri, S., & Reza Hekmat, M. (2019). Clinical Application of Cell-Free DNA Sequencing-Based Noninvasive Prenatal Testing for Trisomies 21, 18, 13 and Sex Chromosome Aneuploidy in a Mixed-Risk Population in Iran. *Fetal Diagn Ther*, 1-8. https://doi.org/10.1159/000501014

Genetics, I. (2016). *informaSeq® noninvasive prenatal test*. Illumina, Inc. https://www.labcorp.com/tests/related-documents/L13272

GenPath. (2023). CLARITESTTM CORE NON-INVASIVE PRENATAL SCREENING. https://www.genpathdiagnostics.com/hcp/womens-health/prenatal-screening/claritest/

Glenn E Palomaki, P. M. M., PhDJacquelyn V Halliday, MS. (2024, June 19, 2024). *Prenatal screening for common aneuploidies using cell-free DNA*. https://www.uptodate.com/contents/prenatal-screening-for-common-aneuploidies-using-cell-free-dna?

Gregg, A. R., Skotko, B. G., Benkendorf, J. L., Monaghan, K. G., Bajaj, K., Best, R. G., Klugman, S., & Watson, M. S. (2016). Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics. *Genet Med*, *18*(10), 1056-1065. https://doi.org/10.1038/gim.2016.97

Heesterbeek, C. J., Aukema, S. M., Galjaard, R.-J. H., Boon, E. M. J., Srebniak, M. I., Bouman, K., Faas, B. H. W., Govaerts, L. C. P., Hoffer, M. J. V., den Hollander, N. S., Lichtenbelt, K. D., van Maarle, M. C., van Prooyen Schuurman, L., van Rij, M. C., Schuring-Blom, G. H., Stevens, S. J. C., Tan-Sindhunata, G., Zamani Esteki, M., de Die-Smulders, C. E. M., . . . Macville, M. V. E. (2022). Noninvasive Prenatal Test Results

Indicative of Maternal Malignancies: A Nationwide Genetic and Clinical Follow-Up Study. *Journal of Clinical Oncology*, *40*(22), 2426-2435. https://doi.org/10.1200/JCO.21.02260

Illumina. (2023). *Verifi Prenatal Test Services*. https://www.illumina.com/clinical/illumina_clinical_laboratory/verifi-prenatal-tests.html

Invitae. (2023). Invitae Non-invasive Prenatal Screening. https://www.invitae.com/en/noninvasive-prenatal-screening/

ISPD. (2018). Joint Position Statement from the International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM), and the Perinatal Quality Foundation (PQF) on the use of genome-wide sequencing for fetal diagnosis. *Prenat Diagn*, *38*(1), 6-9. https://doi.org/10.1002/pd.5195

LabCorp. (2023a). *Early risk assessment of Down syndrome and other conditions*. Retrieved 10/10/2022 from https://www.integratedgenetics.com/patients/pregnancy/maternit21plus

LabCorp. (2023b). *First and second trimester serum screening*. Laboratory Corporation of America Holdings. Retrieved 11/11 from https://www.integratedgenetics.com/patients/pregnancy/serum-screening

Lee, Chen, L. C., Cheong, M. L., Chou, C. Y., & Tsai, M. S. (2013). First trimester combined test for Down syndrome screening in unselected pregnancies - a report of a 13-year experience. *Taiwan J Obstet Gynecol*, *52*(4), 523-526. https://doi.org/10.1016/j.tjog.2013.10.012

Lee, Kim, H., Park, J., Yun, T., Park, D. Y., Kim, M., & Ryu, H. M. (2019). Clinical Validation of Non-Invasive Prenatal Testing for Fetal Common Aneuploidies in 1,055 Korean Pregnant Women: a Single Center Experience. *J Korean Med Sci*, *34*(24), e172. https://doi.org/10.3346/jkms.2019.34.e172

Liang, D., Cram, D. S., Tan, H., Linpeng, S., Liu, Y., Sun, H., Zhang, Y., Tian, F., Zhu, H., Xu, M., Wang, H., Yu, F., & Wu, L. (2019). Clinical utility of noninvasive prenatal screening for expanded chromosome disease syndromes. *Genetics in Medicine*, *21*(9), 1998-2006. https://doi.org/10.1038/s41436-019-0467-4

Lo, Y. M., Corbetta, N., Chamberlain, P. F., Rai, V., Sargent, I. L., Redman, C. W., & Wainscoat, J. S. (1997). Presence of fetal DNA in maternal plasma and serum. *Lancet*, *350*(9076), 485-487. https://doi.org/10.1016/s0140-6736(97)02174-0

Luna. (2022a). *Luna Prenatal Test*. Luna Genetics. https://cdn.prod.website-files.com/60adc548ba98e3382beed23d/62a91215f4f2bf0f6bbe361f_QSE8.FORM.004%20Luna%20Prenatal%20Test%20Information%20Sheet%20v2.0.pdf

Luna. (2022b). Luna Prenatal Test White Paper. https://assets.website-files.com/60adc548ba98e3382beed23d/62a915e6d02dc869709d0bea_QSE8.FORM.005%20Luna%20Prenatal%20Test%20White%20Paper%20v2.0.pdf

Luo, Y., Hu, H., Zhang, R., Ma, Y., Pan, Y., Long, Y., Hu, B., Yao, H., & Liang, Z. (2021). An assessment of the analytical performance of non-invasive prenatal testing (NIPT) in detecting sex chromosome aneuploidies: 34,717-patient sample in a single prenatal diagnosis Centre in China. *J Gene Med*, *23*(9), e3362. https://doi.org/10.1002/jgm.3362

Malone, F. D., Canick, J. A., Ball, R. H., Nyberg, D. A., Comstock, C. H., Bukowski, R., Berkowitz, R. L., Gross, S. J., Dugoff, L., Craigo, S. D., Timor-Tritsch, I. E., Carr, S. R., Wolfe, H. M., Dukes, K., Bianchi, D. W., Rudnicka, A. R., Hackshaw, A. K., Lambert-Messerlian, G., Wald, N. J., & D'Alton, M. E. (2005). First-trimester or second-trimester screening, or both, for Down's syndrome. *N Engl J Med*, *353*(19), 2001-2011. https://doi.org/10.1056/NEJMoa043693

McKanna, T., Ryan, A., Krinshpun, S., Kareht, S., Marchand, K., Grabarits, C., Ali, M., McElheny, A., Gardiner, K., LeChien, K., Hsu, M., Saltzman, D., Stosic, M., Martin, K., & Benn, P. (2018). Fetal fraction-based risk algorithm for non-invasive prenatal testing: screening for trisomy 13, 18, and triploidy in women with low cell-free fetal DNA. *Ultrasound Obstet Gynecol*. https://doi.org/10.1002/uog.19176

Muzzey, D., Goldberg, J. D., & Haverty, C. (2020). Noninvasive prenatal screening for patients with high body mass index: Evaluating the impact of a customized whole genome sequencing workflow on sensitivity and residual risk. *Prenat Diagn*, *40*(3), 333-341. https://doi.org/10.1002/pd.5603

Myriad. (2023). MYRIAD PREQUELTM PRENATAL SCREEN. https://myriadwomenshealth.com/patient-prequel/

Natera. (2023). *Panorama Test*. https://www.natera.com/panorama-test

Neufeld-Kaiser, W. A., Cheng, E. Y., & Liu, Y. J. (2015). Positive predictive value of non-invasive prenatal screening for fetal chromosome disorders using cell-free DNA in maternal serum: independent clinical experience of a tertiary referral center. *BMC Med*, *13*, 129. https://doi.org/10.1186/s12916-015-0374-8

NIPD. (2023, 2021). *NIPD Genetics VERACITY new generation NIPT*. NIPD Genetics. https://nipd.com/products/prenatal/veracity-patients/

Norton, M. E., Jacobsson, B., Swamy, G. K., Laurent, L. C., Ranzini, A. C., Brar, H., Tomlinson, M. W., Pereira, L., Spitz, J. L., Hollemon, D., Cuckle, H., Musci, T. J., & Wapner, R. J. (2015). Cell-free DNA analysis for noninvasive examination of trisomy. *N Engl J Med*, *372*(17), 1589-1597. https://doi.org/10.1056/NEJMoa1407349

Palomaki, G. E., Chiu, R. W. K., Pertile, M. D., Sistermans, E. A., Yaron, Y., Vermeesch, J. R., Vora, N. L., Best, R. G., & Wilkins-Haug, L. (2020). International Society for Prenatal Diagnosis (ISPD) Position Statement: Cell free (cf)DNA screening for Down syndrome in multiple pregnancies. *Prenatal Diagnosis*, *Accepted for publication*(n/a). https://doi.org/10.1002/pd.5832

Palomaki, G. E., Deciu, C., Kloza, E. M., Lambert-Messerlian, G. M., Haddow, J. E., Neveux, L. M., Ehrich, M., van den Boom, D., Bombard, A. T., Grody, W. W., Nelson, S. F., &

Canick, J. A. (2012). DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. *Genet Med*, *14*(3), 296-305. https://doi.org/10.1038/gim.2011.73

Palomaki, G. E., Kloza, E. M., Lambert-Messerlian, G. M., Haddow, J. E., Neveux, L. M., Ehrich, M., van den Boom, D., Bombard, A. T., Deciu, C., Grody, W. W., Nelson, S. F., & Canick, J. A. (2011). DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. *Genet Med*, *13*(11), 913-920. https://doi.org/10.1097/GIM.0b013e3182368a0e

Palomaki, G. E., Messerlian, G. M., & Halliday, J. V. (2024, Jun 19, 2024). *Prenatal screening for common aneuploidies using cell-free DNA*. https://www.uptodate.com/contents/prenatal-screening-for-common-aneuploidies-using-cell-free-dna

Palomaki, G. E., Neveux, L. M., Knight, G. J., Haddow, J. E., & Pandian, R. (2004). Maternal Serum Invasive Trophoblast Antigen (Hyperglycosylated hCG) as a Screening Marker for Down Syndrome during the Second Trimester. *Clinical Chemistry*, *50*(10), 1804-1808. https://doi.org/10.1373/clinchem.2004.038059

Park, S. Y., Jang, I. A., Lee, M. A., Kim, Y. J., Chun, S. H., & Park, M. H. (2016). Screening for chromosomal abnormalities using combined test in the first trimester of pregnancy. *Obstet Gynecol Sci*, *59*(5), 357-366. https://doi.org/10.5468/ogs.2016.59.5.357

PerkinElmer. (2023). *VANADIS® NIPT SYSTEM A Whole New Way to NIPT*. https://rh.perkinelmer.com/products/vanadis-nipt-system/

QuestDiagnostics. (2022). Prenatal Screening and Diagnosis of Neural Tube Defects, Down Syndrome, Trisomy 18, and Trisomy 13. https://testdirectory.questdiagnostics.com/test/test-guides/TS_Quad_Screen/quad-screen

QuestDiagnostics. (2023). QNatal® Advanced. http://education.questdiagnostics.com/faq/FAQ167

Reddy, U. M., Page, G. P., Saade, G. R., Silver, R. M., Thorsten, V. R., Parker, C. B., Pinar, H., Willinger, M., Stoll, B. J., Heim-Hall, J., Varner, M. W., Goldenberg, R. L., Bukowski, R., Wapner, R. J., Drews-Botsch, C. D., O'Brien, B. M., Dudley, D. J., & Levy, B. (2012). Karyotype versus Microarray Testing for Genetic Abnormalities after Stillbirth. *New England Journal of Medicine*, *367*(23), 2185-2193. https://doi.org/10.1056/NEJMoa1201569

Robson, S. C., Chitty, L. S., Morris, S., Verhoef, T., Ambler, G., Wellesley, D. G., Graham, R., Leader, C., Fisher, J., & Crolla, J. A. (2017). Efficacy and Mechanism Evaluation. In *Evaluation of Array Comparative genomic Hybridisation in prenatal diagnosis of fetal anomalies: a multicentre cohort study with cost analysis and assessment of patient, health professional and commissioner preferences for array comparative genomic hybridisation*. NIHR Journals Library. Copyright © Queen's Printer and Controller of HMSO 2017. . https://doi.org/10.3310/eme04010

Roche. (2023). *Harmony Prenatal Test*. Retrieved 10/10/2022 from https://harmonytest.roche.com/global/en/home.html

Shaw, J., Scotchman, E., Chandler, N., & Chitty, L. S. (2020). PREIMPLANTATION GENETIC TESTING: Non-invasive prenatal testing for aneuploidy, copy-number variants and single-gene disorders. *Reproduction*, *160*(5), A1-A11. https://doi.org/10.1530/rep-19-0591

Shiefa, S., Amargandhi, M., Bhupendra, J., Moulali, S., & Kristine, T. (2013). First Trimester Maternal Serum Screening Using Biochemical Markers PAPP-A and Free β-hCG for Down Syndrome, Patau Syndrome and Edward Syndrome. *Indian J Clin Biochem*, *28*(1), 3-12. https://doi.org/10.1007/s12291-012-0269-9

Strom, C. M., Anderson, B., Tsao, D., Zhang, K., Liu, Y., Livingston, K., Elzinga, C., Evans, M., Nguyen, Q., Wolfson, D., Rowland, C., Kolacki, P., Maxwell, M., Wang, J. C., Rabin, D., Catanese, J., Owen, R., Braastad, C., & Sun, W. (2017). Improving the Positive Predictive Value of Non-Invasive Prenatal Screening (NIPS). *PLoS One*, *12*(3), e0167130. https://doi.org/10.1371/journal.pone.0167130

Taneja, P. A., Snyder, H. L., de Feo, E., Kruglyak, K. M., Halks-Miller, M., Curnow, K. J., & Bhatt, S. (2016). Noninvasive prenatal testing in the general obstetric population: clinical performance and counseling considerations in over 85 000 cases. *Prenat Diagn*, *36*(3), 237-243. https://doi.org/10.1002/pd.4766

van der Meij, K. R. M., Sistermans, E. A., Macville, M. V. E., Stevens, S. J. C., Bax, C. J., Bekker, M. N., Bilardo, C. M., Boon, E. M. J., Boter, M., Diderich, K. E. M., de Die-Smulders, C. E. M., Duin, L. K., Faas, B. H. W., Feenstra, I., Haak, M. C., Hoffer, M. J. V., den Hollander, N. S., Hollink, I. H. I. M., Jehee, F. S., . . . Weiss, M. M. (2019). TRIDENT-2: National Implementation of Genome-wide Non-invasive Prenatal Testing as a First-Tier Screening Test in the Netherlands. *The American Journal of Human Genetics*, *105*(6), 1091-1101. https://doi.org/10.1016/j.ajhg.2019.10.005

Wald, N. J., Huttly, W. J., Murphy, K. W., Ali, K., Bestwick, J. P., & Rodeck, C. H. (2009). Antenatal screening for Down's syndrome using the Integrated test at two London hospitals. *J Med Screen*, *16*(1), 7-10. https://doi.org/10.1258/jms.2009.008094

Wald, N. J., Rodeck, C., Hackshaw, A. K., Walters, J., Chitty, L., & Mackinson, A. M. (2003). First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). *J Med Screen*, *10*(2), 56-104. https://doi.org/10.1258/096914103321824133

Westerfield, L., Darilek, S., & van den Veyver, I. B. (2014). Counseling Challenges with Variants of Uncertain Significance and Incidental Findings in Prenatal Genetic Screening and Diagnosis. *J Clin Med*, *3*(3), 1018-1032. https://doi.org/10.3390/jcm3031018

Wilson, K. L., Czerwinski, J. L., Hoskovec, J. M., Noblin, S. J., Sullivan, C. M., Harbison, A., Campion, M. W., Devary, K., Devers, P., & Singletary, C. N. (2013). NSGC practice

guideline: prenatal screening and diagnostic testing options for chromosome aneuploidy. *J Genet Couns*, *22*(1), 4-15. https://doi.org/10.1007/s10897-012-9545-3

Witters, G., Van Robays, J., Willekes, C., Coumans, A., Peeters, H., Gyselaers, W., & Fryns, J. P. (2011). Trisomy 13, 18, 21, Triploidy and Turner syndrome: the 5T's. Look at the hands. *Facts Views Vis Obgyn*, *3*(1), 15-21. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3991414/

Wu, X., Li, Y., Xie, X., Su, L., Cai, M., Lin, N., Du, S., Xu, L., & Huang, H. (2020). Clinical Review of Noninvasive Prenatal Testing: Experience from 551 Pregnancies with Noninvasive Prenatal Testing–Positive Results in a Tertiary Referral Center. *The Journal of Molecular Diagnostics*. https://doi.org/10.1016/j.jmoldx.2020.09.008

YourgeneHealth. (2023). Non-Invasive Prenatal Testing (NIPT). https://www.yourgene-health.com/yourgeneproducts/nipt

Proteogenomic Testing of Individuals with Cancer

Policy Number: AHS – M2168 – Proteogenomic Testing of Individuals with Cancer	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Proteogenomic testing (encompassing analysis of both the genome and the proteome) is emerging as a new discipline in clinical settings. Until recently, genomic and proteomic analyses have remained in relative isolation. However, as techniques continue to improve, integrated analysis of both large-scale items has become more and more feasible. With precise and personalized medicine continuing to be a point of emphasis in clinical evaluation, proteogenomic analysis has received significant attention in treating cancer (Ang et al., 2019).

II. Related Policies

Policy Number	Policy Title
AHS-G2054	Liquid Biopsy
AHS-M2178	Microsatellite Instability and Tumor Mutational Burden Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with cancer, integrated comprehensive tumor profiling to calculate a tumor mutational burden score using genomic and/or transcriptomic data in conjunction with immunohistochemistry analysis (e.g., OncoExTra™, Caris MI Tumor Seek™, Caris MI Profile™ Comprehensive Testing) **MEETS COVERAGE CRITERIA** as

defined within AHS-M2178-Microsatellite Instability and Tumor Mutational Burden Testing.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

2) For individuals with cancer, integrated analysis of proteomic, genomic, **and/or** transcriptomic testing with or without algorithmic analysis (e.g., GPS Cancer®, DarwinOncoTarget™/DarwinOncoTreat™) **DOES NOT MEET COVERAGE CRITERIA**.

3) Tumor gene expression profiling with algorithmic analysis providing gene pathway activity scores **DOES NOT MEET COVERAGE CRITERIA**.

4) Optical genome mapping with or without whole genome sequencing and transcriptome analysis **DOES NOT MEET COVERAGE CRITERIA**.

IV. Table of Terminology

Term	Definition
ACMG	American College of Medical Genetics
AKT1	Protein kinase B
ALK	Anaplastic lymphoma kinase
AML	Acute myeloid leukemia
AMP	Adenosine monophosphate
APC	*Adenomatous polyposis coli*
APOLLO	Applied Proteogenomics Organization Learning and Outcomes
ASCO	American Society of Clinical Oncology
AURKB	Aurora kinase b
BCCs	Basal cell carcinomas
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
BRDs	Bromodomain
CAP	College of American Pathologists
CAPN2	Calpain 2
CDK4/6	Cyclin-dependent kinases 4 and 6
CEBPA	*CCAAT Enhancer Binding Protein Alpha*
CLIA'88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid
CPTAC	Clinical Proteomic Tumor Analysis Consortium

CREBBP	cAMP response element-binding protein
CRISPR	*Clustered regularly interspaced short palindromic repeats*
CTDSP2	*Carboxy-terminal domain, RNA polymerase II, polypeptide A*
DBNL	Drebrin like
dMMR	Mismatch repair deficiency
DNA	Deoxyribose nucleic acid
DoD	Department of Defense
EGFR	Epidermal growth factor receptor
EP300	E1A binding protein P300
ER+	Estrogen receptor-positive
ERBB2	Erb-B2 receptor tyrosine kinase 2
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
FLT3	*Fms related receptor tyrosine kinase 3*
GBMs	Glioblastomas
GEM	Georgia Estoteric and Molecular Laboratory
GRP	Glucose related protein
GRP78	Glucose regulated protein 78
H2B	Histone H2b
HCC	Hepatocellular carcinoma
HER2	Human epidermal growth factor receptor 2
HSP90β	Heat shock protein HSP 90-beta
HSPB1	Heat shock protein beta member 1
ICIs	Immune checkpoint inhibitors
INTS7	*Integrator complex subunit 7*
ISR	Illumina short read
KMT2A-PTD	*Lysine (K)-specific methyltransferase 2A- partial tandem duplication*
KRAS	*Kirsten rat sarcoma viral oncogene homolog*
LDTs	Laboratory developed tests
LNCaP	Lymph node carcinoma of the prostate
METABRIC	Molecular Taxonomy of Breast Cancer International Consortium
mRNA	Messenger ribonucleic acid
MSI	Microsatellite instability
NCCN	National Comprehensive Cancer Network
NFKBIZ	*NF-kappa-B inhibitor zeta*
NGS	Next generation sequencing

NICE	The National Institute for Health and Care Excellence
NPM1	*Nucleophosmin 1*
NRAS	*Neuroblastoma RAS viral oncogene homolog*
OGM	Optical genome mapping
PC-3	Prostate cancer cell line 3
PC-3M	Prostate cancer cell 3M
PCOs	Provisional clinical opinions
PD-L1	Programmed death ligand 1
postM	Post-menopausal
preM	Pre-menopausal
pre-mRNA	Messenger ribonucleic acid precursors
Rb	Retinoblastoma
RB1	*Retinoblastoma transcriptional corepressor 1*
RNA	Ribonucleic acid
rRNAs	Ribosomal ribonucleic acids
RUNX1	*Runt related transcription factor 1*
SeC	Sebaceous carcinomas
SCCs	Squamous cell carcinomas
SH3BGRL	*SH3 domain binding glutamate rich protein like*
siRNAs	Short-interfering ribonucleic acids
SNPs	Single nucleotide polymorphisms
TCGA	The Cancer Genome Atlas
TGFβ	Transforming growth factor beta
TMB	Tumor mutational burden
TML	Tumor mutational load
TP53	*Tumor protein p53*
UV	Ultraviolet
VA	Department of Veterans Affairs
WGS	Whole genome sequencing

V. Scientific Background

As newer and faster technology makes evaluating enormous amounts of molecular information possible, proteogenomic testing is on the rise. Techniques such as whole genome sequencing, transcriptome sequencing, and proteomic analysis, previously not clinically viable, are now within the clinical laboratory landscape. Information yielded from these tests may be used for a variety of purposes, including prognosis, diagnosis, identifying targeted treatments, and more. Proteogenomic testing typically revolves around three different sets of analytes: DNA, RNA, and proteins (Fancello & Burger, 2022).

DNA is the first stage in the genetic flow of information, represented by genes, including both exons and introns. All the protein-coding genes are represented in the exome, where at least 85% of pathogenic mutations are found. The exome represents a mere 1.5%-2% of the genome, thereby typically making it more cost-effective to sequence than the whole genome (Hulick, 2022). The entire exome includes approximately 30 megabases compared to the 3.3 gigabases of the genome. However, a pathogenic mutation may be in a noncoding region of the genome, such as mutations that result in dysfunction of gene regulation, resulting in situations where sequencing of the entire genome may be useful (Hulick, 2022).

RNA is the second stage in the genetic flow of information, as DNA is transcribed into RNA. Transcriptome sequencing refers to "digital counts" of each RNA molecule, or direct sequencing and quantification of RNA. The RNA transcript being quantified is not a perfect complement of the original DNA sequence; certain regulatory processes and post-transcriptional modifications, such as splicing, polyadenylation, and capping, alter the pre-mRNA sequence. Furthermore, additional regulatory RNA classes are not translated into a protein product, including but not limited to, ribosomal RNAs (rRNAs facilitate the translation of mRNA to protein) or short-interfering RNAs (siRNAs, capable of downregulating the translation of mRNA to protein). Transcriptome sequencing identifies these regulatory RNA sequences that are not otherwise identified at the DNA or protein level (Steiling, 2023).

Proteins are the third stage in the genetic flow of information, as most RNA is translated into protein products. Proteomics is a qualitative and quantitative assessment of the protein constituents in each biological sample. Mass spectrometry is typically used to identify peptide sequences, and these sequences are used to infer protein identity and quantity. "Shotgun" proteomics is the most common method of identifying and labeling large amounts of proteins, analyzing both the "parent" ions eluting from the liquid chromatographer and the "daughter" ions, which are comprised of fragments of the parent ion. The apparatus then attempts to match the ions using several features, such as signal intensity and mass to charge ratio. From here, the peptide sequences (and therefore proteins) are inferred (Ang et al., 2019).

Integration of all three disciplines may be termed "proteogenomic" testing. The drive for "precision" and "personalized" medicine has encouraged more in-depth research on the genetic landscape, particularly for heterogenous conditions such as cancer. Proteogenomic testing has been proposed to fill clinical gaps that exist with disciplines in isolation, including finding the connections from genotype to phenotype. Identifying targeted therapies, drug resistance mechanisms, and other potentially crucial clinical factors are all questions that may be answered with proteogenomic testing. Although the individual methodologies used to perform proteogenomic testing are well-

validated in research settings (next generation sequencing [NGS], mass spectrometry), numerous challenges and limitations exist in translating them to the clinical realm. For example, there is currently no amplification technique available for proteins that would allow for smaller samples to be used. Additionally, reproducibility issues presented by the current techniques used in proteomic research can occur. Overall, validation of the enormous database of proteomics, as well as development of the bioinformatic infrastructure required to connect proteogenomics to the clinic, is still in progress (Ang et al., 2019).

In 2016, the Clinical Proteomic Tumor Analysis Consortium (CPTAC) created the Applied Proteogenomics Organizational Learning and Outcomes (APOLLO) network. The APOLLO network was launched to incorporate proteogenomics into patient care and is a collaborative effort between the Department of Defense (DoD) and the Department of Veterans Affairs (VA). APOLLO is currently analyzing proteogenomic data from 8,000 human tissue samples; this data will be publicly available once curated (NIH, 2016). As of 2022, the CPTAC has begun to release some individual genomic, proteomic, and imaging data sets from this ongoing research but combined proteogenomic analysis from this data is just beginning to emerge(Dong et al., 2022; Krug et al., 2020; Wang et al., 2021).

Proprietary Testing

Several proteogenomic testing platforms are commercially available.

GPS Cancer®

The GPS Cancer® test from NantOmics, a member of the NantWorks family, utilizes quantitative proteomics through mass spectrometry, whole genome sequencing (over 20,000 genes across three billion base pairs), and whole transcriptome sequencing technologies (over 200,000 RNA transcripts). These three factors combined provide oncologists with a comprehensive molecular profile of a patient's cancer. The test is intended to provide information about targeted therapies, such as which therapies a patient may benefit from or which therapies a patient may resist (NantOmics, 2023). Finally, the third component of GPS Cancer® is the tumor "normal" sequencing. This component provides a comparison of a patient's healthy, unaffected genome to the genome affected by the tumor. It is intended to provide "pharmacogenomic analysis for potential drug toxicity and/or interactions" and to separate mutations caused by cancer from those that were present prior to cancer (NantOmics, 2023).

DarwinOncoTarget™ and DarwinOncoTreat

DarwinOncoTarget™ and DarwinOncoTreat™ are synergistic proteogenomic tests offered by Columbia University. DarwinOncoTarget™ identifies 193 potentially targetable proteins while DarwinOncoTreat™ assesses the regulatory activity of 6293 proteins ("tumor-checkpoints"). DarwinOncoTreat™ then "prioritizes" drugs based on their ability to revert the activity of these checkpoints. DarwinOncoTarget™ is available for all malignancies whereas DarwinOncoTreat™ is only available for certain cancer subtypes (Columbia, 2023).

Caris Molecular Intelligence

Other proprietary proteogenomic platforms are offered by Caris Molecular Intelligence. The MI Cancer Seek utilizes an NGS technique to identify "Whole exome sequencing for DNA mutations, copy number alterations, insertions/deletions, genomic signatures MSI [microsatellite instability] and TMB [tumor mutational burden], and whole transcriptome sequencing for RNA fusions and variant transcripts" (Caris, 2023b). The Caris Molecular Intelligence Comprehensive Tumor Profiling test uses precision medicine to assess DNA, RNA, and proteins to aid individualized treatment regimens (Caris, 2023a).

Praxis Genomics Optical Genome Mapping, Whole Genome Sequencing, and Transcriptome Analysis

Praxis Genomics offers a proteogenomic approach via combined testing with their Optical Genome Mapping, Whole Genome Sequencing, and Transcriptome Analysis. Optimal Genome Mapping (OGM), developed by Bionano Genomics LLC, evaluates DNA samples for large-scale changes such as chromosomal transfer of DNA fragments, chromosomal inversion or complex rearrangement, measurement of repetitive regions that control adjacent gene expression, and measurement of tandem repeat expansions. Whole Genome Sequencing obtains sequence from the entire genome (roughly 3 billion units). Praxis uses the Dragen alignment and variant calling pipeline to identify genomic changes. Highly repetitive DNA content and genomic rearrangements cannot be detected with whole genome sequencing. Transcriptome analysis allows for the evaluation of the functional consequences of DNA mutations found in Optical Genome Mapping or Whole Genome Sequencing. "Only by building on the diverse strength of OGM and ISR [Illumina Short Read] WGS and transcriptome sequencing can we increase the sensitivity and specificity of genetic diagnosis until novel technologies even more precise and faster come along" (Praxis, 2022).

OncoSignal™

Philips' OncoSignal™ technology is used by Protean BioDiagnostics as an "innovative and unique test to expand the information that can be obtained from cancer tissue

analysis," as part of their proprietary Oncology MAPS™ solution. OncoSignal™ uses advanced molecular and bioinformatic systems to measure mRNA expression patterns, calculating the specific activity of seven key oncogenic drive signal pathways (Estrogen receptor, Androgen receptor, Phosphoinositide 3-kinase, Hedgehog pathway, Notch signal, transforming growth factor receptor beta, and Mitogen activated protean kinase). These pathways "measure key oncogenic drivers of numerous distinct cancer types including but not limited to breast cancer, prostate cancer, ovarian cancer, colon cancer, lymphoma and more." Each pathway is given a score based on molecular and bioinformatic findings and pathway activity is interpreted as low, normal, or high in comparison to the normal physiological range. The clinically-used Oncology MAPS™ report then provides targeted treatment recommendations (AccessWire, 2022; Protean, 2023).

MosaicNeedle™

Nanomosaic Inc. developed MosaicNeedle™ technology for proteomic and multi-omic investigation and application (PRNewswire, 2022). The commercial launch of their Tessie™ platform for proteomics and multi-omics is slated to occur in 2022. According to Nanomosaic, their proteomics technology "bridges the gaps of functional information lost due to post-transcriptional and post-translational modifications in a genomic approach." Because the traditional proteogenomic approach typically requires multiple diverse assays and workflows, this novel technology simplifies by using "densely integrated nanoneedle sensors on a planar substrate that integrates proteogenomic analysis in one platform." Each nanoneedle binds a single molecule and detects both nucleic acids and proteins in a single assay process. The result is a full proteogenomic quantification in one single reaction, on one platform, with high sensitivity and lower cost (Quan et al., 2022).

Clinical Utility and Validity

North et al. (2018) used NantHealth's profile to characterize 32 cases of sebaceous carcinomas (SeC). The authors identified ultraviolet (UV) damage in ten samples and microsatellite instability in nine samples. UV cases of SeC were shown to have more severe histopathologic features, including poorer differentiation and an "infiltrative" growth pattern. The authors also noted that the transcriptomes of the UV SeC cases were like cutaneous squamous cell carcinomas (SCCs) and basal cell carcinomas (BCCs). Overall, three distinct classes of sebaceous carcinoma were identified based on mutation pattern and cell of origin (North et al., 2018).

Liao et al. (2015) used two genetic datasets (TCGA and METABRIC) to characterize over 2500 cases of breast cancer in pre-menopausal (preM) and post-menopausal individuals (postM) (defined as ≤45 years and ≥55 years respectively, individuals of

ages 45-55 were not included). The following molecular features were examined: gene expression, somatic mutation, copy number, methylation, and protein expression. In preM tumors, the authors identified unique methylation patterns, copy numbers, and somatic mutations in estrogen receptor-positive (ER+) tumors. Further investigation of this subset revealed "elevated integrin/laminin and EGFR signaling, with enrichment for upstream TGFβ-regulation." The authors concluded that preM ER+ tumors have "distinct molecular characteristics" compared to postM ER+ tumors (Liao et al., 2015).

Rabizadeh et al. (2018) compared tumor-only DNA sequencing to tumor-normal DNA (containing controls for germline mutations) plus RNA sequencing. A total of 621 patients with 30 different cancer types were studied using a 35-gene sequencer and the precision of somatic variant calling was evaluated. Without the germline controls, 94% of the variants were single nucleotide polymorphisms (SNPs) and considered false positives. Removing these SNPs resulted in a 48% false-positive rate. Tumor-only sequencing ultimately led to a 29% false-positive rate in "at least one of twelve genes with directly targetable drugs" and RNA analysis revealed that 18% of variants were not expressed (Rabizadeh et al., 2018).

Sjostrom et al. (2020) evaluated the utility of transcriptomic profiling in breast cancer and whether these results could safely allow patients to decline systemic therapy. A 141-gene signature was derived from a node-negative cohort previously untreated with chemotherapy. This signature was used to evaluate 454 node-negative, ER+, and systemically untreated cancer patients. The authors noted that this was a low-risk subgroup but did find that of the patients in the lowest 25th percentile of signature scores, 95% of patients were metastasis-free after 15 years despite lack of endocrine therapy. The authors concluded that "transcriptomic profiling identifies patients with an excellent outcome without any systemic adjuvant therapy in clinically low-risk patients of…two separate cohorts" (Sjostrom et al., 2020).

Feng et al. (2019) evaluated the proteomic profile of sorafenib resistance in hepatocellular carcinoma patients. Tumor samples from 60 patients were examined. The authors identified three proteins that were overexpressed in sorafenib-resistant cells: 78 kDa glucose related protein (GRP78), 14-3-3ε, and heat shock protein 90β (HSP90β). Approximately 73% of cells had high GRP78 expression, 18% had high 14-3-3ε expression, and 85% had high HSP90β expression. The authors also noted that elevated GRP78 was associated with the shortest progression-free survival of patients treated with sorafenib. The authors concluded that "GRP78 can be a predictive biomarker in HCC patients treated with sorafenib" (Feng et al., 2019).

Shiba et al. (2019) evaluated the genetic landscape of pediatric acute myeloid leukemia. The authors performed a transcriptome analysis in 139 patients (of 369 in

the total cohort). Fifty-four in-frame gene mutations and one *RUNX1* out-of-frame fusion were found in 53 of the 139 patients. Moreover, 258 gene fusions were found in the 369 total patients. Five novel gene fusions were found, and several fewer common gene rearrangements were identified. Out of the 111 remaining patients, "*KMT2A-PTD*, biallelic *CEBPA*, and *NPM1* gene mutations were found in 11, 23, and 17 patients, respectively." The authors noted these mutations were mutually exclusive compared with other gene fusions. The authors remark that risk stratification should be reconsidered (Shiba et al., 2019).

Yang et al. (2019) evaluated the genomic landscape of rectal cancer patients who did not respond to chemotherapy. The authors performed whole exome sequencing on 28 paired tumors collected before and after chemotherapy. Several mutations were identified (*CTDSP2, APC, KRAS, TP53* and *NFKBIZ*) that appeared to confer selective advantages to cancer cells and the authors noted that chemotherapy altered the genomic landscape of these tumors and that high intratumoral heterogeneity in any stage of cancer contributed to poor survival in patients (Yang et al., 2019).

Tredan et al. (2019) evaluated the impact of broad molecular profiling on identifying targeted therapies. A "molecular tumor board," consisting of molecular biologists, medical oncologists, and pathologists, selected the genes to be included in the profile and a total of 69 genes were included on the final panel. A total of 1980 molecular profiles were constructed. Of these profiles, 948 had no actionable mutations (leaving 1032 with at least one actionable mutation) and a targeted therapy was recommended for 699 of these patients. A total of 182 targeted therapies were initiated, with only 23 patients experiencing an objective response (13% of patients receiving therapy, 0.9% of the total cohort of 2579 patients). The authors concluded that "molecular screening should not be used at present to guide decision-making in routine clinical practice outside of clinical trials" (Tredan et al., 2019).

Kwon et al. (2019) identified and analyzed mutant peptides in prostate cancer cell lines. The authors obtained four cell lines of varying aggression (LNCaP, LNCaP-LN3, PC-3, and PC-3M) and profiled the resulting mutant peptides. A total of 70 mutant peptides were identified. Expression of seven mutant peptides were found to be altered in tumors, with "CAPN2 D22E" as the most significantly up-regulated peptide. Increased levels of the *INTS7* gene and decreased levels of the *SH3BGRL* gene were found to be correlated with aggressiveness of prostate cancer (Kwon et al., 2019).

Wang et al. (2019) constructed a "quantitative proteome and transcriptome abundance atlas" of 29 paired healthy human tissues. A total of 18,072 transcripts and 13,640 proteins (including 37 without "prior protein-level evidence") were represented. However, the authors concluded that proteogenomics remains challenging. The

authors noted that out of 9848 amino acid variants found by exome sequencing, only 238 could be confidently detected at the protein level. The authors also remarked that many proteins could not be detected despite highly expressed mRNA, that very few proteins showed tissue-specific expression, and that strong differences existed between mRNA and protein quantities. Overall, the writers determined that proteogenomics "needs better computational methods and requires rigorous validation" (Wang et al., 2019).

Treue et al. (2019) performed analysis on a model of a drug-resistant *EGFR*-mutated non-small cell lung cancer case. The authors integrated several proteogenomic techniques, including whole exome sequencing and "global time-course discovery phosphoproteomics" to identify molecular alterations. The writers remarked that this allowed them to reduce the complexity of the model down to 44 "predicted" phosphoproteins and 35 "topologically close" genetic alterations. From here, the authors found that targeting of HSPB1, DBNL, and AKT1 showed "potent antiproliferative effects overcoming resistance against EGFR-inhibitory therapy" (Treue et al., 2019).

Salem et al. (2018) evaluated the correlation of tumor mutational load (TML; "high" defined as greater than or equal to 17 mutations/MB), PD-L1 expression, and mismatch repair deficiency (dMMR) status with response to immune checkpoint inhibitors (ICIs). A total of 4125 tumors from 14 different gastrointestinal sites were examined. A 592 gene panel was used to calculate the TML. Microsatellite instability (MSI), PD-L1 expression, and dMMR status were all evaluated. The authors found that high-TML was "strongly associated" with high MSI. Right-sided colon and small-bowel adenocarcinomas had the highest rates of high-TML tumors (14.6% and 10.2% respectively) whereas pancreatic neuroendocrine and gastrointestinal stromal tumors had the lowest (1.3%, 0%). The authors noted that high-TML rate varied "widely" among gastrointestinal cancers (Salem et al., 2018).

Gillette et al. (2020) utilized proteogenomics to reveal therapeutic vulnerabilities in lung adenocarcinoma. Comprehensive proteogenomic characterization was performed on 110 tumors. "Multi-omics clustering revealed four subgroups defined by key driver mutations, country, and gender" (Gillette et al., 2020). Therapeutic vulnerabilities were identified in the *KRAS, EGFR,* and *ALK* genes and the authors note that "this proteogenomics dataset represents a unique public resource for researchers and clinicians seeking to better understand and treat lung adenocarcinomas" (Gillette et al., 2020).

Krug et al. (2020) integrated mass spectrometry-based proteomics and next generation DNA and RNA sequencing to create a proteogenomic profile of 122 treatment-naïve

primary breast cancer tumors. They found that "proteogenomics challenged standard breast cancer diagnoses, provided detailed analysis of the ERBB2 amplicon, defined tumor subsets that could benefit from immune checkpoint therapy, and allowed more accurate assessment of Rb status for prediction of CDK4/6 inhibitor responsiveness." The authors note that their results "underscore the potential of proteogenomics for clinical investigation of breast cancer through more accurate annotation of targetable pathways and biological features of this remarkably heterogeneous malignancy" (Krug et al., 2020).

Wang et al. (2021) integrated genomic, proteomic, post-translational modification, and metabolomic data to examine 99 treatment-naïve glioblastomas (GBMs), where they identified key phosphorylation events as potential mediators of oncogenic pathway activation and potential targets for *EGFR*-, *TP53*-, and *RB1*-altered tumors. The identified "immune subtypes with distinct immune cell types are discovered using bulk omics methodologies" and note that "histone H2B acetylation in classical-like and immune-low GBM is driven largely by BRDs, CREBBP, and EP300." Their work highlights the important of an integrated proteogenomic approach in GBM and "highlights biological relationships that could contribute to stratification of GBM patients for more effective treatment" (Wang et al., 2021).

Joshi et al. (2021) examined the stepwise evolution of gilteritinib resistance in *FLT3*-mutated acute myeloid leukemia (AML). To mechanistically define both early and late resistance in AML, they integrated whole exome sequencing, CRISPR-CAS9, metabolomics, proteomics, and pharmacologic approaches. They found that "early resistant cells undergo metabolic reprogramming, grow more slowly, and are dependent upon Aurora kinase B (AURKB). Late resistant cells are characterized by expansion of pre-existing NRAS mutant subclones and continued metabolic reprogramming," creating a model that closely mirrors the timing, and mutations of AML patients treated with gilteritinib. They also note that "pharmacological inhibition of AURKB resensitizes both early resistant cell cultures and primary leukemia cells from gilteritinib-treated AML patients." Their findings support a "combinatorial strategy to target early resistant AML cells with AURKB inhibitors and gilteritinib before the expansion of pre-existing resistance mutations occurs" (Joshi et al., 2021).

Akcakanat et al. (2021) integrated DNA, RNA, and functional proteogenomics from tumor samples of 52 patients with metastatic breast cancer (inclusive of ten patients with both primary and metastatic tumors to chart the evolution of the tumor's profile). The aim was to determine potentially actionable alterations in metastatic breast cancer and analyze the molecular evolution of the tumors. Regarding proteomic profiling, needle biopsy samples were evaluated via reverse phase protein arrays. The panel was composed of 295 antibodies and the PI3K pathway activity score was "defined as the

sum of the normalized values of the 13phosphor-protein levels of Akt, 4E-BP1, S6K, and S6." Samples were considered PI3K activated if their PI3K scores were in the top quartile. In discussion, the authors noted: "Neither genomic alterations predicted gene or protein expression nor was there a strong correlation between proteomic and transcriptomic data. [However] we cannot exclude the possibility that the lack of concordance between DNA alterations and RNA and protein and protein phosphorylation are not merely the result of tumor heterogeneity and the tumor cellularity of the samples" (Akcakanat et al., 2021).

VI. Guidelines and Recommendations

Since this is an emerging field, there is limited guidance from applicable professional societies. As of publication date, no specific guidance was found from professional medical societies, including NCCN, ACMG, NICE, AMP, and CAP.

National Comprehensive Cancer Network

Currently, the NCCN does not list proteogenomic testing as a recommended technique for any type of cancer. Furthermore, proprietary comprehensive genomic profiles have been submitted for inclusion in guidelines for several types of cancer, but they have never been included as a recommended technique as of June 23, 2022 (NCCN, 2023).

American Society of Clinical Oncology

In 2020, the American Society of Clinical Oncology (ASCO) published a clinical oncology educational book which included an article on integrating genetic and genomic testing into oncology practice. Transcriptome and proteomic sequencing were not mentioned in the article. However, the authors note that "Examples of the integration of genomic information into the care of patients with cancer include germline testing for *BRCA1/2* in breast, ovarian, pancreatic, and prostate cancer; evaluation of mismatch repair in endometrial cancer; and somatic sequencing in lung cancer" (Domchek et al., 2020). In 2022, they published a guideline on "Neoadjuvant Chemotherapy, Endocrine Therapy, and Targeted Therapy for Breast Cancer," where it was noted that "although tumor histology, grade, stage, and estrogen, progesterone, and human epidermal growth factor receptor 2 (HER2) expression should routinely be used to guide clinical decisions, there is insufficient evidence to support the use of other markers or genomic profiles" (ASCO, 2022).

In 2022, ASCO published a provisional clinical opinion titled, "Somatic Genomic Testing in Patients with Metastatic or Advanced Cancer." They note an increasing number of

therapies have been approved to treat cancers harboring specific genomic biomarkers. The following represents their recommendations (Chakravarty et al., 2022):

- "Patients with metastatic or advanced cancer should undergo genomic sequencing in a certified laboratory if the presence of one or more specific genomic alterations has regulatory approval as biomarkers to guide the use of or exclusion from certain treatments for their disease."
- "Multigene panel–based assays should be used if more than one biomarker-linked therapy is approved for the patient's disease."
- "Site-agnostic approvals for any cancer with a high tumor mutation burden, mismatch repair deficiency, or neurotrophic tyrosine receptor kinase (NTRK) fusions provide a rationale for genomic testing for all solid tumors."
- "Multigene testing may also assist in treatment selection by identifying additional targets when there are few or no genotype-based therapy approvals for the patient's disease."
- "For treatment planning, the clinician should consider the functional impact of the targeted alteration and expected efficacy of genomic biomarker–linked options relative to other approved or investigational treatments."

Regarding proteogenomic or protein profiling, ASCO recommended:

- "Several hundred genes and their mutant protein products have been linked to increased cell signaling, proliferation, and survival in cell lines or mouse models and thus proposed to be cancer drivers. However, at this time, few genomic alterations have been clinically proven as therapeutic targets. Therefore, in addition to the functional effects of mutations on protein chemistry and signaling, evidence of clinical relevance should be considered."
- "Clinical decision making should incorporate (1) the known or predicted impact of a specific genomic alteration on protein expression or function and (2) clinical data on the efficacy of targeting that genomic alteration with a particular agent (strength of recommendation: strong)" (ASCO, 2021; Chakravarty et al., 2022).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-

database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration

Many labs have developed specific tests that they must validate and perform in house. These laboratory developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81479	Unlisted molecular pathology procedure
81599	Unlisted multianalyte assay with algorithmic analysis
0019U	Oncology, RNA, gene expression by whole transcriptome sequencing, formalin-fixed paraffin-embedded tissue or fresh frozen tissue, predictive algorithm reported as potential targets for therapeutic agents Proprietary test: DarwinOncoTarget™/DarwinOncoTreat™ Lab/Manufacturer: Columbia University Department of Pathology and Cell Biology/Darwin Health
0260U	Rare diseases (constitutional/heritable disorders), identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping Proprietary test: Augusta Optical Genome Mapping Lab/Manufacturer: Georgia Esoteric and Molecular (GEM) Laboratory, LLC/Bionano Genomics Inc
0262U	Oncology (solid tumor), gene expression profiling by real-time RT-PCR of 7 gene pathways (ER, AR, PI3K, MAPK, HH, TGFB, Notch), formalin-fixed paraffin-embedded (FFPE), algorithm reported as gene pathway activity score Proprietary test: OncoSignal 7 Pathway Signal Lab/Manufacturer: Protean BioDiagnostics/Philips Electronics Nederland BV
0264U	Rare diseases (constitutional/heritable disorders), identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping Proprietary test: Praxis Optical Genome Mapping Lab/Manufacturer: Praxis Genomics LLC

CPT	Code Description
0266U	Unexplained constitutional or other heritable disorders or syndromes, tissue-specific gene expression by whole-transcriptome and next-generation sequencing, blood, formalin-fixed paraffin-embedded (FFPE) tissue or fresh frozen tissue, reported as presence or absence of splicing or expression changes Proprietary test: Praxis Transcriptome Lab/Manufacturer: Praxis Genomics LLC
0267U	Rare constitutional and other heritable disorders, identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping and whole genome sequencing Proprietary test: Praxis Combined Whole Genome Sequencing and Optical Genome Mapping Lab/Manufacturer: Praxis Genomics LLC
0298U	Oncology (pan tumor), whole transcriptome sequencing of paired malignant and normal RNA specimens, fresh or formalin-fixed paraffin-embedded (FFPE) tissue, blood or bone marrow, comparative sequence analyses and expression level and chimeric transcript identification Proprietary test: Praxis Somatic Transcriptome Lab/Manufacturer: Praxis Genomics LLC
0299U	Oncology (pan tumor), whole genome optical genome mapping of paired malignant and normal DNA specimens, fresh frozen tissue, blood, or bone marrow, comparative structural variant identification Proprietary test: Praxis Somatic Optical Genome Mapping Lab/Manufacturer: Praxis Genomics LLC
0300U	Oncology (pan tumor), whole genome sequencing and optical genome mapping of paired malignant and normal DNA specimens, fresh tissue, blood, or bone marrow, comparative sequence analyses and variant identification Proprietary test: Praxis Somatic Combined Whole Genome Sequencing and Optical Genome Mapping Lab/Manufacturer: Praxis Genomics LLC
0436U	Oncology (lung), plasma analysis of 388 proteins, using aptamer-based proteomics technology, predictive algorithm reported as clinical benefit from immune checkpoint inhibitor therapy. Proprietary test: PROphet® NSCLC Test Lab/Manufacturer: OncoHost, Inc
0454U	Rare diseases (constitutional/heritable disorders), identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping

CPT	Code Description
	Proprietary test: Chromosome Genome Mapping Lab/Manufacturer: UR Medicine Lab, Bionano Genomics, Inc
0485U	Oncology (solid tumor), cell-free DNA and RNA by next-generation sequencing, interpretative report for germline mutations, clonal hematopoiesis of indeterminate potential, and tumor-derived single-nucleotide variants, small insertions/deletions, copy number alterations, fusions, microsatellite instability, and tumor mutational burden Proprietary test: Caris Assure Lab/Manufacturer: Caris MPI, Inc d/b/a Caris Life Sciences

Current Procedural Terminology© American Medical Association. All Rights reserved.
Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

AccessWire. (2022). *Protean BioDiagnostics to Present at AACR 2021 Annual Meeting*. https://www.accesswire.com/635791/Protean-BioDiagnostics-to-Present-at-AACR-2021-Annual-Meeting

Akcakanat, A., Zheng, X., Cruz Pico, C. X., Kim, T.-B., Chen, K., Korkut, A., Sahin, A., Holla, V., Tarco, E., Singh, G., Damodaran, S., Mills, G. B., Gonzalez-Angulo, A. M., & Meric-Bernstam, F. (2021). Genomic, Transcriptomic, and Proteomic Profiling of Metastatic Breast Cancer. *Clinical Cancer Research*, *27*(11), 3243-3252. https://doi.org/10.1158/1078-0432.CCR-20-4048

Ang, M. Y., Low, T. Y., Lee, P. Y., Wan Mohamad Nazarie, W. F., Guryev, V., & Jamal, R. (2019). Proteogenomics: From next-generation sequencing (NGS) and mass spectrometry-based proteomics to precision medicine. *Clin Chim Acta*, *498*, 38-46. https://doi.org/10.1016/j.cca.2019.08.010

ASCO. (2021). *Guidelines/Provisional Clinical Opinions (PCOs)/Endorsements in Development*. https://old-prod.asco.org/practice-patients/guidelines/guidelines-recently-published-development

ASCO. (2022). *Neoadjuvant Chemotherapy, Endocrine Therapy, and Targeted Therapy for Breast Cancer*. Retrieved 08/05/2021 from https://www.asco.org/research-guidelines/quality-guidelines/guidelines/breast-cancer#/150037

Caris. (2023a). *Comprehensive Tumor Profiling*. https://www.carismolecularintelligence.com/comprehensivetumorprofiling/

Caris. (2023b). *MI Cancer Seek*. https://www.carismolecularintelligence.com/mi-cancer-seek/

Chakravarty, D., Johnson, A., Sklar, J., Lindeman, N. I., Moore, K., Ganesan, S., Lovly, C. M., Perlmutter, J., Gray, S. W., Hwang, J., Lieu, C., André, F., Azad, N., Borad, M., Tafe, L.,

Messersmith, H., Robson, M., & Meric-Bernstam, F. (2022). Somatic Genomic Testing in Patients With Metastatic or Advanced Cancer: ASCO Provisional Clinical Opinion. *Journal of Clinical Oncology*, *40*(11), 1231-1258. https://doi.org/10.1200/jco.21.02767

Columbia. (2023). *Darwin OncoTarget™/OncoTreat™*. https://www.pathology.columbia.edu/diagnostic-specialties/personalized-genomic-medicine/oncology-testing/darwin-oncotarget-tm-oncotreat

Domchek, S. M., Mardis, E., Carlisle, J. W., & Owonikoko, T. K. (2020). *American Society of Clinical Oncology Educational Book* (Vol. 40). https://ascopubs.org/doi/pdf/10.1200/EDBK_280607

Dong, L., Lu, D., Chen, R., Lin, Y., Zhu, H., Zhang, Z., Cai, S., Cui, P., Song, G., Rao, D., Yi, X., Wu, Y., Song, N., Liu, F., Zou, Y., Zhang, S., Zhang, X., Wang, X., Qiu, S., . . . Fan, J. (2022). Proteogenomic characterization identifies clinically relevant subgroups of intrahepatic cholangiocarcinoma. *Cancer Cell*, *40*(1), 70-87.e15. https://doi.org/10.1016/j.ccell.2021.12.006

Fancello, L., & Burger, T. (2022). An analysis of proteogenomics and how and when transcriptome-informed reduction of protein databases can enhance eukaryotic proteomics. *Genome Biology*, *23*(1), 132. https://doi.org/10.1186/s13059-022-02701-2

Feng, Y. H., Tung, C. L., Su, Y. C., Tsao, C. J., & Wu, T. F. (2019). Proteomic Profile of Sorafenib Resistance in Hepatocellular Carcinoma; GRP78 Expression Is Associated With Inferior Response to Sorafenib. *Cancer Genomics Proteomics*, *16*(6), 569-576. https://doi.org/10.21873/cgp.20159

Gillette, M. A., Satpathy, S., Cao, S., Dhanasekaran, S. M., Vasaikar, S. V., Krug, K., Petralia, F., Li, Y., Liang, W. W., Reva, B., Krek, A., Ji, J., Song, X., Liu, W., Hong, R., Yao, L., Blumenberg, L., Savage, S. R., Wendl, M. C., . . . Carr, S. A. (2020). Proteogenomic Characterization Reveals Therapeutic Vulnerabilities in Lung Adenocarcinoma. *Cell*, *182*(1), 200-225.e235. https://doi.org/10.1016/j.cell.2020.06.013

Hulick, P. (2022, Oct 25, 2024). *Next-generation DNA sequencing (NGS): Principles and clinical applications*. https://www.uptodate.com/contents/next-generation-dna-sequencing-ngs-principles-and-clinical-applications

Joshi, S. K., Nechiporuk, T., Bottomly, D., Piehowski, P. D., Reisz, J. A., Pittsenbarger, J., Kaempf, A., Gosline, S. J. C., Wang, Y. T., Hansen, J. R., Gritsenko, M. A., Hutchinson, C., Weitz, K. K., Moon, J., Cendali, F., Fillmore, T. L., Tsai, C. F., Schepmoes, A. A., Shi, T., . . . Traer, E. (2021). The AML microenvironment catalyzes a stepwise evolution to gilteritinib resistance. *Cancer Cell*, *39*(7), 999-1014 e1018. https://doi.org/10.1016/j.ccell.2021.06.003

Krug, K., Jaehnig, E. J., Satpathy, S., Blumenberg, L., Karpova, A., Anurag, M., Miles, G., Mertins, P., Geffen, Y., Tang, L. C., Heiman, D. I., Cao, S., Maruvka, Y. E., Lei, J. T., Huang, C., Kothadia, R. B., Colaprico, A., Birger, C., Wang, J., . . . Clinical Proteomic Tumor Analysis, C. (2020). Proteogenomic Landscape of Breast Cancer Tumorigenesis

and Targeted Therapy. *Cell*, *183*(5), 1436-1456 e1431. https://doi.org/10.1016/j.cell.2020.10.036

Kwon, O. K., Ha, Y. S., Lee, J. N., Kim, S., Lee, H., Chun, S. Y., Kwon, T. G., & Lee, S. (2019). Comparative Proteome Profiling and Mutant Protein Identification in Metastatic Prostate Cancer Cells by Quantitative Mass Spectrometry-based Proteogenomics. *Cancer Genomics Proteomics*, *16*(4), 273-286. https://doi.org/10.21873/cgp.20132

Liao, S., Hartmaier, R. J., McGuire, K. P., Puhalla, S. L., Luthra, S., Chandran, U. R., Ma, T., Bhargava, R., Modugno, F., Davidson, N. E., Benz, S., Lee, A. V., Tseng, G. C., & Oesterreich, S. (2015). The molecular landscape of premenopausal breast cancer. *Breast Cancer Res*, *17*, 104. https://doi.org/10.1186/s13058-015-0618-8

NantOmics. (2023). *GPS Cancer*. https://nantomics.com/gpscancer/

NCCN. (2023). *Transparency: Process and Recommendations*. https://www.nccn.org/disclosures/transparency.aspx

NIH. (2016). *APOLLO Network*. https://proteomics.cancer.gov/programs/apollo-network#:~:text=The%20Applied%20Proteogenomics%20OrganizationaL%20Learning,activity%20and%20expression%20of%20the

North, J. P., Golovato, J., Vaske, C. J., Sanborn, J. Z., Nguyen, A., Wu, W., Goode, B., Stevers, M., McMullen, K., Perez White, B. E., Collisson, E. A., Bloomer, M., Solomon, D. A., Benz, S. C., & Cho, R. J. (2018). Cell of origin and mutation pattern define three clinically distinct classes of sebaceous carcinoma. *Nature Communications*, *9*(1), 1894. https://doi.org/10.1038/s41467-018-04008-y

Praxis. (2022). https://www.praxisgenomics.com/test-list

PRNewswire. (2022). *NanoMosaic Closes a $40.75MM Over-Subscribed Series A Round*. https://www.insightpartners.com/about-us/news-press/nanomosaic-closes-a-40-75mm-over-subscribed-series-a-round/

Protean. (2023). *Protean MAPS*. https://www.proteanbiodx.com/proteanmaps

Quan, Q., Ritchey, J., Wilkinson, J., Kaiser, A., Geanacopoulos, J., & Boyce, J. (2022). Proteogenomic detection of circulating biomarkers for clinical oncology. *Journal of Clinical Oncology*, *40*(16_suppl), e15010-e15010. https://doi.org/10.1200/JCO.2022.40.16_suppl.e15010

Rabizadeh, S., Garner, C., Sanborn, J. Z., Benz, S. C., Reddy, S., & Soon-Shiong, P. (2018). Comprehensive genomic transcriptomic tumor-normal gene panel analysis for enhanced precision in patients with lung cancer. *Oncotarget*, *9*(27), 19223-19232. https://doi.org/10.18632/oncotarget.24973

Salem, M. E., Puccini, A., Grothey, A., Raghavan, D., Goldberg, R. M., Xiu, J., Korn, W. M., Weinberg, B. A., Hwang, J. J., Shields, A. F., Marshall, J. L., Philip, P. A., & Lenz, H. J. (2018). Landscape of Tumor Mutation Load, Mismatch Repair Deficiency, and PD-L1 Expression in a Large Patient Cohort of Gastrointestinal Cancers. *Mol Cancer Res*, *16*(5), 805-812. https://doi.org/10.1158/1541-7786.Mcr-17-0735

Shiba, N., Yoshida, K., Hara, Y., Yamato, G., Shiraishi, Y., Matsuo, H., Okuno, Y., Chiba, K., Tanaka, H., Kaburagi, T., Takeuchi, M., Ohki, K., Sanada, M., Okubo, J., Tomizawa, D., Taki, T., Shimada, A., Sotomatsu, M., Horibe, K., . . . Hayashi, Y. (2019). Transcriptome analysis offers a comprehensive illustration of the genetic background of pediatric acute myeloid leukemia. *Blood Adv*, *3*(20), 3157-3169. https://doi.org/10.1182/bloodadvances.2019000404

Sjostrom, M., Chang, S. L., Fishbane, N., Davicioni, E., Hartman, L., Holmberg, E., Feng, F. Y., Speers, C., Pierce, L. J., Malmstrom, P., Ferno, M., & Karlsson, P. (2020). Comprehensive transcriptomic profiling identifies breast cancer patients who may be spared adjuvant systemic therapy. *Clin Cancer Res*. https://doi.org/10.1158/1078-0432.Ccr-19-1038

Steiling, K., Christenson, Stephanie. (2023). *Tools for genetics and genomics: Gene expression profiling*. https://www.uptodate.com/contents/tools-for-genetics-and-genomics-gene-expression-profiling

Tredan, O., Wang, Q., Pissaloux, D., Cassier, P., de la Fouchardiere, A., Fayette, J., Desseigne, F., Ray-Coquard, I., de la Fouchardiere, C., Frappaz, D., Heudel, P. E., Bonneville-Levard, A., Flechon, A., Sarabi, M., Guibert, P., Bachelot, T., Perol, M., You, B., Bonnin, N., . . . Blay, J. Y. (2019). Molecular screening program to select molecular-based recommended therapies for metastatic cancer patients: analysis from the ProfiLER trial. *Ann Oncol*, *30*(5), 757-765. https://doi.org/10.1093/annonc/mdz080

Treue, D., Bockmayr, M., Stenzinger, A., Heim, D., Hester, S., & Klauschen, F. (2019). Proteogenomic systems analysis identifies targeted therapy resistance mechanisms in EGFR-mutated lung cancer. *Int J Cancer*, *144*(3), 545-557. https://doi.org/10.1002/ijc.31845

Wang, D., Eraslan, B., Wieland, T., Hallstrom, B., Hopf, T., Zolg, D. P., Zecha, J., Asplund, A., Li, L. H., Meng, C., Frejno, M., Schmidt, T., Schnatbaum, K., Wilhelm, M., Ponten, F., Uhlen, M., Gagneur, J., Hahne, H., & Kuster, B. (2019). A deep proteome and transcriptome abundance atlas of 29 healthy human tissues. *Mol Syst Biol*, *15*(2), e8503. https://doi.org/10.15252/msb.20188503

Wang, L. B., Karpova, A., Gritsenko, M. A., Kyle, J. E., Cao, S., Li, Y., Rykunov, D., Colaprico, A., Rothstein, J. H., Hong, R., Stathias, V., Cornwell, M., Petralia, F., Wu, Y., Reva, B., Krug, K., Pugliese, P., Kawaler, E., Olsen, L. K., . . . Clinical Proteomic Tumor Analysis, C. (2021). Proteogenomic and metabolomic characterization of human glioblastoma. *Cancer Cell*, *39*(4), 509-528 e520. https://doi.org/10.1016/j.ccell.2021.01.006

Yang, J., Lin, Y., Huang, Y., Jin, J., Zou, S., Zhang, X., Li, H., Feng, T., Chen, J., Zuo, Z., Zheng, J., Li, Y., Gao, G., Wu, C., Tan, W., & Lin, D. (2019). Genome landscapes of rectal cancer before and after preoperative chemoradiotherapy. *Theranostics*, *9*(23), 6856-6866. https://doi.org/10.7150/thno.37794

Red Blood Cell Molecular Testing

Policy Number: AHS – M2170 – Red Blood Cell Molecular Testing	Prior Policy Name and Number, as applicable:
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

The first successful blood transfusion can be dated back to the 1600s (Osterman & Arora, 2017), and molecular testing methods to analyze blood samples were introduced to the transfusion medicine community in the 1990s (Westhoff, 2006). Several ailments may warrant a blood transfusion and the phenotypic and genotypic determination of red blood cell antigens assist in limiting immune responses in transfusion patients. Agglutination tests via serology have been the gold standard for determining blood group antigens for more than 100 years (Boccoz et al., 2015), yet newer molecular techniques may grant added specificity (Elite, 2015).

For guidance regarding fetal RHD genotyping in RHD negative pregnant individuals, please refer to AHS- M2179-Prenatal Screening (Genetic).

II. Related Policies

Policy Number	Policy Title
AHS-M2131	Genetic Testing for Alpha- and Beta-Thalassemia
AHS-M2179	Prenatal Screening (Genetic)

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) Red cell genotyping (including C, c, D, E, e, K, k, Jka, Jkb, Fya, Fyb, S, s, U) **MEETS COVERAGE CRITERIA** in **any** of the following situations:

a) For individuals with sickle cell disease, thalassemia syndromes, hemoglobinopathies, and/or other medical conditions requiring recurring transfusions.

b) For individuals with post-transfusion hemolysis, when no antibodies are detectable and no other possible cause is known (e.g., sickle cell crisis, mechanical hemolysis due to heart valve failure).

c) For individuals with autoimmune hemolytic anemia.

d) For multiply transfused individuals and/or individuals who are direct antiglobulin test positive.

e) For pregnant individuals who, prior to their first transfusion, had non-transfusion-dependent thalassemia.

f) To help manage hemolytic disease of the fetus and newborn.

g) To resolve conflicting serological antibody results.

h) For individuals with suspected Duffy-null neutropenia.

2) Red cell genotyping (including C, c, D, E, e, K, k, Jka, Jkb, Fya, Fyb, S, s, U) **DOES NOT MEET COVERAGE CRITERIA** in **any** of the following situations:

a) For individuals who have had allogenic hematopoietic stem cell transplants.

b) For the diagnosis of sickle cell disease.

c) As a routine pre-transfusion test.

d) As a routine screen for individuals receiving a solid organ transplant.

NOTES:

Note: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
ABOi	ABO-Incompatible
ASH	American Society of Hematology
BCSH	British Committee for Standards in Hematology
CIBMTR	Center for International Blood and Marrow Transplant Research
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare and Medicaid Services

DAT	Direct antiglobulin test
DAT+	Direct antiglobulin test positive
EAU	European Association of Urology
EDQM	European Directorate for the Quality of Medicines & HealthCare
FDA	Food and Drug Administration
FUT2	*Fucosyltransferase 2*
HDFN	Hemolytic Disease of The Fetus and Newborn
HEA	Human erythrocyte antigen
HLA	Human leukocyte antigen
HSCT	Hematopoietic Stem Cell Transplantation
HTRs	Hemolytic transfusion reactions
ICTM	International Collaboration for Transfusion Medicine
ISBT	International Society of Blood Transfusion
KDIGO	Kidney disease improving global outcomes
LAMP	Loop-mediated isothermal amplification method
LDTs	Laboratory-developed tests
MHC	Major histocompatibility complex
MT SC	Molecular testing standards committee
NGS	Next generation sequencing
NHLBI	National Heart, Lung, And Blood Institute
NMDP	National marrow donor program
NTDT	Non-Transfusion-Dependent Thalassemia
PCR	Polymerase chain reaction
PCR-RFLP	PCR restriction fragment length polymorphism
RBC	Red blood cell
Rh	Rhesus
RT-q	Reverse Transcription-Quantitative
SCD	Sickle cell disease
SNPs	Single-nucleotide polymorphisms
SSP-PCR	Sequence-Specific PCR
TDT	Transfusion Dependent Thalassemia
TIF	Thalassemia International Federation

V. Scientific Background

Transfusion medicine requires extensive blood type knowledge to ensure a safe and efficient transfusion process. Blood type differs throughout the population based on an individual's genome, and genes can vary due to allelic variations. Alleles are alternative forms of the same gene that arise by mutation. Alleles cause genomic

changes that ultimately lead to a discrepancy in the proteins produced in the body. Single-nucleotide polymorphisms (SNPs) are also found in the genome and describe a location where the nucleotide that is present differs between individuals because a substitution has been made. Alleles are sometimes caused by SNPs but can also be caused by other genetic mutations as well, such as additions, deletions, and insertions. Further, SNPs do not always lead to new alleles, as they could occur in non-coding areas of the genome. These alleles and SNPs play an important role in blood type and, therefore, in blood transfusion outcomes. Genetic variations will always affect an organism's genotype, which is the set of genes carried by the individual's cells, but may or may not affect an organism's phenotype, which are the observable characteristics or physical expression of those genes.

Blood Groups

Blood is quite complex and can be categorized into several different groups. Historically, blood groups have been classified by the antigens found "on or in the surface of erythrocytes which are present in certain individuals of a species but are lacking in others" (Kabat, 1956). These antigens are proteins with the ability to promote an immune response in the body by inducing the formation of antibodies.

To date, dozens of blood groups have been identified. As of July 2023, the International Society of Blood Transfusion has recognized 45 blood group systems (ISBT, 2023). This number seems to be constantly changing as new discoveries are made. Only a few years earlier, McBean et al. (2014) reported that 35 blood group systems had been identified, as well as 44 blood group genes and 1568 alleles. Most of these blood group antigens result from SNPs (Boccoz et al., 2015).

Karl Landsteiner, an Austrian biologist in the twentieth century, received the Nobel Prize for the discovery of the first human blood grouping system, ABO, in the early 1900s (Harmening et al., 2012). The ABO group was later classified into four antigens (A, B, O and AB) and six genotypes (AA, AO, BB, BO, OO and AB) in 1924 (Zhang et al., 2015). Examples of these are shown below in Table 1. As likely inferred from the name, the ABO blood group has three main allelic forms at its chromosomal locus: A, B and O. An individual's ABO blood type is genetically inherited, and A and B alleles are inherited in a codominant manner over O (Harmening et al., 2012). The ABO locus has been identified on chromosome 9 at 9q34.1-q34.2; differences in the A and B alleles have been identified and include variations in seven nucleotides via substitution, with four translating into different amino acids (Bethesda, 2005).

Another inherited antigen found on the surface of red blood cells is the Rhesus (Shih et al.) antigen (+/-); after the ABO blood group, the Rh factor is the most clinically significant factor (Písačka, 2018). The Rh blood group system, also identified by Karl

Landsteiner alongside Alexander Weiner, was named for the rhesus monkey from which this blood group was originally discovered (Agre et al., 1990). The Rh+ genotype is completely dominant over Rh-. It was recently reported that the Rh blood group system is comprised of 54 antigens and is "the most complex and polymorphic system" (Raud et al., 2017). Several journal articles have identified genetic variants in the RH (*RHD* and *RHCE*) genes, including SNP substitutions, insertions, deletions, and rearrangements; however, the expression of these variants require further research to determine how overall blood type is affected (Raud et al., 2017). Westhoff (2006) states that more than 100 different *RHD* genes have been identified which encode "proteins with single amino acid changes, or rearranged genes encoding hybrid proteins, and over 50 different *RHCE* genes with single or multiple amino acid changes." Genetic variation in the *RHD* and *RHCE* genes, including partial D antigens, are common, especially in individuals with African descent; further, the weak D phenotype is commonly identified in Caucasians (Elite, 2015). Other minor blood group systems include Kell, Kidd, Duffy, MNS, P, Lewis, Junior, and Lutheran (Kahar & Patel, 2014; Raud et al., 2017).

Red blood cell genotyping is necessary to confirm the presence or absence of Duffy antigens (Fy(a) and Fy(b)) on red blood cells. The Duffy-null phenotype is more common in individuals of African descent and can be associated with resistance to malaria, particularly *Plasmodium vivax*. Genetic variation determines Duffy blood group status, which has impacts that may encompass: (1) malaria resistance, (2) hemolytic transfusion reactions, and (3) hemolytic disease of the fetus and newborn (HDFN). Duffy antigen expression also affects baseline neutrophil counts. However, the Duffy-null phenotype can also be linked to a benign form of neutropenia (i.e., low neutrophil count), which is prevalent in individuals of African ancestry. This condition (known as benign ethnic neutropenia) does not typically result in increased susceptibility to infections (Merz & Achebe, 2021; Merz et al., 2023). By confirming the Duffy-null phenotype through genotyping, it is possible to differentiate between benign ethnic neutropenia and other causes of neutropenia (that may be cause for heightened concern). Additionally, in transfusion settings, genotyping is essential to ensure compatibility and prevent alloimmunization (Quinn & Berliner, 2023).

Blood Transfusions

Certain antibodies are considered more clinically significant than others for blood transfusion purposes. Clinically significant antibodies (with antigens listed in the subsequent parentheses) include "ABO (A, B), Rh (D, C, c, E, e), Duffy (Fya, Fyb), Kidd (Jka, Jkb), Kell (K, k), and SsU (S, s, U)" and less clinically significant antibodies include "Lewis (Le^a, Le^b), MN, P1, Xg^a, Cartwright (Yt^a), Bg, Knops (Kn^a, McC^a, Yk^a),

Chido/Rodgers (Ch1/Rg1), Sda; as well as high titer low avidity (HTLA) antibodies" (Uhl, 2023).

The ABO blood group is widely accepted as the most important of all blood systems for blood transfusion and transplant purposes. This is because the ABO group is responsible for most immune responses due to blood type incompatibility (Hu et al., 2019). Further, ABO is "the only blood group system in which individuals predictably have antibodies in their serum to antigens" absent from their red blood cells (Harmening et al., 2012). It is known that individuals with type A blood (containing A antigens) have anti-B antibodies in their serum, individuals with type B blood (containing B antigens) have anti-A antibodies in their serum, individuals with type O blood (containing no antigens) have anti-A and anti-B antibodies in their serum, and individuals with type AB blood (containing A and B antigens) do not have any anti-A or anti-B antibodies in their serum (Bethesda, 2005). ABO antibodies form in the serum naturally and are stimulated when the immune system encounters the "missing" ABO blood group antigen from food or in micro-organisms; "This happens at an early age because sugars that are identical to, or very similar to, the ABO blood group antigens are found throughout nature" (Bethesda, 2005). Although every red blood cell reportedly expresses about two million ABO blood group antigens, ABO antigens are not only present on red blood cells; these antigens are also found on many types of human tissue and most endothelial and epithelial cells (Bethesda, 2005). In certain individuals, these antigens can also be found in saliva and most bodily fluids as well (Bethesda, 2005).

Table 1: Phenotypes, Genotypes and Antibodies of the ABO Blood Group

Blood Type	Phenotype (i.e. antigen)	Potential Genotype(s)	Antibodies found in serum
A	A	AA, AO	Anti-B
B	B	BB, BO	Anti-A
AB	A and B	AB	None
O	Neither A nor B	OO	Anti-A and Anti-B

Due to the presence of antigens and antibodies on red blood cells, safe blood transfusions rely heavily on blood type knowledge. Administration of the wrong blood type can immediately lead to red blood cell lysis and death, and it is a leading cause of transfusion-related death as reported to the Food and Drug Administration (FDA) (Harmening et al., 2012). If the incorrect blood type is given to a patient, antibodies begin complement causing "rapid intravascular hemolysis, triggering an acute hemolytic transfusion reaction that can cause disseminated intravascular coagulation, shock, acute renal failure, and death" (Bethesda, 2005).

Several medical procedures utilize blood antigen typing such as blood transfusions, bone marrow transplants, stem cell transplants, and organ transplants. Blood transfusions may be administered for a multitude of reasons, including severe blood loss, leukemia, sickle cell disease, thalassemia, anemia, and kidney disease. Bone marrow, which is found in several adult bones including those of the pelvis, can assist with the transplant of healthy blood stem cells to replace others that have been destroyed by disease or infection; bone marrow transplants are known to assist in the treatment of several cancers, including leukemia, myeloma and lymphoma (Miller et al., 2019; Shah et al., 2018). Several factors—including age, illness severity, co-morbidities, and hemorrhage amount and rate—should be taken into account before a blood transfusion occurs (Osterman & Arora, 2017; Yaddanapudi & Yaddanapudi, 2014). Still, it has been reported that the use of blood transfusions for medical practice has been notoriously overused since the 20th century for the treatment of anemia and hemorrhage (Yaddanapudi & Yaddanapudi, 2014). Based on data distributed by the Red Cross, more than 30 million blood components are transfused annually in the United States (Osterman & Arora, 2017). In addition to whole blood transfusions, several types of blood product transfusions are available, including platelet, plasma and cryoprecipitate (Yaddanapudi & Yaddanapudi, 2014).

Hematopoietic Stem Cell Transplantation (HSCT)

Hematopoietic stem cells are immature cells with the ability to develop into all types of blood cells including red blood cells, white blood cells, and platelets. The transplantation of these cells, known as a hematopoietic stem cell transplantation (HSCT), is a medical procedure which involves the intravenous transfusion of hematopoietic stem cells obtained from the same (autologous) or different (allogenic) individual. These stem cells can be obtained from the umbilical cord, bone marrow, or peripheral blood and may be used to improve hematopoietic function or fight diseases such as leukemias, lymphoid neoplasia, solid tumors, and non-malignant disorders (Passweg et al., 2016).

In contrast to the requirement of ABO match for other types of blood transfusions and solid organ transplants, ABO mismatch is acceptable in HSCTs (Shokrgozar & Tamaddon, 2018). HSCTs can be safely matched by the human leukocyte antigen (Mandelbrot et al.), which is inherited independently of the ABO antigens (Booth et al., 2013; Shokrgozar & Tamaddon, 2018). Approximately 40-50% of all HSCTs will involve recipient-donor ABO incompatibility (Booth et al., 2013; Worel, 2016). However, an ABO mismatch may cause the recipient to experience pure red blood cell aplasia, delayed hemolytic reactions, or delayed red blood cell engraftment (Booth et al., 2013). Researchers note that outcomes in ABO-incompatible HSCT have yielded inconsistent

results, and pre-transplant antigen evaluation may be important to determine the extent of the incompatibility (Booth et al., 2013; Parkhideh et al., 2019).

Solid Organ Transplants

Solid Organ transplants are common between blood group- compatible individuals, with this process currently considered the most effective treatment for end-stage organ failure (Hu et al., 2019). To date, most important tissues in the body have been successfully transplanted, with exception of the brain.

Solid organ transplant is generally completed with ABO identical donor organs; however, minor ABO-incompatible solid organ transplants may be performed (Uhl, 2023). While ABO compatibility is widely accepted as the primary immunologic barrier for solid organ transplants, human leukocyte antigen (Mandelbrot et al.) compatibility is also very salient (Fasano et al., 2017). The HLA complex is a key part of the immune system controlled by genes on chromosome six; the HLA loci are part of the major histocompatibility complex (MHC) which helps the immune system to recognize foreign molecules (Shankarkumar et al., 2002). Some HLA antigens are recognized on all tissues in the body (as opposed to mainly red blood cells), making these antigens an integral part of organ and tissue transplants. The importance of HLA antibodies have been studied extensively in renal transplants "where recipient antibodies directed against mismatched donor HLA antigens are associated with antibody mediated rejection and/or graft loss" (Fasano et al., 2017).

Hemoglobinopathies (Sickle Cell Disease and Thalassemia)

Hemoglobinopathies, such as sickle cell disease and thalassemia, are red blood cell disorders. Sickle cell disease is a collection of inherited blood disorders which cause the development of abnormal sickle-shaped red blood cells that disrupt blood flow and lead to a plethora of problems, including ischemia and inflammation (Ware et al., 2017). Approximately 300,000 new blood disorder cases are reported globally each year due to sickle cell disease (Strouse, 2016). Blood transfusions for those with sickle cell disease can assist with an increase in oxygenation to tissues in the body, restoration of blood volume, reduction of sickle erythropoiesis, and prevention of acute vaso-occlusion (Ware et al., 2017). Alloimmunization, which is when the immune system responds to foreign antigens after a blood transfusion, occurs in a percentage of transfusion cases. "Extended red blood cell (RBC) antigen matching is recommended to limit alloimmunization in patients with sickle cell disease" (Casas et al., 2015). Thalassemia is an inherited blood disorder in which an abnormal or inadequate amount of hemoglobin is produced. Thalassemia can be categorized as α-or β-thalassemia depending on which hemoglobin chain is affected (Brancaleoni et al.,

2016). Blood transfusions are a potential treatment option for individuals with this disease (Dhawan et al., 2014).

However, post-transfusion complications are frequent with hemoglobinopathies due to "subtle antigenic differences between donors and patients" (Belsito et al., 2017). For sickle cell disease, red blood cell alloimmunization is common, and red blood cell antigen discrepancies are particularly important to identify because delayed transfusion reactions may be life threatening (Ware et al., 2017). Regarding thalassemia, alloimmunization rates emphasize "the need for RBC antigen typing before first transfusion and issue of antigen matched blood (at least for Rh and Kell antigen)" (Dhawan et al., 2014). Patients with sickle cell disease and thalassemia develop alloantibodies at a much higher rate than typical transfusions; patients with diseases such as these, or other patients who receive blood transfusions often, will benefit from an extended phenotype determination either with serological methods or genotypic methods (Elite, 2015). Fasano and Chou (2016) have used red blood cell antigen genotyping for sickle cell disease, thalassemia and other complications that may arise from transfusions; "Genotyping can be used to determine RBC antigen phenotypes in patients recently transfused or with interfering allo- or autoantibodies, to resolve discrepant serologic typing, and/or when typing antisera are not readily available" (Fasano & Chou, 2016).

Blood Transfusion Molecular Testing

For more than 100 years, agglutination tests using serology have been considered the gold standard for determining blood group antigens (Boccoz et al., 2015). These agglutination tests are simple to perform and identify antibodies or antigens in a sample, allowing for blood type to be inferred. Antiglobulin (Coombs) testing is a common laboratory technique that utilizes a secondary antibody to detect antibodies in the patient's serum; direct antiglobulin testing detects antibodies on the surface of red blood cells, and indirect antiglobulin testing identifies alloantibodies in patient serum (Uhl, 2023). However, these methods have limitations, such as inadequate immunological reagent availability, rising reagent costs, and specificity discrepancies between minor and rare blood types (Boccoz et al., 2015; Westhoff, 2006). Further, a recent red blood cell transfusion may also affect serologic results.

Certain immunohematological tests, such as adsorption/elution techniques, enzyme tests and indirect antiglobulin tests, are important for the detection of specific weak D phenotypes, but other weak D phenotypes cannot be detected by serological techniques (Guzijan et al., 2019). Weak or partial blood type antigens such as Rh, Fy^b, Jk^a, and U have been known to cause blood typing discrepancies (Elite, 2015). Moreover, certain blood type antigens exist for which "there is no commercially

available antisera and poorly characterized patient-source antisera (e,g., hrB, hrS, Joa, Hy, U)" (Elite, 2015). These limitations are significant because researchers have noted that "In practice, serological matching for ABO blood group antigens, Rhesus antigens (RhD, RhC, and RhE), and Kell (K) antigens does not fully prevent alloimmunisation, because of RH alleles with reduced or altered antigen expression" (Ware et al., 2017).

To combat serologic discrepancies, several DNA-based molecular testing methods may now be used for blood transfusion purposes. These methods can resolve the red blood cell inconsistencies often caused by weak and partial antigens (Belsito et al., 2017). Nonetheless, molecular typing is expensive, and results may take a few days or weeks to be returned. Further, for the majority of transfusion recipients, matching beyond ABO and D blood type antigens is not necessary (Anstee, 2009). It is, therefore, unlikely that these methods will become a substitute for serological testing but may be used instead when more detailed results are required (Sapatnekar, 2015).

These molecular techniques include Sanger sequencing of both genomic DNA and complimentary DNA as well as SNP assay testing; an SNP assay may consist of polymerase chain reaction (PCR)-restriction fragment length polymorphism (PCR-RFLP) or sequence-specific PCR (SSP-PCR) (Elite, 2015). Additional DNA amplification techniques may also be used for red blood cell genotyping, including "PCR-single-strand conformation polymorphism, allele-specific-PCR, PCR-amplified product length polymorphism, reverse transcription-quantitative (RT-q)PCR and DNA chip" (Zhang et al., 2015). Further, next generation sequencing is considered very promising for HLA typing, but more research is required to determine if this method is useful in the identification of blood group antigens (Elite, 2015). Other methods include array-based techniques which allow for multi-parallel testing where numerous samples can be searched at once for SNPs or other analytes (Elite, 2015). Microarrays, which may be planar arrays or suspended bead arrays, help to overcome several serology limitations by "replacing the immunochemical reagents by synthetic and controlled probes" (Boccoz et al., 2015).

Genotyping assays from whole-blood samples, which use an on-chip test to identify up to 24 blood group antigens, are also available (Boccoz et al., 2015), and next generation sequencing (NGS) methods can detect *de novo* genetic polymorphisms, SNPs, and other genetic variants (Belsito et al., 2017). Liu et al. (2014) claim that NGS methods "assess genetic variations that cannot be achieved by traditional Sanger sequencing or other genotyping platforms." More recently, it has been reported that molecular diagnostics for red blood cell genotypes can now be determined in the clinical laboratory and that the majority of allelic variations can be contributed to SNPs (Wilkinson, 2016).

A few panel tests have been developed that identify several blood antigens simultaneously. The BLOODchip® ID CORE XT™ by Grifols Diagnostic Solutions Inc. is an FDA-approved test. The BLOODchip® ID CORE XT™ can simultaneously identify 37 blood antigens in the following ten blood group systems: Rh, Kell, Kidd, Duffy, MNS, Diego, Dombrock, Colton, Cartwright and Lutheran (Grifols, 2018). This kit will be particularly beneficial to patients who require ongoing blood transfusions, such as individuals with sickle cell disease and thalassemia, as well as for cancer patients who may require very thorough blood typing. This kit uses PCR to amplify DNA samples and can provide results in approximately four hours. A study of 1000 samples reported a sensitivity of 100% and, after bidirectional sequencing, a 100% specificity for the BLOODchip® ID CORE XT blood typing assay; one discrepancy was identified in the E-antigen and 33 discrepancies were identified in the Fy^b- antigen (Lopez et al., 2018).

The PreciseType® HEA Test by Immuncor, Inc. is an FDA-approved multiplexed molecular assay which provides information on 35 red blood cell antigens from eleven different blood groups (Rh, Kell, Duffy, Kidd, MNS, Lutheran, Dombrock, Landsteiner-Wiener, Diego, Colton and Scianna) (Immuncor, 2024). This test can also identify 24 gene mutations and one SNP commonly associated with hemoglobinopathies. This test uses PCR and requires no confirmation of results with antisera (Immuncor, 2024). Another test by One Lamda, LinkSēq™ Blood Genotyping, was released which is a real-time PCR for RBC, ABO and HPA Genotyping (Lambda, 2024).

There are many other assays not yet FDA-approved but are used for research and investigational purposes only. The chart below displays various blood group genotyping methodologies which detect polymorphisms and antigens (Sippert, 2019).

Name/Manufacturer	Principle	Number of polymorphisms and antigens identified
Immucor PreciseType Molecular BeadChip by BioArray Solutions (FDA approved)	Multiplex PCR followed by hybridization of amplified DNA to probes attached to spectrally distinguishable beads and elongation	24 polymorphisms associated with 38 antigens plus phenotypic variants and Hemoglobin S
BioArray RHCE and RHD BeadChip by BioArray Solutions	Multiplex PCR followed by hybridization of amplified DNA to probes attached to spectrally distinguishable beads and elongation	35+ *RHCE* variants; 80+ *RHD* variants

ID CORE XT by Progenika Biopharma (FDA-approved)	Multiplex biotinylating PCR followed by hybridization to probes on color-coded microspheres	29 polymorphisms associated with 37 antigens and phenotypic variants
ID RHD XT by Progenika Biopharma	Multiplex biotinylating PCR followed by hybridization to probes coupled to color-coded microspheres	6 *RHD* variants and HPA-1
Hemo ID DQS Panel by Agena Bioscience	Multiplex end-point PCR and single base primer extension	101 antigens associated with 16 systems, and 23 platelet and neutrophil antigens, in modules
HIFI Blood 96 by AXO Science	Automated multiplex PCR and microarray-based assay	15 polymorphisms associated with 24 antigens
RBC-FluoGene vERYfy eXtend by inno-train diagnostik GMBH	Automated TaqMan-based assay	70 antigens and phenotypic variants associated with 12 systems
RBC-ReadyGene by inno-train diagnostic GMBH	PCR-SSP-based assay	16 modules encompassing antigens and variant phenotypes within 13 systems
TaqMan genotyping on OpenArray by BioTrove	Nanofluidic TaqMan assays performed in OpenArray plates	16, 32 or 64 assays (custom designed)
Pre-designed TaqMan assays Thermo Fisher Scientific	Real-time allelic discrimination PCR	Polymorphisms associated with eight systems

Analytical Validity

A novel method for ABO genotyping was developed by Muro et al. (2012) which used allele-specific primers and real-time PCR; this method can be completed in less than two hours, detects SNPs at three different nucleotide positions in the ABO gene, and can determine six main ABO genotypes. The authors state that "this method could be applicable for rapid and simple screening of forensic samples" (Muro et al., 2012).

However, this method is limited for transfusion medicinal purposes as it only identifies ABO genotypes.

Zhang et al. (2015) developed a method of determining ABO blood group genotypes with a real-time loop-mediated isothermal amplification method (LAMP); this LAMP method used two SNPs at nucleotides 261 and 803 to differentiate six ABO genotypes in less than an hour. A total of 101 samples were tested in this study, and each sample resulted in an ABO genotype; "The results were compared with the phenotypes determined by serological assay and the genotypes determined by direct sequencing, and no discrepancies were observed" (Zhang et al., 2015). This shows that this LAMP method is an accurate and efficient method to assist in ABO blood type genotyping.

Krog et al. (2019) have developed 35 PCR assays for blood donor genotyping. This high-throughput PCR genotyping method analyzed samples from 1034 patients, identifying 62 discrepancies between initial results, 43 of which are due to serology techniques (Krog et al., 2019). The accuracy of the genotyping method was determined at 99.9% with only 17 errors; "Of 17 discrepancies caused by the genotype, three were incorrect antigen-negative predictions and could potentially, as the solitary analysis, have caused an adverse transfusion reaction" (Krog et al., 2019). This is an extremely sensitive and cost-efficient method with a high capacity for screening many donors.

Another set of researchers developed a method of amplifying DNA via PCR "directly from plasma or serum of blood donors followed by a melting curve analysis in a capillary rapid-cycle PCR assay" (Wagner et al., 2017). This method allows the DNA extraction step to be skipped, permitting this assay to be completed in only 40 minutes. A positive predictive value was identified at 100%, and a negative predictive value at 84%. The authors state that "With fast turnaround times, the rapid-cycle PCR assay may eventually be developed and applied to red cell genotyping in the hospital setting" (Wagner et al., 2017).

It has been reported that the genetic Rh complexity among sickle cell disease patients is so great that molecular assays are not detailed enough to identify all possible genetic variants; therefore, next generation sequencing has been employed to identify all Rh genetic variants in an effort to improve transfusion outcomes in sickle cell disease patients (Dezan et al., 2017). This study used samples from 35 sickle cell disease patients with "unexplained Rh antibodies"; a genotypic method which used *RHD* and *RHCE* gene-specific primers was compared to traditional serological data. It was reported that "Ten and 25 variant *RHD* and *RHCE* alleles were identified, respectively. Among all cases of unexplained Rh antibodies, 62% had been inaccurately classified by serological analysis and, of these, 73.1% were considered as relevant" (Dezan et al., 2017). This next generation sequencing assay is therefore an effective

measure to identify genetic variants and improve blood transfusion outcomes in the sickle cell disease population.

Molecular *RHD* typing was implemented at two institutions in Europe via PCR-SSP; this was completed to assist in blood transfusion cases (Guzijan et al., 2019). This molecular method confirmed the majority of serologically identified weak D variants with more detail, and it also identified a discrepancy in one sample (Guzijan et al., 2019). This study has verified the accuracy of this genotyping method to confirm *RHD* variants, which will assist in the implementation of safer blood transfusion practices.

Shah et al. (2019) compared serological and molecular detection methods for common red cell antigen groups in thalassemia major and sickle cell disease patients. Specifically, the authors compared the molecular genotyping of Rh (D, C, c, E, e), Kell (K, k), Duffy (Fy^a, Fy^b) and Kidd (Jk^a, Jk^b) blood group antigens using PCR and PCR-RFLP to red blood cell antigen phenotypes identified by serology; serological testing was completed by tube agglutination. The authors note that the serological testing for extended RHCcEe, Kell, Kidd and Duffy testing for multi-transfused patients may not generate the correct blood grouping of the recipient. The authors report that "In more than 80% of the cases recipient's molecular testing blood groups were at variance with serologically tested blood groups ($p < 0.0001$). Mixed field reactions in serological typing were common. In sickle cell anemia patients, no discrepancy was found" (Shah et al., 2019). This shows that molecular detection methods to determine the genotype was particularly useful in multi-transfused thalassemia patients.

Sheppard et al. (2020) investigated the diagnostic accuracy of two PCR-based group genotyping platforms for patients with sickle cell disease (SCD) including the human erythrocyte antigen (Shah et al.) BeadChip (Immucor, Norcross, GA) and ID CORE XT (Progenika-Grifols, Barcelona, Spain). DNA samples were extracted from 138 patients with SCD to compare predicted phenotype results and calculate a concordance rate. As a result, predicted phenotype results were comparable across platforms for 31 antigens and the concordance rate was 99.9%. The study also identified discrepancies between the two assays in the highly variable Rh blood group system. In some cases, the two assays interrogated different positions in the gene, and the software made different assumptions based on the population frequency of the alleles. The ID CORE XT was able to identify the phenotype in two of three samples which had discrepancies in the Rh blood group while the HEA BeadChip identified the phenotype in one of three samples due to lack of interrogation in the gene. The authors conclude that genotyping provides valuable information not available by serologic methods and both methods are beneficial due to the high concordance rate (Sheppard et al., 2020).

Jadhao et al. (2022) studied the use of RBCeq, an algorithm for accurate genetic blood typing which identifies 36 blood group systems. "The RBCeq algorithm demonstrated 99.07% concordance when validated on 402 samples which included 29 antigens with serology and nine antigens with SNP-array validation in 14 blood group systems and 59 antigens validation on manual predicted phenotype from variant call files." The authors conclude that the use of RBCeq can reduce pre-transfusion testing time and increase sample processing throughput (Jadhao et al., 2022).

Clinical Utility and Validity

It is imperative that patients with sickle cell anemia receive correct red blood cell transfusions. In an effort to compare the accuracy of new DNA-based testing methods to traditional agglutination methods, Casas et al. (2015) compared 494 red blood cell phenotypes of sickle cell disease patients determined by hemagglutination methods to genotypic predictions made by DNA-based testing methods. If any discrepancies were identified, repeat serologic testing was performed to confirm these results. It was found that "Seventy-one typing discrepancies were identified among 6360 antigen comparisons (1.1%). New specimens for repeat serologic testing were obtained for 66 discrepancies and retyping agreed with the genotype in 64 cases" (Casas et al., 2015). This shows that DNA-based red blood cell genotyping can provide more accurate results when compared to traditional serologic methods, leading to more safe and efficient treatments for sickle cell disease patients.

Flegel et al. (2015) completed a large-scale blood genotyping project; samples were analyzed from 43,066 blood donors from the Blood Center of Wisconsin. Red blood cell genotyping was completed with "a nanofluidic microarray system, using 32 single nucleotide polymorphisms to predict 42 blood group antigens" (Flegel et al., 2015). This microarray technique allowed researchers to fill 95% of antigen-negative red blood cell requests for hospitals served by the Blood Center of Wisconsin; the remining requests were filled via serology identification. This genotyping system is therefore efficient and should be used along with conventional serology methods.

The frequency of red blood cell autoimmunization and alloimmunization development in β-thalassemia major patients was studied by Dhawan et al. (2014); clinical and transfusion records from 319 patients were analyzed. A total of 18 patients were found to have alloantibodies, 90 patients developed autoantibodies, and nine patients had both alloantibodies and autoantibodies; "Alloimmunization was detected in 5.64% of multitransfused thalassemia patients. Rh and Kell blood group system antibodies accounted for more than 80% of alloantibodies. This study re-emphasizes the need for RBC antigen typing before first transfusion and issue of antigen matched blood (at least for Rh and Kell antigen)" (Dhawan et al., 2014).

Parkhideh et al. (2019) studied the effects of ABO incompatibility on the outcome of allogenic HSCTs. This retrospective study includes data from 186 different patients with 108 identical, 38 minor, 32 major and eight bidirectional ABO incompatible scenarios. The researchers did not identify any significant associations between ABO incompatibility and white blood cell or platelet engraftment, or graft-versus-host disease; however, white blood cell engraftment was significantly lower in patients with minor ABO incompatibilities (Parkhideh et al., 2019).

Shih et al. (2020) discussed using red cell antigen genotyping and serological phenotyping in SCD patients to risk-stratify patients for alloimmunization risk. In this study, 106 SCD patients underwent genotyping to assess whether partial antigen expression may lead to increased risk of alloimmunization and hemolytic transfusion reactions (HTRs). A total of 91% of the 106 patients showed additional clinically relevant genotyping information when compared to serological phenotyping alone. The FY*02N.01 was found in 90% of patients and RH variant alleles were found in 49% of patients, with most of them having putative partial antigen expression. Overall, genotyping helped discover more clinically relevant information and "genotyping SCD patients before transfusion may prevent alloimmunization and HTRs." Additionally, "knowledge of the FY*02N.01 variant allele increases feasibility of finding compatible blood" (Shih et al., 2020).

Vege et al. (2021) studied the impact of *RHD* genotyping on transfusion practice and identification of novel *RHD* alleles. A total of 57 samples from Denmark and 353 samples from the US were genotyped with *RHD* Beadchip in the US and CDE in Denmark. "Of Caucasian samples from Denmark, 90% (n = 51) had weak D types 1, 2, or 3; two had other weak D, two partial D, and two new alleles. In diverse ethnic U.S. samples, 44% (n = 155) had weak D types 1, 2, or 3 and 56% (n = 198) had other alleles. Most samples with weak or variable D typing from Denmark had alleles without risk for anti-D. In U.S. samples, 48% could safely be treated as D+, 18% may require consideration if pregnancy possible, and 34% could potentially benefit from being treated as D" (Vege et al., 2021).

VI. Guidelines and Recommendations

The AABB and The Molecular Testing Standards Committee (MT SC)

The second edition of the MT SC for red cell, platelet and neutrophil antigen guidelines states that "When a serological reagent is not available, molecular results shall be confirmed by another method (e.g., DNA sequencing) or an external laboratory" (MTSC, 2014). This committee is spearheaded by the AABB (formerly known as the American Association of Blood Banks).

American Society of Hematology (Jadhao et al.)

The ASH has published guidelines for sickle cell disease transfusion support. These guidelines give the following recommendations:

- "The ASH guideline panel suggests an extended red cell antigen profile by genotype or serology over only ABO/RhD typing for all patients with SCD (all genotypes) at the earliest opportunity (optimally before the first transfusion) (conditional recommendation based on very low certainty in the evidence about effects).
- The ASH guideline panel recommends prophylactic red cell antigen matching for Rh (C, E or C/c, E/e) and K antigens over only ABO/RhD matching for patients with SCD (all genotypes) receiving transfusions (strong recommendation based on moderate certainty in the evidence about effects" (Chou et al., 2020).

British Committee for Standards in Hematology (BCSH)

The BCSH has published guidelines on pre-transfusion compatibility procedures in blood transfusion laboratories. These guidelines state the following:

"If a patient shows signs of active haemolysis following transfusion but no antibodies are detectable and no other possible cause is known (e.g. sickle cell crisis or mechanical haemolysis due to heart valve failure), this can be due to very rare examples of antibodies which are not detectable by normal serological techniques. In this case:

i. Serological investigation should be undertaken using more sensitive techniques such as Polyethylene Glycol or enzyme IAT, which may require referral to a reference center.
ii. It may be appropriate to fully red cell genotype the patient and select donor blood matched as closely as possible.
iii. Advice should be sought from a clinical haematologist or from a reference laboratory at the local Blood Centre" (Milkins et al., 2013).

Regarding antibody screening, the BCSH states that "Antibody screening should always be performed as part of pre-transfusion testing as it provides the laboratory with a more reliable and sensitive method of detecting a red cell antibody than serological crossmatching" (Milkins et al., 2013).

For patients who have received a transfusion in the previous three months, the BCSH recommends:

i. "Phenotyping may be misleading due to the presence of transfused cells; In this case genotyping can be used as an alternative and is available from specialist laboratories.
ii. When an Rh antibody is suspected, the C, c, E, e types should be determined to aid in the selection of appropriately phenotyped red cells.
iii. Where antibody exclusion is problematic, more extensive phenotyping may be informative to identify antigens to which the patient may be alloimmunised" (Milkins et al., 2013).

For patients with autoimmune hemolytic anemia, BCSH guidelines state that the "Risk of transfusion reaction due to underlying alloantibodies can further be reduced by matching blood with the patient's own type. Determination of the phenotype in multiply transfused and/or DAT+ patients may be problematic and determination of genotype offers useful information in managing these complex cases; this particularly applies to regularly transfused cases and those with autoantibodies resistant to removal by adsorption" (Milkins et al., 2013).

Finally, for patients with sickle cell disease, the BCSH recommends the following:

"The patient's red cells should be phenotyped as fully as possible prior to transfusion. Where patients have already been transfused, the genotype can be determined.

i. An extended phenotype (or genotype) should include C, c, E, e, K, k, Jka, Jkb, Fya, Fyb, S, s.
ii. If S- s-, then U typing should be performed.
iii. As a minimum, red cells should be matched for Rh and K antigens" (Milkins et al., 2013).

The BCSH also published guidelines on red blood cell transfusion in sickle cell disease. The following recommendations are given for investigating a hemolytic transfusion reaction:

"Serological testing on pre-transfusion and post-transfusion blood samples.

- Repeat ABO/Rh D typing
- Check antibody screen on both samples
- Red cell units transfused within 12–24 h should be crossmatched against both the pre- and post-reaction samples
- Check the direct antiglobulin test (DAT). A positive DAT may be encountered as part of an investigation

Selection of red cell units for further transfusion

- Select ABO extended Rh and K matched units negative for the relevant antigen(s) to which there are current or historical antibodies
- Undertake serological crossmatch to check compatibility; electronic issue should not be used
- If the identity of the new alloantibody is in doubt despite further specialist testing, consider providing extended antigen matched blood (if serological phenotyping cannot be used because of the presence of transfused donor red blood cells, the sample should be sent to an appropriate reference laboratory for molecular red cell genotyping)" (Davis et al., 2017).

These guidelines later stated that "Fetal RHD typing using a high-throughput methodology in pregnant [individuals] who have not formed anti-D, as part of a screening program to target anti-D Ig prophylaxis, is sufficiently accurate for implementation from 11 weeks gestation. The cost effectiveness of such testing is dependent on provision at a point in the antenatal care pathway that does not necessitate an additional visit (Grade 1C)" (White et al., 2016).

Regarding serological methods, the guidelines state that samples from pregnant individuals "with immune anti-D or anti-c should be assessed serologically at 4 weekly intervals to 28 weeks gestation and at fortnightly intervals thereafter until delivery. Such cases should be referred to a fetal medicine specialist if the antibody reaches the critical level and/or the level is rising significantly, where assessment of the need for further monitoring will be made (Grade 1B)" and pregnant individuals "with anti-K or other Kell system antibodies (unless the father is confirmed to be negative for the corresponding antigen) should be assessed serologically at monthly intervals to 28 weeks gestation and at fortnightly intervals thereafter until delivery and referred to a fetal medicine specialist when the antibody is first identified (Grade 1B)" (White et al., 2016).

European Directorate for the Quality of Medicines & HealthCare (EDQM)

The EDQM has published a guide for the preparation, use and quality assurance of blood components. Regarding blood group testing for donor blood, it is stated that "Each donation should be tested for ABO and RhD blood groups and at least all first-time donors should be tested for clinically-significant irregular red-cell antibodies" and that "ABO and RhD blood groups should be verified on each subsequent donation" (EDQM, 2023). The guidelines also give laboratory testing steps for both serological and NAT testing.

The EDQM also recommends that serological testing is the standard procedure used in most laboratories and "molecular testing is becoming increasingly available and used as an alternate or supplemental technique to serological testing. In time, molecular

testing may replace the need for routine serological testing." Molecular testing should be performed when the results of serological testing of blood donors and patients are unclear, when there is a suspicion of weak antigens or variants, or when serological reagents directed to specific antigens do not exist or are not readily available. "It can also be useful in chronically transfused patients to predict their red cell phenotype and aid the selection of phenotyped or genotyped red cell components. Testing can be undertaken on samples from blood, amniocentesis, biopsy of chorionic villi, and plasma" (EDQM, 2023).

International Collaboration for Transfusion Medicine (ICTM)

The ICTM published red blood cell related guidelines for patients with hemoglobinopathies. These background of these guidelines state that "No international consensus exists for antigen matching in patients with SCD and b-thalassemia. Some transfusion medicine services provide preventive phenotype (or genotype) extensive matching for C, c, E, e, and K antigens in addition to routine ABO and D. Additional extended matching for Jka, Jkb, Fya, Fyb, and S, s is offered at some centers. In contrast, others provide ABO and D matched RBCs and switch to more extensive matching only if alloantibodies are detected" (Compernolle et al., 2018).

The following recommendations were given by the ICTM:

- "Patients with SCD who do not have alloantibodies and who are anticipated to have a transfusion (simple or exchange transfusion) should probably be transfused with CcEe and K-matched RBCs to reduce the risk of alloimmunization (low quality of evidence, weak recommendation). RBCs matched for CcEe and K can be provided by phenotyping or genotyping RBCs. The use of phenotyping or genotyping will depend on the costs of each method in each jurisdiction. Genotyping appears to be more accurate.
- Patients with thalassemia syndromes who do not have alloantibodies and who require RBC transfusion should probably be transfused with CcEe and K-matched RBCs to reduce the risk of alloimmunization (low quality of evidence, weak recommendation). RBCs matched for CcEe and K can be provided by phenotyping or genotyping RBCs. The use of phenotyping or genotyping will depend on the costs of each method in each jurisdiction. Genotyping appears to be more accurate" (Compernolle et al., 2018).

In 2020, the British Journal of Haematology released a position paper reviewing ICTMG guidelines and were in agreement with the above recommendations (Trompeter et al., 2020).

National Heart, Lung, and Blood Institute (NHLBI)

An expert panel from the NHLBI state that "RBC units that are to be transfused to individuals with SCD should include matching for C, E, and K antigens" (NHLBI, 2014). The laboratory method to perform the antigen matching is not mentioned in these guidelines.

Thalassemia International Federation (TIF)

The TIF published guidelines for the management of non-transfusion dependent thalassemia (NTDT) (Taher et al., 2018). These guidelines recommend that pregnant individuals "with NTDT who were previously never- or minimally-transfused, should be considered at high risk of alloimmunization if blood transfusions are to be administered during pregnancy. If blood transfusion is deemed necessary, extended genotype and antibody screening should be performed before giving any transfusion and fully-phenotyped matched blood should be given" (Taher et al., 2018).

The TIF also published guidelines for the management of transfusion dependent thalassemia (TDT) in 2014 which was updated in 2021 (Cappellini et al., 2014; TIF, 2021). These guidelines recommend the following for compatibility testing:

"Before embarking on transfusion therapy, patients should have extended red cell antigen typing that includes at least C, c, D, E, e, and Kell, (though preferably a full red cell phenotype/genotype panel) in order to help identify and characterize antibodies in case of later immunization.

If the patient is already transfused, antigen typing can be performed using molecular rather than serologic testing.

All patients with thalassaemia should be transfused with ABO and Rh(C, c, D, E, e) and Kell compatible blood in order to avoid alloimmunisation against these antigens.

There should be a valid group and antibody screen available prior to transfusion being administered.

At each transfusion, give ABO, Rh(D) compatible blood. Choosing units compatible units for ABO, C,c, E,e and Kell antigens is highly recommended.

Before each transfusion, perform a screen for new antibodies and an IAT cross-match, or in centres that meet regulatory requirements, perform an electronic cross-match where allowed" (TIF, 2021).

European Association of Urology (EAU)

The EAU updated their guidelines on renal transplantation in 2024. These guidelines include the following strong recommendations:

- "Determine the ABO blood group and the human leukocyte antigen A, B, C and DR phenotypes for all candidates awaiting kidney transplantation.
- Test both the donor and recipient for human leukocyte antigen DQ. Human leukocyte antigen DP testing may be performed for sensitised patients.
- Perform thorough testing for HLA antibodies before transplantation
- Perform adequate cross-match tests to avoid hyper-acute rejection, before each kidney and combined kidney/pancreas transplantation
- Prevent hyper-acute rejection by adequate ABO blood group and HLA matching of donor and recipients" (A. Breda, 2024).

The article also states that "compatibility for ABO blood group antigens and HLA antigens was of critical importance in kidney transplantation. This may change in the future, e.g. in the new U.S. allocation system A2 and A2B donors are transplanted into B recipients. To avoid an increasing imbalance between demand and supply in deceased-donor kidney transplantation in O recipients, ABO identity is demanded by several organ allocation organisations with a few exceptions, e.g. as in zero HLA-A+B+DR-mismatch kidneys. With the introduction of antibody elimination methods, potent immunosuppression and novel agents (e.g. anti B-cell drugs), successful ABO-incompatible living donor transplantations, with good long-term outcomes are possible" (A. Breda, 2024). ABO genotyping is not mentioned in these guidelines.

Kidney Disease Improving Global Outcomes (KDIGO)

The KDIGO published guidelines on the evaluation and care of living kidney donors. The following recommendations were given:

- "Donor ABO blood typing should be performed twice before donation to reduce the risk of unintended blood type incompatible transplantation.
- Human leukocyte antigen (Mandelbrot et al.) typing for major histocompatibility complex (MHC) Class I (A, B, C) and Class II (DP, DQ, DR) should be performed in donor candidates and their intended recipients, and donor-specific anti-HLA antibodies should be assessed in intended recipients
- Donor candidates who are ABO blood group or HLA incompatible with their intended recipient should be informed of availability, risks, and benefits of treatment options, including kidney paired donation and incompatibility management strategies" (Mandelbrot et al., 2020).

The guidelines also mention in a rationale section that "ABO blood typing should be performed in living donor candidates before donation, including routine duplicate testing, to reduce the risk of unintended blood type-incompatible transplantation. Unintended ABO-incompatible (ABOi) transplantation should be avoidable with ABO typing of the donor and the recipient; however, human errors have led to cases of

accidental ABOi organ transplantation in contemporary practice. ABO-subtype testing should be performed when donation is planned to recipients with anti-A antibodies" (Mandelbrot et al., 2020).

The authors later state that "While recipient care is out of the scope of this guideline, it is important to emphasize that recipient candidates should undergo antidonor antibody examinations, including complement-dependent cytotoxicity or flow cytometry crossmatching and Luminex (Bio-Rad Laboratories, Inc., Hercules, CA) assays to determine the history of sensitization, and this testing should be current before proceeding with donor nephrectomy and living donor transplantation" (Mandelbrot et al., 2020).

Eurotransplant Manual

Version 5.4 (Chapter 10) of the Eurotransplant Manual states that "In case of stem cell transplantation before organ transplantation, a new HLA typing and ABO blood group typing should be performed." The specific methodology is not mentioned (Eurotransplant, 2023).

National Marrow Donor Program (NMDP) and Center for International Blood and Marrow Transplant Research (CIBMTR)

The NMDP and CIBMTR have published guidelines on unreleated donors and cord blood units for HSCTs. These guidelines state that "Sufficient high-resolution donor-recipient HLA match is of primary importance in transplantation with adult unrelated donors"; further, "While HLA matching remains the primary criteria for donor selection, non-HLA factors are often considered when selecting donors, including cytomegalovirus serostatus, sex, age, ABO compatibility, prior pregnancies, and larger body weight" (Dehn et al., 2019).

American Red Cross

The American Red Cross published transfusion practice guidelines in 2021. They recommended RHD genotyping be used in individuals of childbearing potential to resolve serologic weak D phenotypes, or discrepant D types and that extended RBC phenotyping (i.e. C/c, E/e, K, Jka/Jkb, Fya/Fyb, M/N, and S/s) be performed routinely on all SCD patients by either serologic or molecular methods (Cross, 2021).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state

coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

The Immucor PreciseType Human Erythrocyte Antigen Molecular BeadChip Test

This test was approved by the FDA in 2014 and is an in vitro diagnostic test that is able to molecularly determine allele variants which assist in the prediction of erythrocyte antigen phenotypes (FDA, 2020). This test is known as the first FDA approved device for molecular typing of red blood cells.

ID CORE XT by Progenika Biopharma

This molecular based assay tests for non-ABO red blood cell types. This is a PCR hybridization-based genotyping test and can identify alleles which encode for human erythrocyte antigens in DNA and was approved by the FDA in 2018 (FDA, 2018).

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81403	Molecular pathology procedure, Level 4 (eg, analysis of single exon by DNA sequence analysis, analysis of >10 amplicons using multiplex PCR in 2 or more independent reactions, mutation scanning or duplication/deletion variants of 2-5 exons)
81479	Unlisted molecular pathology procedure
0001U	Red blood cell antigen typing, DNA, human erythrocyte antigen gene analysis of 35 antigens from 11 blood groups, utilizing whole blood, common RBC alleles reported Proprietary test: PreciseType® HEA Test Lab/Manufacturer: Immucor, Inc

CPT	Code Description
0084U	Red blood cell antigen typing, DNA, genotyping of 10 blood groups with phenotype prediction of 37 red blood cell antigens Proprietary test: BLOODchip® ID CORE XT™ Lab/Manufacturer: Grifols Diagnostic Solutions Inc.
0180U	Red cell antigen (ABO blood group) genotyping (ABO), gene analysis Sanger/chain termination/conventional sequencing, ABO (ABO, alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase) gene, including subtyping, 7 exons Proprietary test: Navigator ABO Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0181U	Red cell antigen (Colton blood group) genotyping (CO), gene analysis, AQP1 (aquaporin 1 [Colton blood group]) exon 1 Proprietary test: Navigator CO Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0182U	Red cell antigen (Cromer blood group) genotyping (CROM), gene analysis, CD55 (CD55 molecule [Cromer blood group]) exons 1-10 Proprietary test: Navigator CROM Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0183U	Red cell antigen (Diego blood group) genotyping (DI), gene analysis, SLC4A1 (solute carrier family 4 member 1 [Diego blood group]) exon 19 Proprietary test: Navigator DI Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0184U	Red cell antigen (Dombrock blood group) genotyping (DO), gene analysis, ART4 (ADP-ribosyltransferase 4 [Dombrock blood group]) exon 2 Proprietary test: Navigator DO Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0185U	Red cell antigen (H blood group) genotyping (FUT1), gene analysis, FUT1 (fucosyltransferase 1 [H blood group]) exon 4 Proprietary test: Navigator FUT1 Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0186U	Red cell antigen (H blood group) genotyping (FUT2), gene analysis, FUT2 (fucosyltransferase 2) exon 2 Proprietary test: Navigator FUT2 Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0187U	Red cell antigen (Duffy blood group) genotyping (FY), gene analysis, ACKR1 (atypical chemokine receptor 1 [Duffy blood group]) exons 1-2 Proprietary test: Navigator FY Sequencing Lab/Manufacturer: Grifols Immunohematology Center

CPT	Code Description
0188U	Red cell antigen (Gerbich blood group) genotyping (GE), gene analysis, GYPC (glycophorin C [Gerbich blood group]) exons 1-4 Proprietary test: Navigator GE Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0189U	Red cell antigen (MNS blood group) genotyping (GYPA), gene analysis, GYPA (glycophorin A [MNS blood group]) introns 1, 5, exon 2 Proprietary test: Navigator GYPA Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0190U	Red cell antigen (MNS blood group) genotyping (GYPB), gene analysis, GYPB (glycophorin B [MNS blood group]) introns 1, 5, pseudoexon 3 Proprietary test: Navigator GYPB Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0191U	Red cell antigen (Indian blood group) genotyping (IN), gene analysis, CD44 (CD44 molecule [Indian blood group]) exons 2, 3, 6 Proprietary test: Navigator IN Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0192U	Red cell antigen (Kidd blood group) genotyping (JK), gene analysis, SLC14A1 (solute carrier family 14 member 1 [Kidd blood group]) gene promoter, exon 9 Proprietary test: Navigator JK Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0193U	Red cell antigen (JR blood group) genotyping (JR), gene analysis, ABCG2 (ATP binding cassette subfamily G member 2 [Junior blood group]) exons 2-26 Proprietary test: Navigator JR Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0194U	Red cell antigen (Kell blood group) genotyping (KEL), gene analysis, KEL (Kell metallo-endopeptidase [Kell blood group]) exon 8 Proprietary test: Navigator KEL Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0195U	KLF1 (Kruppel-like factor 1), targeted sequencing (ie, exon 13) Proprietary test: Navigator KLF1 Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0196U	Red cell antigen (Lutheran blood group) genotyping (Boccoz et al.), gene analysis, BCAM (basal cell adhesion molecule [Lutheran blood group]) exon 3 Proprietary test: Navigator LU Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0197U	Red cell antigen (Landsteiner-Wiener blood group) genotyping (LW), gene analysis, ICAM4 (intercellular adhesion molecule 4 [Landsteiner-Wiener

CPT	Code Description
	blood group]) exon 1 Proprietary test: Navigator LW Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0198U	Red cell antigen (RH blood group) genotyping (RHD and RHCE), gene analysis Sanger/chain termination/conventional sequencing, RHD (Rh blood group D antigen) exons 1-10 and RHCE (Rh blood group CcEe antigens) exon 5 Proprietary test: Navigator RHD/CE Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0199U	Red cell antigen (Scianna blood group) genotyping (SC), gene analysis, ERMAP (erythroblast membrane associated protein [Scianna blood group]) exons 4, 12 Proprietary test: Navigator SC Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0200U	Red cell antigen (Kx blood group) genotyping (XK), gene analysis, XK (X-linked Kx blood group) exons 1-3 Proprietary test: Navigator XK Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0201U	Red cell antigen (Yt blood group) genotyping (YT), gene analysis, ACHE (acetylcholinesterase [Cartwright blood group]) exon 2 Proprietary test: Navigator YT Sequencing Lab/Manufacturer: Grifols Immunohematology Center
0221U	Red cell antigen (ABO blood group) genotyping (ABO), gene analysis, next-generation sequencing, ABO (ABO, alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase) gene Proprietary test: Navigator ABO Blood Group NGS Lab/Manufacturer: Grifols Immunohematology Center
0222U	Red cell antigen (RH blood group) genotyping (RHD and RHCE), gene analysis, next-generation sequencing, RH proximal promoter, exons 1-10, portions of introns 2-3 Proprietary test: Navigator Rh Blood Group NGS Lab/Manufacturer: Grifols Immunohematology Center
0246U	Red blood cell antigen typing, DNA, genotyping of at least 16 blood groups with phenotype prediction of at least 51 red blood cell antigens Proprietary test: PrecisionBlood™ Lab/Manufacturer: San Diego Blood Bank
0282U	Red blood cell antigen typing, DNA, genotyping of 12 blood group system genes to predict 44 red blood cell antigen phenotypes

CPT	Code Description
	Proprietary test: Versiti™ Red Cell Genotyping Panel Lab/Manufacturer: Versiti™ Comprehensive Bleeding Disorder Panel

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

A. Breda, K. B., A. Figueiredo, E. Lledó García, J. Olsburgh (Vice-chair), H. Regele R. Boissier, V. Hevia, O. Rodríguez Faba, R.H. Zakri C. Bezuidenhout. (2024). European Association of Urology Guidelines on Renal Transplantation. *Eur Urol Focus*. https://d56bochluxqnz.cloudfront.net/documents/full-guideline/EAU-Guidelines-on-Renal-Transplantation-2024.pdf

Agre, P., Smith, B. L., & Hartel-Schenk, S. (1990). Biochemistry of the erythrocyte Rh polypeptides: a review. *Yale J Biol Med*, *63*(5), 461-467. https://www.ncbi.nlm.nih.gov/pubmed/2127333

Anstee, D. J. (2009). Red cell genotyping and the future of pretransfusion testing. *Blood*, *114*(2), 248-256. https://doi.org/10.1182/blood-2008-11-146860

Belsito, A., Magnussen, K., & Napoli, C. (2017). Emerging strategies of blood group genotyping for patients with hemoglobinopathies. *Transfus Apher Sci*, *56*(2), 206-213. https://doi.org/10.1016/j.transci.2016.11.007

Bethesda, D. L. (2005). *Blood Groups and Red Cell Antigens* https://www.ncbi.nlm.nih.gov/books/NBK2267/

Boccoz, S. A., Le Goff, G., Blum, L. J., & Marquette, C. A. (2015). Microarrays in blood group genotyping. *Methods Mol Biol*, *1310*, 105-113. https://doi.org/10.1007/978-1-4939-2690-9_9

Booth, G. S., Gehrie, E. A., Bolan, C. D., & Savani, B. N. (2013). Clinical guide to ABO-incompatible allogeneic stem cell transplantation. *Biol Blood Marrow Transplant*, *19*(8), 1152-1158. https://doi.org/10.1016/j.bbmt.2013.03.018

Brancaleoni, V., Di Pierro, E., Motta, I., & Cappellini, M. D. (2016). Laboratory diagnosis of thalassemia. *Int J Lab Hematol*, *38 Suppl 1*, 32-40. https://doi.org/10.1111/ijlh.12527

Cappellini, M. D., Cohen, A., Porter, J., Taher, A., & Viprakasit, V. (2014). *Guidelines for the Management of Transfusion Dependent Thalassaemia (TDT)* Thalassaemia International Federation. https://thalassaemia.org.cy/wp-content/uploads/2017/08/GuidelinesTDT_English.pdf

Casas, J., Friedman, D. F., Jackson, T., Vege, S., Westhoff, C. M., & Chou, S. T. (2015). Changing practice: red blood cell typing by molecular methods for patients with sickle cell disease. *Transfusion*, *55*(6 Pt 2), 1388-1393. https://doi.org/10.1111/trf.12987

Chou, S. T., Alsawas, M., Fasano, R. M., Field, J. J., Hendrickson, J. E., Howard, J., Kameka, M., Kwiatkowski, J. L., Pirenne, F., Shi, P. A., Stowell, S. R., Thein, S. L., Westhoff, C. M., Wong, T. E., & Akl, E. A. (2020). American Society of Hematology 2020 guidelines for sickle cell disease: transfusion support. *Blood Adv*, *4*(2), 327-355. https://doi.org/10.1182/bloodadvances.2019001143

Compernolle, V., Chou, S. T., Tanael, S., Savage, W., Howard, J., Josephson, C. D., Odame, I., Hogan, C., Denomme, G., & Shehata, N. (2018). Red blood cell specifications for patients with hemoglobinopathies: a systematic review and guideline. *Transfusion*, *58*(6), 1555-1566. https://doi.org/10.1111/trf.14611

Cross, A. R. (2021). A Compendium of Transfusion Practice Guidelines. https://www.redcrossblood.org/content/dam/redcrossblood/rcb/biomedical-services/components/compendium_v_4.0.pdf

Davis, B. A., Allard, S., Qureshi, A., Porter, J. B., Pancham, S., Win, N., Cho, G., & Ryan, K. (2017). Guidelines on red cell transfusion in sickle cell disease. Part I: principles and laboratory aspects. *Br J Haematol*, *176*(2), 179-191. https://doi.org/10.1111/bjh.14346

Dehn, J., Spellman, S., Hurley, C. K., Shaw, B. E., Barker, J. N., Burns, L. J., Confer, D. L., Eapen, M., Fernandez-Vina, M., Hartzman, R., Maiers, M., Marino, S. R., Mueller, C., Perales, M. A., Rajalingam, R., & Pidala, J. (2019). Selection of unrelated donors and cord blood units for hematopoietic cell transplantation: guidelines from the NMDP/CIBMTR. *Blood*, *134*(12), 924-934. https://doi.org/10.1182/blood.2019001212

Dezan, M. R., Ribeiro, I. H., Oliveira, V. B., Vieira, J. B., Gomes, F. C., Franco, L. A. M., Varuzza, L., Ribeiro, R., Chinoca, K. Z., Levi, J. E., Krieger, J. E., Pereira, A. C., Gualandro, S. F. M., Rocha, V. G., Mendrone-Junior, A., Sabino, E. C., & Dinardo, C. L. (2017). RHD and RHCE genotyping by next-generation sequencing is an effective strategy to identify molecular variants within sickle cell disease patients. *Blood Cells Mol Dis*, *65*, 8-15. https://doi.org/10.1016/j.bcmd.2017.03.014

Dhawan, H. K., Kumawat, V., Marwaha, N., Sharma, R. R., Sachdev, S., Bansal, D., Marwaha, R. K., & Arora, S. (2014). Alloimmunization and autoimmunization in transfusion dependent thalassemia major patients: Study on 319 patients. *Asian J Transfus Sci*, *8*(2), 84-88. https://doi.org/10.4103/0973-6247.137438

EDQM. (2023). Guide to the preparation, use and quality assurance of BLOOD COMPONENTS. *21*. https://www.avis.it/wp-content/uploads/2023/06/Blood_Guide_21st_edistion.pdf

Elite. (2015). *RBC Genotyping - Elite Healthcare*. https://www.elitecme.com/resource-center/laboratory/rbc-genotyping/

Eurotransplant. (2023). Chapter 10 Histocompatibility Testing. *5.4*. https://www.eurotransplant.org/wp-content/uploads/2023/09/H10-Histocompatibility-202309.pdf

Fasano, R. M., & Chou, S. T. (2016). Red Blood Cell Antigen Genotyping for Sickle Cell Disease, Thalassemia, and Other Transfusion Complications. *Transfus Med Rev*, *30*(4), 197-201. https://doi.org/10.1016/j.tmrv.2016.05.011

Fasano, R. M., Sullivan, H. C., Bray, R. A., Gebel, H. M., Meyer, E. K., Winkler, A. M., Josephson, C. D., Stowell, S. R., Sandy Duncan, A., & Roback, J. D. (2017). Genotyping Applications for Transplantation and Transfusion Management: The Emory Experience. *Arch Pathol Lab Med*, *141*(3), 329-340. https://doi.org/10.5858/arpa.2016-0277-SA

FDA. (2018). *ID CORE XT (Reagents and Analysis Software)*. https://www.fda.gov/vaccines-blood-biologics/approved-blood-products/id-core-xt-reagents-and-analysis-software

FDA. (2020). *Immucor PreciseType Human Erythrocyte Antigen (HEA) Molecular BeadChip Test*. https://www.fda.gov/vaccines-blood-biologics/approved-blood-products/immucor-precisetype-human-erythrocyte-antigen-hea-molecular-beadchip-test

Flegel, W. A., Gottschall, J. L., & Denomme, G. A. (2015). Integration of red cell genotyping into the blood supply chain: a population-based study. *Lancet Haematol*, *2*(7), e282-289. https://doi.org/10.1016/s2352-3026(15)00090-3

Grifols. (2018). *FDA Approves Grifols ID CORE XT Test For Molecular Red Blood Cell Typing*. https://www.prnewswire.com/news-releases/fda-approves-grifols-id-core-xt-test-for-molecular-red-blood-cell-typing-300730173.html

Guzijan, G., Jovanovic Srzentic, S., Pavlovic Jankovic, N., Djilas, I., & Lilic, M. (2019). Implementation of Molecular RHD Typing at Two Blood Transfusion Institutes from Southeastern Europe. *Transfus Med Hemother*, *46*(2), 114-120. https://doi.org/10.1159/000496751

Harmening, D. M., Forneris, G., & Tubby, B. J. (2012). *Modern Blood Banking & Transufsion Practices* (Sixth ed.). F.A. Davis Company.

Hu, X., Zhang, Z., & Zeng, S. (2019). *ABO-incompatible Organ Transplantation*. https://link.springer.com/chapter/10.1007/978-981-13-3399-6_2

Immuncor. (2024). *PreciseTypeTM HEA (human erythrocyte antigen) - NEW Test of Record for Extended Blood Cell Antigen Typing.* https://www.immucor.com/en-us/Products/Pages/PreciseType-HEA.aspx

ISBT. (2023, 31 July 2023). *201 Table of blood group systems*. https://www.isbtweb.org/resource/tableofbloodgroupsystems.html

Jadhao, S., Davison, C. L., Roulis, E. V., Schoeman, E. M., Divate, M., Haring, M., Williams, C., Shankar, A. J., Lee, S., Pecheniuk, N. M., Irving, D. O., Hyland, C. A., Flower, R. L., & Nagaraj, S. H. (2022). RBCeq: A robust and scalable algorithm for accurate genetic blood typing. *eBioMedicine*, *76*, 103759. https://doi.org/https://doi.org/10.1016/j.ebiom.2021.103759

Kabat, E. (1956). *Blood Group Substances: Their Chemistry and Immunochemistry*. Academic Press Inc. Publishers.

Kahar, M. A., & Patel, R. D. (2014). Phenotype frequencies of blood group systems (Rh, Kell, Kidd, Duffy, MNS, P, Lewis, and Lutheran) in blood donors of south Gujarat, India. *Asian J Transfus Sci*, *8*(1), 51-55. https://doi.org/10.4103/0973-6247.126693

Krog, G. R., Rieneck, K., Clausen, F. B., Steffensen, R., & Dziegiel, M. H. (2019). Blood group genotyping of blood donors: validation of a highly accurate routine method. *Transfusion*, *59*(10), 3264-3274. https://doi.org/10.1111/trf.15474

Lambda, O. (2024). LinkSēq™ PCR Typing Kits. https://www.thermofisher.com/onelambda/us/en/pre-transplant/hla-typing/linkseq.html

Liu, Z., Liu, M., Mercado, T., Illoh, O., & Davey, R. (2014). Extended blood group molecular typing and next-generation sequencing. *Transfus Med Rev*, *28*(4), 177-186. https://doi.org/10.1016/j.tmrv.2014.08.003

Lopez, M., Apraiz, I., Rubia, M., Piedrabuena, M., Azkarate, M., Veldhuisen, B., Vesga, M. A., Van Der Schoot, E., Puente, F., & Tejedor, D. (2018). Performance evaluation study of ID CORE XT, a high throughput blood group genotyping platform. *Blood Transfus*, *16*(2), 193-199. https://doi.org/10.2450/2016.0146-16

Mandelbrot, D. A., Reese, P. P., Garg, N., Thomas, C. P., Rodrigue, J. R., Schinstock, C., Doshi, M., Cooper, M., Friedewald, J., Naik, A. S., Kaul, D. R., Ison, M. G., Rocco, M. V., Verbesey, J., Hladunewich, M. A., Ibrahim, H. N., & Poggio, E. D. (2020). KDOQI US Commentary on the 2017 KDIGO Clinical Practice Guideline on the Evaluation and Care of Living Kidney Donors. *Am J Kidney Dis*, *75*(3), 299-316. https://doi.org/10.1053/j.ajkd.2019.10.005

McBean, R. S., Hyland, C. A., & Flower, R. L. (2014). Approaches to determination of a full profile of blood group genotypes: single nucleotide variant mapping and massively parallel sequencing. *Comput Struct Biotechnol J*, *11*(19), 147-151. https://doi.org/10.1016/j.csbj.2014.09.009

Merz, L. E., & Achebe, M. (2021). When non-Whiteness becomes a condition. *Blood*, *137*(1), 13-15. https://doi.org/10.1182/blood.2020008600

Merz, L. E., Story, C. M., Osei, M. A., Jolley, K., Ren, S., Park, H. S., Yefidoff Freedman, R., Neuberg, D., Smeland-Wagman, R., Kaufman, R. M., & Achebe, M. O. (2023). Absolute neutrophil count by Duffy status among healthy Black and African American adults. *Blood Adv*, *7*(3), 317-320. https://doi.org/10.1182/bloodadvances.2022007679

Milkins, C., Berryman, J., Cantwell, C., Elliott, C., Haggas, R., Jones, J., Rowley, M., Williams, M., & Win, N. (2013). Guidelines for pre-transfusion compatibility procedures in blood transfusion laboratories. British Committee for Standards in Haematology. *Transfus Med*, *23*(1), 3-35. https://doi.org/10.1111/j.1365-3148.2012.01199.x

Miller, K. C., Gertz, M. A., Buadi, F. K., Hayman, S. R., Wolf, R. C., Lacy, M. Q., Dispenzieri, A. A., Dingli, D., Kapoor, P., Gonsalves, W. I., Kourelis, T., Hogan, W. J., & Kumar, S. K. (2019). Comparable outcomes using propylene glycol-free melphalan for autologous stem cell transplantation in multiple myeloma. *Bone Marrow Transplant*, *54*(4), 587-594. https://doi.org/10.1038/s41409-018-0302-6

MTSC. (2014). *Standards for Molecular Testing for Red Cell, Platelet and Neutrophil Antigens, 2nd edition*. https://www.aabb.org/sa/standards/Documents/sigchngmtstds2ed.pdf

Muro, T., Fujihara, J., Imamura, S., Nakamura, H., Kimura-Kataoka, K., Toga, T., Iida, R., Yasuda, T., & Takeshita, H. (2012). Determination of ABO genotypes by real-time PCR using allele-specific primers. *Leg Med (Tokyo)*, *14*(1), 47-50. https://doi.org/10.1016/j.legalmed.2011.10.002

NHLBI. (2014). *Evidence-Based Management of Sickle Cell Disease*. https://www.nhlbi.nih.gov/sites/default/files/media/docs/sickle-cell-disease-report%20020816_0.pdf

Osterman, J. L., & Arora, S. (2017). Blood Product Transfusions and Reactions. *Hematol Oncol Clin North Am*, *31*(6), 1159-1170. https://doi.org/10.1016/j.hoc.2017.08.014

Parkhideh, S., Chegeni, R., Mehdizadeh, M., Roshandel, E., Tavakoli, F., & Hajifathali, A. (2019). Effects of ABO incompatibility on the outcome of allogeneic hematopoietic stem cell transplantation. *Transfus Apher Sci*, 102696. https://doi.org/10.1016/j.transci.2019.102696

Passweg, J. R., Baldomero, H., Bader, P., Bonini, C., Cesaro, S., Dreger, P., Duarte, R. F., Dufour, C., Kuball, J., Farge-Bancel, D., Gennery, A., Kroger, N., Lanza, F., Nagler, A., Sureda, A., & Mohty, M. (2016). Hematopoietic stem cell transplantation in Europe 2014: more than 40 000 transplants annually. *Bone Marrow Transplant*, *51*(6), 786-792. https://doi.org/10.1038/bmt.2016.20

Písačka, M. (2018). After 70 years, serological RhD determination remains a challenge: DNA to the rescue. *ISBT Science Series*. https://onlinelibrary.wiley.com/doi/full/10.1111/voxs.12475

Quinn, C. T., & Berliner, N. (2023). *Gene test interpretation: ACKR1 (Duffy blood group gene),*. https://www.uptodate.com/contents/gene-test-interpretation-ackr1-duffy-blood-group-gene/print

Raud, L., Ferec, C., & Fichou, Y. (2017). From genetic variability to phenotypic expression of blood group systems. *Transfus Clin Biol*, *24*(4), 472-475. https://doi.org/10.1016/j.tracli.2017.06.011

Sapatnekar, S. (2015). *Molecular Typing for Red Blood Cell Antigens*. https://www.aacc.org/publications/cln/articles/2015/march/molecular-typing-for-red-blood-cell-antigens

Shah, A., Patel, P., Patel, K., Patel, B., Jariwala, K., Sharma, P., Mishra, K., & Ghosh, K. (2019). Comparison of serology and molecular detection of common red cell

antigens in multitransfused thalassemia major and sickle cell disease patients. *Transfus Apher Sci*, 102599. https://doi.org/10.1016/j.transci.2019.06.026

Shah, G. L., Landau, H., Sarubbi, C., Schofield, R., Lin, A., Bhatt, V., Harnicar, S., Devlin, S. M., Castro-Malaspina, H., Chung, D. J., Dahi, P. B., Gyurkocza, B., Koehne, G., Matasar, M. J., Moskowitz, C. H., Papadopoulos, E. B., Sauter, C. S., Shaffer, B. C., Tamari, R., . . . Giralt, S. A. (2018). Pharmacokinetics and Toxicities after Evomela® (Propylene Glycol Free Melphalan) with Hematopoietic Stem Cell Transplant (HCT) for Multiple Myeloma (MM), AL Amyloidosis (AL), Lymphoma, Acute Myeloid Leukemia (AML), and Myelodysplastic Syndrome (MDS). *Biology of Blood and Marrow Transplantation*, *24*(3), S79–S80. https://www.bbmt.org/article/S1083-8791(17)31563-X/abstract

Shankarkumar, U., Ghosh, K., & Mohanty, D. (2002). The human leucocyte antigen (HLA) system. *J Assoc Physicians India*, *50*, 916-926. http://www.krepublishers.com/02-Journals/IJHG/IJHG-04-0-000-000-2004-Web/IJHG-04-2-091-166-2004-Abst-PDF/IJHG-04-2-090-103-2004-Shankar/IJHG-04-2-091-103-2004-Shankar.pdf

Sheppard, C. A., Bolen, N. L., Meny, G., Kalvelage, M., & Ochoa-Garay, G. (2020). Concordance of two polymerase chain reaction-based blood group genotyping platforms for patients with sickle cell disease. *Immunohematology*, *36*(4), 123-128. https://www.ncbi.nlm.nih.gov/pubmed/33544616

Shih, A. W., Yan, M. T. S., Elahie, A. L., Barty, R. L., Liu, Y., Berardi, P., Azzam, M., Siddiqui, R., Parvizian, M. K., Mcdougall, T., Heddle, N. M., Al-Habsi, K. S., Goldman, M., Cote, J., Athale, U., & Verhovsek, M. M. (2020). Utilising red cell antigen genotyping and serological phenotyping in sickle cell disease patients to risk-stratify patients for alloimmunisation risk. *Transfusion Medicine*, *30*(4), 263-274. https://doi.org/https://doi.org/10.1111/tme.12685

Shokrgozar, N., & Tamaddon, G. (2018). ABO Blood Grouping Mismatch in Hematopoietic Stem Cell Transplantation and Clinical Guides. *Int J Hematol Oncol Stem Cell Res*, *12*(4), 322-328. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6375375/pdf/IJHOSCR-12-322.pdf

Sippert. (2019). Accuracy of Blood Group Typing in the Management and Prevention of Alloimmunization. *Human Blood Group Systems and Haemoglobinopathies*. https://www.intechopen.com/books/human-blood-group-systems-and-haemoglobinopathies/accuracy-of-blood-group-typing-in-the-management-and-prevention-of-alloimmunization

Strouse, J. (2016). Sickle cell disease. *Handb Clin Neurol*, *138*, 311-324. https://doi.org/10.1016/b978-0-12-802973-2.00018-5

Taher, A., Musallam, K., & Cappellini, M. D. (2018). In D. Weatherall (Ed.), *Guidelines for the Management of Non Transfusion Dependent Thalassaemia (NTDT) 2nd Edition*. Thalassaemia International Federation (c) 2013 Thalassaemia International Federation.

TIF. (2021). 2021 GUIDELINES FOR THE MANAGEMENT OF TRANSFUSION DEPENDENT THALASSAEMIA (TDT). https://www.thalassemia.org/boduw/wp-content/uploads/2021/06/TIF-2021-Guidelines-for-Mgmt-of-TDT.pdf

Trompeter, S., Massey, E., Robinson, S., & Committee, t. T. T. F. o. t. B. S. o. H. G. (2020). Position paper on International Collaboration for Transfusion Medicine (ICTM) Guideline 'Red blood cell specifications for patients with hemoglobinopathies: a systematic review and guideline'. *British Journal of Haematology*, *189*(3), 424-427. https://doi.org/https://doi.org/10.1111/bjh.16405

Uhl, L. (2023, Dec 14, 2023). *Pretransfusion testing for red blood cell transfusion* https://www.uptodate.com/contents/pretransfusion-testing-for-red-blood-cell-transfusion

Vege, S., Sprogøe, U., Lomas-Francis, C., Jakobsen, M. A., Antonsen, B., Aeschlimann, J., Yazer, M., & Westhoff, C. M. (2021). Impact of RHD genotyping on transfusion practice in Denmark and the United States and identification of novel RHD alleles. *Transfusion*, *61*(1), 256-265. https://doi.org/https://doi.org/10.1111/trf.16100

Wagner, F. F., Flegel, W. A., Bittner, R., & Doscher, A. (2017). Molecular typing for blood group antigens within 40 min by direct polymerase chain reaction from plasma or serum. *Br J Haematol*, *176*(5), 814-821. https://doi.org/10.1111/bjh.14469

Ware, R. E., de Montalembert, M., Tshilolo, L., & Abboud, M. R. (2017). Sickle cell disease. *Lancet*, *390*(10091), 311-323. https://doi.org/10.1016/s0140-6736(17)30193-9

Westhoff, C. M. (2006). Molecular testing for transfusion medicine. *Curr Opin Hematol*, *13*(6), 471-475. https://doi.org/10.1097/01.moh.0000245695.77758.3d

White, J., Qureshi, H., Massey, E., Needs, M., Byrne, G., Daniels, G., & Allard, S. (2016). Guideline for blood grouping and red cell antibody testing in pregnancy. *Transfus Med*, *26*(4), 246-263. https://doi.org/10.1111/tme.12299

Wilkinson, D. S. (2016). Clinical Utility of Genotyping Human Erythrocyte Antigens. *Lab Med*, *47*(3), e28-31. https://doi.org/10.1093/labmed/lmw014

Worel, N. (2016). ABO-Mismatched Allogeneic Hematopoietic Stem Cell Transplantation. *Transfus Med Hemother*, *43*(1), 3-12. https://doi.org/10.1159/000441507

Yaddanapudi, S., & Yaddanapudi, L. (2014). Indications for blood and blood product transfusion. *Indian J Anaesth*, *58*(5), 538-542. https://doi.org/10.4103/0019-5049.144648

Zhang, C., Zhu, J., Yang, J., Wan, Y., Ma, T., & Cui, Y. (2015). Determination of ABO blood group genotypes using the real-time loop-mediated isothermal amplification method. *Molecular Medicine Reports*, *12*, 5963–5966. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4581819/

Testing for Alpha-1 Antitrypsin Deficiency

Policy Number: AHS – M2068 – Testing for Alpha-1 Antitrypsin Deficiency	Prior Policy Name and Number, as applicable: • M2068 Genetic Testing for Alpha-1 Antitrypsin Deficiency
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Alpha 1-antitrypsin deficiency (AATD) is a genetic disease that causes deficient or defective production of the alpha-1 antitrypsin (AAT) protease inhibitor that can affect the lungs, liver, and skin (Stoller, 2024). AAT deficiency results in unbalanced rapid breakdown of proteins, especially in the supporting elastic tissue of the lungs (NORD, 2024a).

II. Related Policies

Policy Number	Policy Title
	Not applicable

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals who are suspected of having alpha-1 antitrypsin (AAT) deficiency, serum quantification of alpha-1 antitrypsin (AAT) protein **and** AAT phenotyping **or** AAT proteotyping **or** genetic testing for AAT deficiency (see Note 1) **MEETS COVERAGE CRITERIA** once per lifetime in **any** of the following situations:
   a) For symptomatic individuals 18 years or older with emphysema, COPD, or asthma.

b) For individuals with unexplained liver disease (e.g., chronic hepatitis with or without cirrhosis, chronically elevated aminotransferase levels, portal hypertension, primary liver cancer).

c) For individuals with persistent obstruction on pulmonary function tests without identifiable risk factors (e.g., cigarette smoking, occupational exposure).

d) For individuals 18 years or older with necrotizing panniculitis.

e) For the siblings of an individual with known alpha-1 antitrypsin (AAT) deficiency.

f) For individuals with anti-proteinase three-positive vasculitis (C-ANCA [anti-neutrophil cytoplasmic antibody]-positive vasculitis).

g) For individuals with bronchiectasis without evident etiology.

h) For individuals with neonatal cholestasis.

2) For individuals who have negative genotype results for common variants or who have discordant results between AAT serum levels and proteotype, but for whom a clinical suspicion of AAT deficiency remains, isoelectric focusing/phenotyping **MEETS COVERAGE CRITERIA**.

3) For individuals with a first-degree relative (see Note 2) with AAT deficiency, comprehensive *SERPINA1* gene sequencing **MEETS COVERAGE CRITERIA**.

4) For individuals with negative genotype testing for common variants **or** discordant results between AAT serum levels and proteotype or phenotype, but for whom the clinical suspicion of AAT deficiency remains, comprehensive *SERPINA1* gene sequencing **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

5) For all other situations not described above, testing for AAT deficiency **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: AAT phenotyping should be performed using isoelectric focusing. AAT proteotyping (Pi-typing or protease inhibitor typing) for Z and S alleles should be performed using liquid chromatography-tandem mass spectrometry. Initial genetic testing should be restricted to genotyping of *SERPINA1* S and Z allele mutations.

Note 2: First-degree relatives include parents, full siblings, and children of the individual.

IV. Table of Terminology

Term	Definition
A1AT	Alpha-1 antitrypsin
AAT	Aspartate aminotransferase
AATD	Alpha 1-antitrypsin deficiency
ACG	American College of Gastroenterology
ALT	Alanine transaminase
AST	Aspartate aminotransferase
ATS/ERS	American Thoracic Society/European Respiratory Society
C-ANCA	C-Anti-neutrophil cytoplasmic antibody
CMS	Centers for Medicare and Medicaid Services
COPD	Chronic obstructive pulmonary disorder
CTS	Canadian Thoracic Society
ERS	European Respiratory Society
GC	Gas chromatography
GOLD	Global Initiative for Chronic Obstructive Lung Disease
HPLC	High-performance liquid chromatography
LBV	Likely benign variants
LDTs	Laboratory developed tests
MALDI	Matrix-assisted laser desorption ionisation
MMP-12	*Matrix metalloproteinase-12* gene
MS-TOF	Time of flight mass spectrometry
NAFLD	Non-alcoholic fatty liver disease
NICE	National Institute Health and Care Excellence
NORD	National Organization for Rare Disorders
ON-CD	Ontario Center
PCR	Polymerase chain reaction
Pi	Protease inhibitor
PiMZ	Protease inhibitor Z allele
PV	Pathogenic variant
QTOF	Quadrupole time of flight
RCTs	Randomized controlled trials
RFLP	Restriction fragment length polymorphism
SERPINA1	Serine protease inhibitor
UMV	Undefined molecular variants
WHO	World Health Organization

V. Scientific Background

Alpha-1 antitrypsin deficiency (AATD) is an underrecognized genetic condition that affects approximately 1 in 2,000 to 1 in 5,000 individuals and predisposes to liver disease and early-onset emphysema (Stoller & Aboussouan, 2012). It is estimated (Campos et al., 2005) that up to 80,000 to 100,000 people in the United States have the severe form of the disease (homozygous in null or abnormal alleles). There is much variation in the disease prevalence in other nations (de Serres et al., 2007), but most current estimates are that three million people worldwide have severe AATD (Stoller, 2023).

Alpha-1 antitrypsin deficiency is a result of abnormal alpha-1 antitrypsin (AAT) protein inherited in an autosomal recessive pattern with codominant expression in which both genes inherited can be active and contribute to the genetic trait they control. AAT is a member of the serine protease inhibitor (Pi) family, referred to as "serpins", and it inhibits the proteolytic enzymes elastase, trypsin, chymotrypsin, and thrombin. AAT is encoded by the gene *SERPINA1* (Stoller, 2023).

The AAT protein is produced in the liver and has a role in protecting lungs from injury by neutrophil elastase, which is secreted by white blood cells as a response to inflammation or infection. If the enzyme remains unchecked by AAT protein, damage to alveoli resulting in chronic obstructive pulmonary disease can occur. This includes emphysema, asthma, bronchiectasis, and spontaneous pneumothorax. Smoking and other environmental exposure can cause further damage (Stoller, 2023, 2024).

Abnormal molecules of AAT protein caused by this illness can also cause liver dysfunction. Pathologic polymerization of the variant AAT can occur, resulting in intrahepatocyte accumulation of AAT molecules, leading to cirrhosis, fibrosis, cholestasis, or hepatomegaly. Liver disease is more common in individuals with certain allele combinations. Gender and obesity may be risk factors for progression to advanced liver disease in adulthood among patients with severe AAT deficiency. In contrast, alcohol use and viral hepatitis do not appear to increase the risk of progressive hepatic failure (Stoller, 2024). AATD is a common genetic cause of liver disease in children (de Serres et al., 2003).

Skin manifestations of AATD are also recognized. The most associated skin condition is necrotizing panniculitis. In this condition, inflammatory skin lesions are thought to be a consequence of the AAT protein loss of function and subsequent unchecked proteolysis enzyme activity in the skin and subcutaneous tissue. Associations between alpha-1 antitrypsin (AAT) and vascular disease, inflammatory bowel disease, glomerulonephritis, and vasculitis have been proposed but not definitively established (Stoller, 2024).

Due to the numerous alleles associated with AAT, each allele has been given a letter code based on the "electrophoretic mobility of the protein produced". The normal allele is the "M" allele, and the most common mutation is the "Z" allele. This system applies for each individual allele; for example, a homozygous Z genotype would be denoted as "ZZ". Similarly, a wildtype (or "normal") genotype would be "MM". Besides the normal phenotype, the three other categories of AAT include "deficient" in which insufficient AAT is produced; "null" in which no AAT is produced at all; and "dysfunctional" in which a typical amount of AAT is produced, but the AAT protein does not function correctly (Stoller, 2023).

Laboratory testing for AATD is comprised of three strategies: serum or plasma AAT quantification, AAT protein phenotyping, and genotyping. Guidelines from national and international societies (e.g., World Health Organization, Spanish Society of Pneumology and Thoracic Surgery, European Respiratory Society) recommend testing for AATD at least once per lifetime for all individuals with liver disease of unknown etiology and for all individuals with chronic obstructive pulmonary disorder (COPD), emphysema, or adults with asthma and irreversible airflow obstruction (Belmonte et al., 2020).

Proprietary Testing

Initial testing often begins with serum quantification of AAT protein. This can be done through several methods, including immune turbidimetry and nephelometry (Stoller, 2023). A low level is generally represented by a serum level below 11 micromol/L (less than 57 mg/dl using nephelometry). Due to the variation of reference ranges in different testing methodologies, most labs will complete isoelectric phenotyping on any individual with a serum AAT levels of < 100 mg/dL (18.4 micromol/L). In fact, the American Thoracic Society suggests persons with borderline serum levels (defined as 12-35 micromoles or 90 to 140 mg/dL) have qualitative testing (ATS/ERS, 2003).

Isoelectric immunophenotype testing uses the difference in migration rates of allele variants under isoelectric focusing. For example, the M variant will migrate to the middle of the gel, Z will migrate the slowest, and F migrates quickly to the side closest to the anode. This is not a genetic test. On occasion the results can be inconclusive or discordant with quantitative testing, requiring genotype testing of the most-common variants (Stoller, 2023).

Genotype testing for the most common allele variants can be utilized where isoelectric immunophenotype testing is inconclusive. Usually polymerase chain reaction (PCR) or restriction fragment length polymorphism (RFLP) techniques are utilized to determine if the most common alleles are present. When dealing with the possibility of a rare

variant or null allele, full gene sequencing can be utilized as a final diagnostic measure (Stoller, 2023).

In 2017, Grifols won FDA approval for AAT Deficiency Test, which is capable of simultaneously analyzing 99% of the most prevalent known mutations causing alpha-1 antitrypsin deficiency. The molecular test analyzes simultaneously 192 samples per kit, and in a single reaction, identifies 14 of the most prevalent known mutations in the SERPINA1 gene, responsible for this genetic disorder (Grifols, 2017b).

Using Progenika's FDA-cleared A1AT Genotyping Test, Matrix Clinical Labs released the proprietary Alpha ID screening test, a comprehensive targeted genetic test assessing 14 common and rare alleles in the *SERPINA1* gene. The Alpha ID screening test utilizes a noninvasive cheek swab screen. If a positive result is found using this test, a follow-up test, Alpha ID Confirm, uses a finger stick and a blood spot card to asses A1AT protein levels as well a potential reflex to next-generation sequencing (NGS) to help physicians achieve an accurate diagnosis of Alpha-1 antitrypsin deficiency (A1ATD) (AlphaID, 2024).

Clinical Utility and Validity

The literature on the analytic and clinical validity of genetic testing for AATD is limited. In addition, few randomized controlled trials (RCTs) have evaluated the impact of AATD testing on patient outcomes. Current evidence-based guidelines (GOLD, 2024) for diagnosis and management of AATD recommend specific interventions for patients with emphysema and AATD. AAT augmentation therapy is often prescribed for patients with AATD and COPD. In addition, several studies have documented that the disease is under-recognized with delay in diagnosis of between five to eight years (Barrecheguren et al., 2016; Stoller et al., 2005).

Snyder et al. (2006) evaluated the laboratory methods of assessing AATD. Samples from 512 individuals were analyzed, and "A1AT concentrations were measured by nephelometry. Phenotype analysis was performed by isoelectric focusing electrophoresis. The genotype assay detected the S and Z deficiency alleles by a melting curve analysis." Of these 512 samples, 10 (2%) were discordant between genotype and phenotype. Of these 10 results, seven were attributed to phenotyping errors. Four percent of the samples submitted to genotype and quantitative analysis were "reflexed" to phenotyping, where phenotyping confirmed the genotype result 85% of the time. The investigators concluded, "The combination of genotyping and quantification, with a reflex to phenotyping, is the optimal strategy for the laboratory evaluation of A1AT deficiency" (Snyder et al., 2006).

Sorroche et al. (2015) examined a cohort of COPD patients and the prevalence of severe AATD. A total of 1002 patients were evaluated, and 785 (78.34%) had normal

AAT levels. The remaining 217 patients had low AAT levels, but only 15 patients had a genotype associated with severe AATD. Of these 15 patients, 12 were ZZ and three were SZ. Of the 202 other patients, 29 were a Z heterozygote, 25 were an S heterozygote, and four were an SS homozygote. Lastly, 144 patients could not be definitively diagnosed (Sorroche et al., 2015).

Corda et al. (2011) examined the prevalence of AATD in a supposed "high-risk" area. A total of 817 residents participated, and 67 had low AAT serum levels. Overall, 118 residents carried AATD-related alleles, 114 of which were heterozygotes "(46 Z, 52 S, 9 P(brescia), 4 M(wurzburg), 2 I, 1 P(lowell)". The authors concluded, "the large number of mostly asymptomatic individuals with AATD identified suggests that in high-risk areas adult population screening programs employing the latest genetic methods are feasible" (Corda et al., 2011).

Soriano et al. (2018) evaluated the prevalence of AATD testing in COPD patients. The patient sample came from "550 UK Optimum Patient Care Research Database general practices". Out of 107,024 COPD patients, only 2.2% had any record of being tested for AATD. Of those tested, 23.7% were diagnosed with AATD. The investigators also noted that between 1994 and 2013, the incidence of AATD diagnosis increased. The authors concluded "that AATD remains markedly underdiagnosed in COPD patients" (Soriano et al., 2018).

Greulich et al. (2016) evaluated the results of a large, targeted screening program for AATD. The samples were distributed by a German AAT laboratory over a period of 12 years, and 18,638 testing kits were obtained. Of this sample, 6919 carried at least one mutation, and 1835 patients were considered to have severe AATD. Overall, 194 of these patients had "rare" genotypes. The authors concluded that "among clinical characteristics, a history of COPD, emphysema, and bronchiectasis were significant predictors for Pi*ZZ, whereas a history of asthma, cough and phlegm were predictors of not carrying the genotype Pi*ZZ" (Greulich et al., 2016).

Mattman et al. (2020) compared the comprehensiveness and efficiency of pathogenic variant (PV) detection of four different protocols from 2011 to 2018 in laboratories across Canada. From 5399 index patients, 396 ZZ genotypes were identified. The protocol for serum A1AT concentration/DNA sequencing in the Ontario center (ON-CD) yielded the highest PV detection – "genotypes with at least one PV, other than S, Z, or F, were identified at 0.67/ZZ as compared to <0.2/ZZ (all others)." However, it also had the highest rates of undefined molecular variants (UMV) (0.16/ZZ vs <0.12/ZZ) or likely benign variants (LBV) compared to all others (0.08/ZZ vs <0.06/ZZ). The authors concluded the "strategies with readily detect variants across the full coding sequence of *SERPINA1* detect more PV as well as more UMV and LBV" (Mattman et al., 2020).

Hamesch et al. (2019) evaluated the clinical landscape of liver symptoms in patients with AATD, specifically the Pi*ZZ genotype. A total of 554 patients (403 exploratory cohort, 151 confirmatory cohort) were included and were compared to 234 controls without pre-existing liver disease. The authors found significantly higher levels of serum liver enzymes in the Pi*ZZ carriers compared to controls, further noting that "significant' fibrosis was suspected in 20%-36% of Pi*ZZ carriers. Signs of advanced fibrosis were 9 to 20 times more common in carriers compared to non-carriers. Controlled attenuation parameter of ≥280 dB/m, which suggests "severe" steatosis was detected in 39% of carriers compared to 31% of controls. Finally, Pi*ZZ carriers were found to have lower serum concentrations of triglyceride, low, and very-low density lipoprotein cholesterol compared to controls, which the authors suggested to represent impaired hepatic secretion of liquid. Overall, the authors concluded that they identified evidence of liver steatosis, impaired liver secretion, liver fibrosis, and that their data could assist in hepatologic management of Pi*ZZ carriers (Hamesch et al., 2019).

Strnad et al. (2019) investigated the impact of the Pi*Z and Pi*S genotypes on subjects with non-alcoholic fatty liver disease (NAFLD) or alcohol misuse. Separate cohorts of 1184 with NAFLD and 2462 with chronic alcohol abuse were included. The authors found Pi*Z genotypes in 13.8% of patients with cirrhotic NAFLD but only 2.4% of patients without liver fibrosis. From there, the increased risk of NAFLD subjects to develop cirrhosis was found to be 7.3 times higher in Pi*Z carriers. The Pi*Z variant was also found in 6.2% of alcohol abusers but only 2.2% of alcohol abusers without significant liver injury. The increased risk was found to be 5.2 times higher in Pi*Z carriers. The Pi*S variant was not associated with NAFLD-related cirrhosis and only mildly with alcohol-related cirrhosis (increased risk = 1.47 times). The authors concluded that the Pi*Z variant was the strongest "single nucleotide polymorphism-based risk factor for cirrhosis in NAFLD and alcohol misuse, whereas the Pi*S variant confers only a weak risk in alcohol misusers" and remarked that this finding should be considered in future genetic counseling of affected individuals (Strnad et al., 2019).

Carreto et al. (2020) examined the utility of routine screening for AATD among patients with bronchiectasis, due to the contradiction in guidelines from the British Thoracic Society, which recommend screening for bronchiectasis among patients with AATD, but not vice versa. After screening 1600 patients with bronchiectasis from two centers in the UK from 2012-2016, they found only eight patients with AATD. They concluded that because of the low prevalence of AATD as an etiology for disease presentation among patients with bronchiectasis, routine screening for AATD would not significantly impact clinical management through augmentation therapy, smoking cessation, and genetic counselling, among other methods. Despite this, the researchers did note that

higher rates of detection may be found in other geographical regions in the UK or in other countries (Carreto et al., 2020).

In 2021, Murray et al. evaluated the efficacy of a liquid chromatography-tandem mass spectrometry (LC-MS/MS) (proteotyping)-based algorithm for AATD detection (n=5474), as compared to the more traditional isoelectric focusing (IEF) phenotyping (n=16147). Here, the authors found that LC-MS/MS reduced the rate of IEF) phenotyping by 97% and the 3% of cases that were reflexed to IEF resulted in and addition 0.2% of phenotype findings. By retrospectively applying the proteotype-based algorithm to the IEF cohort, they demonstrated a 99.9% sensitivity for the detection of deficiency-associated phenotypes. The authors concluded that the "proteotype algorithm is a sensitive and cost-effective approach for the diagnosis of clinical AAT deficiency" (Murray et al., 2021).

Bellemare et al. (2021) studied the clinical utility of determining the allelic background of mutations causing alpha-1 antitrypsin deficiency. *SERPINA1* was DNA sequenced to identify rare variants that could confer the risk of developing emphysema. Seven carriers of a rare variant, Leu353Phe_fsTer24, known to lead to undetectable serum levels of AAT, were studied using an allele-specific DNA sequencing method that they developed. Results demonstrated that Leu353Phe_fsTer24 variant was transmitted on the same allele as the M3 variant in all the patients and two of the seven patients had either a S or Z allele. The lowest AAT serum levels were observed in compound heterozygotes for the S or Z allele, suggesting higher risk of developing emphysema. This study showed that understanding the clinical significance of genetic variants found in SERPINA1 can lead to better clinical outcomes (Bellemare et al., 2021).

Ashenhurst et al. (2022) conducted a study to examine whether direct-to-consumer genetic testing increased the identification of previously undetected individuals specifically with AATD, and whether it impacted clinical care. In this cross-sectional study using a survey from the 23andMe, Inc. research platform with 195,014 participants, researchers found that the allele frequency of PI*S was 15.1%, 6.5% for PI*Z, and 0.63% with PI*ZZ. Half of those with the PI*ZZ allele combination were able to confirm their diagnosis with a physician. Twenty seven percent of the participants were first made aware of their disease status through this test, and among these participants, "the diagnostic delay interval was 22.3 years." As a result of this finding, there was a 1.7 times increased odds of reporting smoking reduction and 4.0 times increased odds of reporting reduced alcohol consumption. This demonstrates that having convenient methods of detecting pathogenic variants of the *SERPINA1* gene in commercial testing could benefit patients in the long run in terms of reducing risks of complications from AATD (Ashenhurst et al., 2022).

Balcar et al. (2022) studied the association between the alpha-1 antitrypsin Pi∗Z allele and liver disease. The study included 1118 patients with advanced chronic liver disease, all of whom had undergone genotyping for the Pi*Z/Pi*S allele. Compared to non-carriers, Pi∗Z carriers had more severe portal hypertension and hepatic dysfunction. "Harbouring the Pi∗Z allele was significantly associated with an increased probability of liver transplantation/liver-related death," but "the Pi∗S allele was unrelated to liver disease severity" and "Pi∗S carriers had no increased risk of events." The authors concluded that "genotyping for the Pi∗Z allele identifies patients with ACLD at increased risk of adverse liver-related outcomes, thereby improving prognostication" (Balcar et al., 2022).

Clark et al. (2018) studied the clinical and histologic features of individuals with AATD. The study included 94 non-cirrhotic adults with Pi*ZZ AATD. "The prevalence of clinically significant liver fibrosis (F ≥ 2) was 35.1%. Alanine aminotransferase, aspartate aminotransferase and gamma-glutamyltransferase values were higher in the F ≥ 2 group." Metabolic syndrome, the presence of accumulated abnormal AAT in hepatocytes, portal inflammation, and hepatocellular degeneration were all associated with clinically significant fibrosis. The authors concluded that "over one-third of asymptomatic and lung affected adults with 'PI*ZZ' AATD have significant underlying liver fibrosis" and that "liver disease in this genetic condition may be related to a "toxic gain of function" from accumulation of AAT in hepatocytes" (Clark et al., 2018).

Eriksson et al. (1986) studied the association between AATD and cirrhosis and primary liver cancer. The study included 17 autopsy cases of individuals with AATD. Each autopsy was matched with four control cases. "The results indicated a strong relation between alpha 1-antitrypsin deficiency and cirrhosis and primary liver cancer" (Eriksson et al., 1986).

Fromme et al. (2022) studied the association between AATD and hepatobiliary phenotypes. The study included 1104 participants (586 Pi*ZZ, 239 Pi*SZ, 279 non-carriers). "Pi*ZZ individuals displayed the highest liver enzyme values, the highest occurrence of liver fibrosis/cirrhosis and primary liver cancer… Pi*SZ participants displayed higher liver enzymes, more frequent liver fibrosis/cirrhosis… Subjects with Pi*MZ genotype had slightly elevated liver enzymes and moderately increased odds for liver fibrosis/cirrhosis…Individuals with homozygous Pi*S mutation (Pi*SS genotype) harboured minimally elevated alanine aminotransferase values." The authors concluded that their findings classify hepatobiliary phenotypes with their most relevant AATD genotypes (Fromme et al., 2022).

The Childhood Liver Disease Research Network Longitudinal Observational Study of Genetic Causes of Intrahepatic Cholestasis (Teckman et al., 2020) is a longitudinal study about pediatric cholestatic liver disease. The study included 350 participants ages zero

to 25 with native livers. Overall, 18 participants developed hypertension, and two died, but "there was no difference in participants with or without preceding neonatal cholestasis progressing to transplantation or death during the study, or in experiencing portal hypertension." The authors concluded that, in youth with AATD, "progression to liver transplantation is slow and death is rare, but the risk of complications and severe liver disease progression persists throughout childhood." The authors also note that "A history of neonatal cholestasis is a weak predictor of severe disease" (Teckman et al., 2020). Teckman et al. (2023) then focused on neonatal cholestasis in children with AATD, using two subgroups: participants with neonatal cholestasis (n=46), and all participants who progressed to livre transplant (n=119). The authors reported "an association of neonatal gamma-glutamyl transpeptidase elevation to more severe disease, and a higher rate of neonatal cholestasis progression to portal hypertension than previously reported (41%) occurring at median age of 5 months." All participants, regardless of neonatal cholestasis, were at risk of progression to liver transplant, but of the participants that progressed to liver transplant, those with neonatal cholestasis were significantly younger at transplant than those without neonatal cholestasis. The authors further concluded that "patients with AATD and neonatal cholestasis are at risk of early progression to severe liver disease, but the risk of severe disease extends throughout childhood" (Teckman et al., 2023).

Lin et al. (2019) completed a systematic literature review of AATD deficiency-associated liver disease in children and put together diagnostic testing recommendations. The literature review revealed that "liver disease occurs in 10% of children, manifested by cholestasis, pruritus, poor feeding, hepatomegaly, and splenomegaly, but the presentation is highly variable." The authors recommend genetic testing for AATD in children with unexplained liver disease or suspected AATD, noting "consensus guidelines recommend diagnostic testing for all patients who have unexplained liver disease" (level of evidence for recommendations: A, based on consistent and good quality patient-oriented evidence) (Lin et al., 2019).

VI. Guidelines and Recommendations

American Thoracic Society/European Respiratory Society (ATS/ERS)

The ATS/ERS released joint guidelines on the "Diagnosis and Management of Individuals with Alpha-1 Antitrypsin Deficiency." These recommendations are as follows (ATS/ERS, 2003):

Policy Guidelines

Recommendations were classified as follows:

Type A: Genetic testing is recommended

Type B: Genetic testing should be discussed and could be accepted or declined

Type C: Genetic testing is not recommended, i.e., should not be encouraged

Type D: Recommend against genetic testing, i.e., should be discouraged

Type A recommendations for diagnostic testing in the following situations:

1. Symptomatic adults with emphysema, COPD or asthma with airflow obstruction that is not completely reversible with aggressive treatment with bronchodilators
2. Individuals with unexplained liver disease, including neonates, children, and adults, particularly the elderly
3. Asymptomatic individuals with persistent obstruction on pulmonary function tests with identifiable risk factors (e.g., cigarette smoking, occupational exposure)
4. Adults with necrotizing panniculitis
5. Siblings of an individual with known alpha-1 antitrypsin (AAT) deficiency

Type B recommendations for diagnostic testing in the following situations:

1. Adults with bronchiectasis without evidence etiology
2. Adolescents with persistent airflow obstruction
3. Asymptomatic individuals with persistent airflow obstruction and no risk factors
4. Adults with C-ANCA positive (anti-proteinase 3-positive) vasculitis
5. Individuals with a family history of COPD or liver disease not known to be attributed to AAT deficiency
6. Distant relatives of an individual who is homozygous for AAT deficiency
7. Offspring or parents of an individual with homozygous AAT deficiency
8. Siblings, offspring, parents or distant relatives of an individual who is heterozygous for AAT deficiency
9. Individuals at high risk of having AAT deficiency-related diseases
10. Individuals who are not at risk themselves of having AAT deficiency but who are partners of individuals who are homozygous or heterozygous for AAT deficiency

Type C recommendations for diagnostic testing in the following situations

1. Adults with asthma in whom airflow obstruction is completely reversible
2. Predispositional testing
3. Population screening of smokers with normal spirometry

Type D recommendations for diagnostic testing in the following situations:

1. Predispositional fetal testing
2. Population screening of either neonates, adolescents or adults*

* Population screening is not recommended currently. However, a possible exception (type B recommendation) may apply in countries satisfying all three of the following conditions: (1) the prevalence of AAT deficiency is high (about 1/1,500, or more); (2) smoking is prevalent; and (3) adequate counseling services are available.

The following features should prompt suspicion by physicians that their patient may be more likely to have AAT deficiency:

Clinical Factors

- Early-onset emphysema (age of 45 years or less)
- Emphysema in the absence of a recognized risk factor (smoking, occupational dust exposure, etc.)
- Emphysema with prominent basilar hyperlucency
- Otherwise unexplained liver disease
- Necrotizing panniculitis
- Anti-proteinase three-positive vasculitis (C-ANCA [anti-neutrophil cytoplasmic antibody]-positive vasculitis)
- Bronchiectasis without evident etiology

The ATS/ERS also made statements on serum testing for AATD. “Serum phenotyping by isoelectric focusing performed by a reliable laboratory is the accepted “gold standard” for diagnosing AAT deficiency”. The guidelines recommend “that all subjects with COPD or asthma characterized by incompletely reversible airflow obstruction should be tested once for quantitative AAT determination. Also, individuals with evidence of cirrhosis of the liver with no known etiology should be tested for candidate phenotypes (e.g., PI*ZZ, PI*MZ, PI*Mmalton) and testing should be considered in individuals with the syndrome of Wegener's granulomatosis (antiproteinase-3 vasculitis)” (ATS/ERS, 2003).

The ATS/ERS states that “Regarding hepatic presentations of AAT deficiency later in childhood, during adolescence, and in adulthood, reports indicate that patients may present with hepatosplenomegaly, ascites, upper gastrointestinal bleeding resulting from esophageal varices, chronic hepatitis, cirrhosis, or hepatic failure. The presentation of AAT deficiency may appear similar to other chronic liver diseases, including autoimmune hepatitis, drug-induced hepatitis, chronic viral hepatitis, and Wilson's disease. The weight of these reports suggests that patients with any unexplained features of chronic liver disease should be evaluated for AAT deficiency” (ATS/ERS, 2003).

American College of Gastroenterology (ACG)

The ACG notes that "defective production of the alpha-1 anti-trypsin protein may result in both panacinar emphysema and chronic obstructive pulmonary disease, as well as progressive liver disease, liver cirrhosis, and hepatocellular carcinoma" and recommends the following for AATD:

- "Patients with persistently elevated aspartate aminotransferase (AST) or alanine aminotransferase (ALT) should undergo screening for alpha-1 antitrypsin (A1AT) deficiency with alpha-1 anti-trypsin phenotype."
- Evaluation of hepatocellular injury (defined by the guidelines as "disproportionate elevation of AST and ALT levels compared with alkaline phosphatase levels") includes testing for A1AT deficiency" (Kwo et al., 2017).

National Organization for Rare Disorders

NORD publishes information on multiple rare disorders that may otherwise lack national guidelines and recommendations. One such disorder is neonatal cholestasis, which refers to impaired flow of bile from the liver cells into the intestine of a newborn. While neonatal cholestasis may be caused by viruses, metabolic disease, or rare disease that affect or impair the function of the liver, it can also be caused by genetic disorders. "The incidence of neonatal cholestasis is estimated to be ~1:2500 live births worldwide, and 25% to 50% are now known to be associated with changes (variants or mutations) in specific genes" (NORD, 2024b). These genetic disorders include "alpha-1-antitrypsin deficiency, PFIC and Alagille syndrome" (NORD, 2024b).

World Health Organization (WHO)

The WHO released a memorandum on AATD regarding AATD's association with conditions such as COPD and asthma. Their recommendation is as follows: "It is therefore recommended that all patients with COPD and adults and adolescents with asthma be screened once for AAT deficiency using a quantitative test. Those with abnormal results on screening should undergo PI typing" (WHO, 1997).

European Respiratory Society (ERS)

The ERS (Miravitlles et al., 2017) published updated guidelines which recommend:

- "The quantitative determination of AAT levels in blood is a crucial first test to identify AATD. Quantitative deficiency must be supported by qualitative tests to identify the genetic mutation(s) causing AATD."
- "Protein phenotyping by isoelectric focusing identifies variants where AAT is present in the sample including the rarer variants F, I and P *etc*."

- "Genotyping allows a rapid and precise identification/exclusion of S and Z alleles and other variants, where specific primers are available."
- "Gene sequencing remains necessary for those cases where a null variant or a deficient variant other than Z or S is suspected."
- "Testing of relatives of identified patients should be considered after appropriate counselling.
- "Genetic testing should be carried out only after informed consent is given and in accordance with the relevant guidelines and legislation."

The ERS has also noted that "there is no evidence to support efficacy of AAT augmentation therapy in PiSZ, PiMZ or current smokers of any protein phenotype" (Miravitlles et al., 2017).

Alpha-1 Foundation

The Alpha-1 Foundation (Sandhaus et al., 2016) sponsored a medical and scientific advisory committee of experts to examine all relevant, recent literature to provide concise recommendations for the diagnosis and management of individuals with AATD.

- "For family testing after a proband is identified, AAT level testing alone is not recommended because it does not fully characterize disease risk from AATD."
- "For diagnostic testing of symptomatic individuals, they recommend genotyping for at least the S and Z alleles. Advanced or confirmatory testing should include Pi-typing, AAT level testing, and/or expanded genotyping."
- "All patients with COPD, unexplained chronic liver disease, necrotizing panniculitis, granulomatosis with polyangiitis, or unexplained bronchiectasis should be tested for AATD."
- "Parents, siblings, and children, as well as extended family of individuals identified with an abnormal gene for AAT, should be provided genetic counseling and offered testing for AATD (see guideline document for special considerations about testing minors)."

The Foundation also noted the following (these statements were not labeled recommendations):

- "For primary diagnosis of AATD the most sensitive and specific method of diagnosis is direct identification of the Z allele by genotyping. By also including the S allele, genotyping for the S and Z allele is greater than 99% specific and sensitive. "

- "AAT levels are insufficient to identify at risk individuals because the AAT level changes with inflammation, pregnancy, and in children. "
- "The range of serum AAT levels among individuals with specific genotypes is sufficiently broad that there is overlap between different genotypes. Thus, serum AAT levels cannot discriminate between different genotypes and additional AAT testing is needed" (Sandhaus et al., 2016).

Global Initiative for Chronic Obstructive Lung Disease (GOLD)

The GOLD guideline notes that "The most relevant (albeit rare) genetic risk factor for COPD identified to date are mutations in *SERPINA1* gene leading to α-1 antitrypsin deficiency. A number of other genetic variables have also been associated with reduced lung function and risk of COPD, but their individual effect size is small."(GOLD, 2024).

Canadian Thoracic Society (CTS)

The CTS released guidelines on genetic testing for AATD, which are as follows:

- "We suggest targeted testing for A1AT deficiency be considered in individuals with COPD diagnosed before 65 years of age or with a smoking history of <20 pack years. (Grade of recommendation: 2C)"
- "We suggest targeted testing for A1AT deficiency not be undertaken in individuals with bronchiectasis or asthma. (Grade of recommendation: 2C)" (Marciniuk et al., 2012)

National Institute Health and Care Excellence (NICE)

NICE published a guideline discussing chronic obstructive pulmonary disease (COPD) in 2019. In it, they note that measurement of serum alpha-1 antitrypsin has a role in identifying deficiencies if the condition is "early onset, [of] minimal smoking history, or [has] family history" (NICE, 2019).

Government of British Columbia

The British Columbia guidelines on the diagnosis and management of COPD state that, "testing for A1AT deficiency is expensive, low yield, often duplicated and may not alter management in a meaningful way. Therefore, refer patients with high pre-test probability to a specialist" (BC Guidelines, 2024).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations

(LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: http://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration

On November 17, 2017, the FDA approved Grifols' (Grifols, 2017a) *SERPINA1* Variant Detection System as a qualitative in vitro molecular diagnostic system used to detect variants in *SERPINA1* gene in genomic DNA isolated from human specimens. On November 7, 2019, the FDA approved Grifols' AlphaID™, a cheek swab that can screen patients with COPD for alpha-1 antitrypsin deficiency. It "utilizes an FDA-approved genotyping assay to screen for the 14 most prevalently reported genetic mutations associated with Alpha-1, including the S, Z, F, I alleles, as well as rare and null alleles, helping detect patients who are at risk for this treatable condition" (Grifols, 2019).

On April 6, 2017 the FDA approved (FDA, 2017) the 23andMe PGS Genetic Health Risk Report for Alpha-1 Antitrypsin Deficiency (AATD) which determines if a person has variants associated with a higher risk of developing AATD-associated lung or liver disease. This report is based on a qualitative genetic test for single nucleotide polymorphism detection of the PI*Z (rs28929474) and PI*S (rs17580) variants in the *SERPINA1* gene by using the 23andMe Personal Genome Service.

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81332	SERPINA1 (serpin peptidase inhibitor, clade A, alpha-1 antiproteinase, antitrypsin, member 1) (eg, alpha-1-antitrypsin deficiency), gene analysis, common variants (eg, *S and *Z)
81479	Unlisted molecular pathology procedure
82103	Alpha-1-antitrypsin; total
82104	Alpha-1-antitrypsin; phenotype

82542	Column chromatography, includes mass spectrometry, if performed (eg, HPLC, LC, LC/MS, LC/MS-MS, GC, GC/MS-MS, GC/MS, HPLC/MS), non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen
83789	Mass spectrometry and tandem mass spectrometry (eg, MS, MS/MS, MALDI, MS-TOF, QTOF), non-drug analyte(s) not elsewhere specified, qualitative or quantitative, each specimen

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

AlphaID. (2024). AlphaID. https://www.alphaid.com/en/hcp/home

Ashenhurst, J. R., Nhan, H., Shelton, J. F., Wu, S., Tung, J. Y., Elson, S. L., & Stoller, J. K. (2022). Prevalence of Alpha-1 Antitrypsin Deficiency, Self-Reported Behavior Change, and Health Care Engagement Among Direct-to-Consumer Recipients of a Personalized Genetic Risk Report. *Chest*, *161*(2), 373-381. https://doi.org/10.1016/j.chest.2021.09.041

ATS/ERS. (2003). American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. *Am J Respir Crit Care Med*, *168*(7), 818-900. https://doi.org/10.1164/rccm.168.7.818

Balcar, L., Scheiner, B., Urheu, M., Weinberger, P., Paternostro, R., Simbrunner, B., Hartl, L., Jachs, M., Bauer, D., Semmler, G., Willheim, C., Pinter, M., Ferenci, P., Trauner, M., Reiberger, T., Stättermayer, A. F., & Mandorfer, M. (2022). Alpha-1 antitrypsin Pi∗Z allele is an independent risk factor for liver transplantation and death in patients with advanced chronic liver disease. *JHEP Rep*, *4*(11), 100562. https://doi.org/10.1016/j.jhepr.2022.100562

Barrecheguren, M., Monteagudo, M., Simonet, P., Llor, C., Rodriguez, E., Ferrer, J., Esquinas, C., & Miravitlles, M. (2016). Diagnosis of alpha-1 antitrypsin deficiency: a population-based study. *Int J Chron Obstruct Pulmon Dis*, *11*, 999-1004. https://doi.org/10.2147/copd.s108505

BC Guidelines. (2024). Chronic Obstructive Pulmonary Disease (COPD): Diagnosis and Management. https://www2.gov.bc.ca/gov/content/health/practitioner-professional-resources/bc-guidelines/copd#diagnosis

Bellemare, J., Gaudreault, N., Valette, K., Belmonte, I., Nuñez, A., Miravitlles, M., Maltais, F., & Bossé, Y. (2021). The Clinical Utility of Determining the Allelic Background of Mutations Causing Alpha-1 Antitrypsin Deficiency: The Case with the Null Variant Q0(Mattawa)/Q0(Ourém). *Chronic Obstr Pulm Dis*, *8*(1), 31-40. https://doi.org/10.15326/jcopdf.8.1.2020.0168

Belmonte, I., Nunez, A., Barrecheguren, M., Esquinas, C., Pons, M., Lopez-Martinez, R. M., Ruiz, G., Blanco-Grau, A., Ferrer, R., Genesca, J., Miravitlles, M., & Rodriguez-Frias, F. (2020). Trends in Diagnosis of Alpha-1 Antitrypsin Deficiency Between 2015 and 2019 in a Reference Laboratory. *Int J Chron Obstruct Pulmon Dis*, *15*, 2421-2431. https://doi.org/10.2147/COPD.S269641

Campos, M. A., Wanner, A., Zhang, G., & Sandhaus, R. A. (2005). Trends in the diagnosis of symptomatic patients with alpha1-antitrypsin deficiency between 1968 and 2003. *Chest*, *128*(3), 1179-1186. https://doi.org/10.1378/chest.128.3.1179

Carreto, L., Morrison, M., Donovan, J., Finch, S., Tan, G. L., Fardon, T., Wilson, R., Furrie, E., Loebinger, M., & Chalmers, J. D. (2020). Utility of routine screening for alpha-1 antitrypsin deficiency in patients with bronchiectasis. *Thorax*, *75*(7), 592-593. https://doi.org/10.1136/thoraxjnl-2019-214195

Clark, V. C., Marek, G., Liu, C., Collinsworth, A., Shuster, J., Kurtz, T., Nolte, J., & Brantly, M. (2018). Clinical and histologic features of adults with alpha-1 antitrypsin deficiency in a non-cirrhotic cohort. *J Hepatol*, *69*(6), 1357-1364. https://doi.org/10.1016/j.jhep.2018.08.005

Corda, L., Medicina, D., La Piana, G. E., Bertella, E., Moretti, G., Bianchi, L., Pinelli, V., Savoldi, G., Baiardi, P., Facchetti, F., Gatta, N., Annesi-Maesano, I., & Balbi, B. (2011). Population genetic screening for alpha1-antitrypsin deficiency in a high-prevalence area. *Respiration*, *82*(5), 418-425. https://doi.org/10.1159/000325067

de Serres, F. J., Blanco, I., & Fernandez-Bustillo, E. (2003). Genetic epidemiology of alpha-1 antitrypsin deficiency in North America and Australia/New Zealand: Australia, Canada, New Zealand and the United States of America. *Clin Genet*, *64*(5), 382-397.

de Serres, F. J., Blanco, I., & Fernandez-Bustillo, E. (2007). PI S and PI Z alpha-1 antitrypsin deficiency worldwide. A review of existing genetic epidemiological data. *Monaldi Arch Chest Dis*, *67*(4), 184-208. https://doi.org/10.4081/monaldi.2007.476

Eriksson, S., Carlson, J., & Velez, R. (1986). Risk of cirrhosis and primary liver cancer in alpha 1-antitrypsin deficiency. *N Engl J Med*, *314*(12), 736-739. https://doi.org/10.1056/nejm198603203141202

FDA. (2017). *Decision Summary for 23andMe PGS Genetic Health Risk Report*. U.S. Food and Drug Administration Retrieved from https://www.accessdata.fda.gov/cdrh_docs/reviews/DEN160026.pdf

Fromme, M., Schneider, C. V., Pereira, V., Hamesch, K., Pons, M., Reichert, M. C., Benini, F., Ellis, P., K, H. T., Mandorfer, M., Burbaum, B., Woditsch, V., Chorostowska-Wynimko, J., Verbeek, J., Nevens, F., Genesca, J., Miravitlles, M., Nuñez, A., Schaefer, B., . . . Strnad, P. (2022). Hepatobiliary phenotypes of adults with alpha-1 antitrypsin deficiency. *Gut*, *71*(2), 415-423. https://doi.org/10.1136/gutjnl-2020-323729

GOLD. (2024). GLOBAL STRATEGY FOR PREVENTION, DIAGNOSIS AND MANAGEMENT OF COPD: 2024 Report. https://goldcopd.org/2024-gold-report/

Greulich, T., Nell, C., Herr, C., Vogelmeier, C., Kotke, V., Wiedmann, S., Wencker, M., Bals, R., & Koczulla, A. R. (2016). Results from a large targeted screening program for

alpha-1-antitrypsin deficiency: 2003 - 2015. *Orphanet J Rare Dis*, *11*(1), 75. https://doi.org/10.1186/s13023-016-0453-8

Grifols. (2017a). *FDA approval of genetic test for alpha-1 deficiency and EMA approval of fibrin sealant*. http://www.grifols.com/en/web/international/view-news/-/new/fda-approval-of-genetic-test-for-alpha-1-deficiency-and-ema-approval-of-fibrin-sealant

Grifols. (2017b). FDA approval of genetic test for alpha-1 deficiency and EMA approval of fibrin sealant. https://www.grifols.com/documents/3627767/3632483/np-20171117-en.pdf

Grifols. (2019, November 7). *Grifols introduces AlphaID™, a free cheek swab to screen for Alpha-1, the most common genetic form of COPD*. Retrieved April 11 from https://www.grifols.com/en/view-news/-/news/grifols-introduces-alphaid-a-free-cheek-swab-to-screen-for-alpha-1-the-most-common-genetic-form-of-copd

Hamesch, K., Mandorfer, M., Pereira, V. M., Moeller, L. S., Pons, M., Dolman, G. E., Reichert, M. C., Schneider, C. V., Woditsch, V., Voss, J., Lindhauer, C., Fromme, M., Spivak, I., Guldiken, N., Zhou, B., Arslanow, A., Schaefer, B., Zoller, H., Aigner, E., . . . Strnad, P. (2019). Liver Fibrosis and Metabolic Alterations in Adults With alpha-1-antitrypsin Deficiency Caused by the Pi*ZZ Mutation. *Gastroenterology*, *157*(3), 705-719.e718. https://doi.org/10.1053/j.gastro.2019.05.013

Kwo, P. Y., Cohen, S. M., & Lim, J. K. (2017). ACG Clinical Guideline: Evaluation of Abnormal Liver Chemistries. *Am J Gastroenterol*, *112*(1), 18-35. https://doi.org/10.1038/ajg.2016.517

Lin, H. C., Kasi, N., & Quiros, J. A. (2019). Alpha1-Antitrypsin Deficiency: Transition of Care for the Child With AAT Deficiency into Adulthood. *Curr Pediatr Rev*, *15*(1), 53-61. https://doi.org/10.2174/1573396314666181113094517

Marciniuk, D. D., Hernandez, P., Balter, M., Bourbeau, J., Chapman, K. R., Ford, G. T., Lauzon, J. L., Maltais, F., O'Donnell, D. E., Goodridge, D., Strange, C., Cave, A. J., Curren, K., & Muthuri, S. (2012). Alpha-1 antitrypsin deficiency targeted testing and augmentation therapy: a Canadian Thoracic Society clinical practice guideline. *Can Respir J*, *19*(2), 109-116. https://doi.org/10.1155/2012/920918

Mattman, A., Gilfix, B. M., Chen, S. X., DeMarco, M. L., Kyle, B. D., Parker, M. L., Agbor, T. A., Jung, B., Selvarajah, S., Barakauskas, V. E., Vaags, A. K., Estey, M. P., Nelson, T. N., & Speevak, M. D. (2020). Alpha-1-antitrypsin molecular testing in Canada: A seven year, multi-centre comparison. *Clin Biochem*, *81*, 27-33. https://doi.org/10.1016/j.clinbiochem.2020.05.001

Miravitlles, M., Dirksen, A., Ferrarotti, I., Koblizek, V., Lange, P., Mahadeva, R., McElvaney, N. G., Parr, D., Piitulainen, E., Roche, N., Stolk, J., Thabut, G., Turner, A., Vogelmeier, C., & Stockley, R. A. (2017). European Respiratory Society statement: diagnosis and treatment of pulmonary disease in alpha1-antitrypsin deficiency. *Eur Respir J*, *50*(5). https://doi.org/10.1183/13993003.00610-2017

Murray, J. D., Willrich, M. A., Krowka, M. J., Bobr, A., Murray, D. L., Halling, K. C., Graham, R. P., & Snyder, M. R. (2021). Liquid Chromatography-Tandem Mass Spectrometry-

Based alpha1-Antitrypsin (AAT) Testing. *Am J Clin Pathol*, *155*(4), 547-552. https://doi.org/10.1093/ajcp/aqaa149
NICE. (2019, July 26). *Chronic obstructive pulmonary disease in over 16s: diagnosis and management*. https://www.nice.org.uk/guidance/ng115/chapter/Recommendations
NORD. (2024a). Alpha-1 Antitrypsin Deficiency. https://rarediseases.org/rare-diseases/alpha-1-antitrypsin-deficiency/
NORD. (2024b, 01/25/2024). *Neonatal Cholestasis*. https://rarediseases.org/rare-diseases/idiopathic-neonatal-hepatitis/
Sandhaus, R. A., Turino, G., Brantly, M. L., Campos, M., Cross, C. E., Goodman, K., Hogarth, D. K., Knight, S. L., Stocks, J. M., Stoller, J. K., Strange, C., & Teckman, J. (2016). The Diagnosis and Management of Alpha-1 Antitrypsin Deficiency in the Adult. *Chronic Obstr Pulm Dis*, *3*(3), 668-682. https://doi.org/10.15326/jcopdf.3.3.2015.0182
Snyder, M. R., Katzmann, J. A., Butz, M. L., Wiley, C., Yang, P., Dawson, D. B., Halling, K. C., Highsmith, W. E., & Thibodeau, S. N. (2006). Diagnosis of alpha-1-antitrypsin deficiency: An algorithm of quantification, genotyping, and phenotyping. *Clin Chem*, *52*(12), 2236-2242. https://doi.org/10.1373/clinchem.2006.072991
Soriano, J. B., Lucas, S. J., Jones, R., Miravitlles, M., Carter, V., Small, I., Price, D., & Mahadeva, R. (2018). Trends of testing for and diagnosis of alpha1-antitrypsin deficiency in the UK: more testing is needed. *Eur Respir J*, *52*(1). https://doi.org/10.1183/13993003.00360-2018
Sorroche, P. B., Fernandez Acquier, M., Lopez Jove, O., Giugno, E., Pace, S., Livellara, B., Legal, S., Oyhamburu, J., & Saez, M. S. (2015). Alpha-1 Antitrypsin Deficiency in COPD Patients: A Cross-Sectional Study. *Arch Bronconeumol*, *51*(11), 539-543. https://doi.org/10.1016/j.arbres.2015.01.008
Stoller, J. (2023, September 13). *Clinical manifestations, diagnosis, and natural history of alpha-1 antitrypsin deficiency*. https://www.uptodate.com/contents/clinical-manifestations-diagnosis-and-natural-history-of-alpha-1-antitrypsin-deficiency
Stoller, J. (2024, July 6). *Extrapulmonary manifestations of alpha-1 antitrypsin deficiency*. https://www.uptodate.com/contents/extrapulmonary-manifestations-of-alpha-1-antitrypsin-deficiency
Stoller, J. K., & Aboussouan, L. S. (2012). A review of alpha1-antitrypsin deficiency. *Am J Respir Crit Care Med*, *185*(3), 246-259. https://doi.org/10.1164/rccm.201108-1428CI
Stoller, J. K., Sandhaus, R. A., Turino, G., Dickson, R., Rodgers, K., & Strange, C. (2005). Delay in diagnosis of alpha1-antitrypsin deficiency: a continuing problem. *Chest*, *128*(4), 1989-1994. https://doi.org/10.1378/chest.128.4.1989
Strnad, P., Buch, S., Hamesch, K., Fischer, J., Rosendahl, J., Schmelz, R., Brueckner, S., Brosch, M., Heimes, C. V., Woditsch, V., Scholten, D., Nischalke, H. D., Janciauskiene, S., Mandorfer, M., Trauner, M., Way, M. J., McQuillin, A., Reichert, M. C., Krawczyk, M., . . . Trautwein, C. (2019). Heterozygous carriage of the alpha1-antitrypsin Pi*Z variant increases the risk to develop liver cirrhosis. *Gut*, *68*(6), 1099-1107. https://doi.org/10.1136/gutjnl-2018-316228

Teckman, J., Rosenthal, P., Hawthorne, K., Spino, C., Bass, L. M., Murray, K. F., Kerkar, N., Magee, J. C., Karpen, S., Heubi, J. E., Molleston, J. P., Squires, R. H., Kamath, B. M., Guthery, S. L., Loomes, K. M., Sherker, A. H., & Sokol, R. J. (2020). Longitudinal Outcomes in Young Patients with Alpha-1-Antitrypsin Deficiency with Native Liver Reveal that Neonatal Cholestasis is a Poor Predictor of Future Portal Hypertension. *J Pediatr*, *227*, 81-86.e84. https://doi.org/10.1016/j.jpeds.2020.07.031

Teckman, J., Rosenthal, P., Ignacio, R. V., Spino, C., Bass, L. M., Horslen, S., Wang, K., Magee, J. C., Karpen, S., Asai, A., Molleston, J. P., Squires, R. H., Kamath, B. M., Guthery, S. L., Loomes, K. M., Shneider, B. L., & Sokol, R. J. (2023). Neonatal cholestasis in children with Alpha-1-AT deficiency is a risk for earlier severe liver disease with male predominance. *Hepatol Commun*, *7*(12). https://doi.org/10.1097/hc9.0000000000000345

WHO. (1997). Alpha 1-antitrypsin deficiency: memorandum from a WHO meeting. *Bull World Health Organ*, *75*(5), 397-415. https://www.ncbi.nlm.nih.gov/pubmed/9447774

Testing for Colorectal Cancer Management

Policy Number: AHS – M2026 – Testing for Colorectal Cancer Management	Prior Policy Name and Number, as applicable: AHS-M2026-*KRAS, NRAS* and *BRAF* Mutation Analysis in Colorectal Cancer AHS-M2026-*KRAS* and *BRAF* Mutation Analysis in Colorectal Cancer
Initial Policy Effective Date:01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Colorectal cancer (CRC) involves the accumulation of genetic and epigenetic modifications within pathways that regulate proliferation, apoptosis, and angiogenesis resulting in carcinoma of the colon and rectum (Bardhan & Liu, 2013). Tumors originate in adenomas or flat dysplasia and evolve into different morphologic patterns with invasion and expansion (Compton, 2023a).

Monoclonal antibodies that bind the epidermal growth factor receptor (*EGFR*), such as cetuximab, and block its activation have led to significant clinical benefits for metastatic colorectal cancer (mCRC) patients (De Roock et al., 2010). Mutations in downstream effectors of the *EGFR* pathway have been associated with resistance to *EGFR* antibody chemotherapies (Allegra et al., 2009; Compton, 2023b; Sepulveda et al., 2017).

For guidance on microsatellite instability or tumor mutational burden testing in colorectal cancer, please refer to AHS-M2178- Microsatellite Instability and Tumor Mutational Burden Testing.

II. Related Policies

Policy Number	Policy Title
AHS-G2181	Colorectal Cancer Screening

AHS-M2004	Lynch Syndrome
AHS-M2024	Genetic Testing for Polyposis Syndromes
AHS-M2029	Molecular Testing for Cutaneous Melanoma
AHS-M2178	Microsatellite Instability and Tumor Mutational Burden Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For all individuals with metastatic colorectal cancer, *KRAS, NRAS*, and *BRAF* mutation genotyping of the primary or the metastatic tumor **MEETS COVERAGE CRITERIA**.

2) For individuals with metastatic colorectal cancer for whom tumor tissue testing did not identify a mutation in *KRAS, NRAS*, **or** *BRAF*, HER2 amplification testing **MEETS COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

3) For all other situations not described above, testing for *KRAS, NRAS*, and *BRAF* mutations **DOES NOT MEET COVERAGE CRITERIA**.

4) For all other situations and/or mutations not described above, genotyping of the colorectal cancer tumor **DOES NOT MEET COVERAGE CRITERIA.**

5) To determine the prognosis of stage II colon cancer following surgery, gene expression profiling **DOES NOT MEET COVERAGE CRITERIA.**

NOTES:

Note: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162-Reimbursement Policy.

IV. Table of Terminology

Term	Definition
ASCO	American Society of Clinical Oncology
ACS	American Cancer Society
BRAF	*B-Raf proto-oncogene*

CEA	Carcinoembryonic antigen
CLIA-1988	Clinical Laboratory Improvement Amendments of 1988
CNA	Copy number alteration
CRC	Colorectal cancer
ctDNA	Circulating tumor deoxyribonucleic acid
DFS	Disease free survival
dMMR	Deficient MMR
EGAPP	Evaluation of Genomic Applications in Practice and Prevention
EGFR	*Epidermal growth factor receptor*
ERBB2	*Erb-B2 Receptor Tyrosine Kinase 2*
ESCP	European Society of Coloproctology
ESMO	European Society for Medical Oncology
EWG	European Working Group
1F1CDx	Foundation One Cdx
FDA	Food and Drug Administration
FFPE	Formalin-fixed paraffin-embedded
FISH	Fluorescence in-situ hybridization
FOLFOX4	5-fluorouracil, leucovorin, and oxaliplatin
HER2	Human epidermal growth factor receptor 2
HR	Hazard ratios
ICI	Immune checkpoint inhibition
IHC	Immunohistochemistry
KRAS	*Kirsten rat sarcoma*
LDTs	Laboratory-developed tests
mAbs	Monoclonal antibodies
mCRC	Metastatic colorectal cancer
MMR	Mismatch repair
MMR-P	Mismatch repair proficient
MPFS	Median progression free survival
MSI	Microsatellite instability
NCCN	National Comprehensive Cancer Network
NGS	Next-generation sequencing
NICE	National Institute for Health and Care Excellence
NRAS	*Neuroblastoma rat sarcoma virus*
NSABP	National Surgical Adjuvant Breast and Bowel Project
OR	Odds ratio
ORR	Objective response rate
OS	Overall survival

PCO	Provisional clinical opinion
PCR	Polymerase chain reaction
PFS	Progression-free survival rate
PIK3CA	*Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha*
PL	Plasma
PTEN	*Phosphatase and TENsin homolog deleted on chromosome 10*
QALY	Quality-adjusted life year
RAF	*Rapidly accelerated fibrosarcoma*
RAS	*Rat sarcoma virus*
RS	Recurrence score
T	Tissue
TMB	Tumor mutational burden
WT	Wild type

V. Scientific Background

Colorectal cancer (CRC) is the third leading cause of cancer-related deaths in the United States following lung cancer. The American Cancer Society (ACS) estimates 106,180 new cases of colon cancer and 44,850 new cases of rectal cancer for 2022. Overall, there is about a four percent lifetime risk of developing colorectal cancer (ACS, 2023). Metastatic colorectal cancer (mCRC), which occurs in 22% of patients with colorectal cancer, has a significantly poorer prognosis than colorectal cancer that hasn't metastasized. The five-year survival is 14% in patients with distant metastases from CRC, as compared to 71% for all CRC patients (El-Deiry et al., 2015; Wang et al., 2020).

Approximately one-quarter of the patients with colon cancer present with stage II disease (Kopetz, 2008). The current National Comprehensive Cancer Network (NCCN) guidelines include adjuvant chemotherapy as a treatment option in this setting, particularly for high-risk stage II patients, as determined by clinical and pathological parameters (NCCN, 2024). Although some of the routinely used parameters for estimating recurrence risk, such as T-stage and mismatch repair (MMR) status, are well established, they may not be reliable predictors of recurrence risk in this population (Gray et al., 2011; Gunderson et al., 2010; Harris et al., 2008; Ribic et al., 2003; Sargent et al., 2010; Venook et al., 2013).

Certain mutations may affect treatment of CRC. For example, the activation of the epidermal growth factor receptor (*EGFR*) signaling cascade is associated with colon tumorigenesis (Therkildsen et al., 2014); therefore, medications such as cetuximab or panitumumab that target the *EGFR* pathway may be used in treatment of CRC.

However, activating mutations in the *KRAS* oncogene will cause anti-*EGFR* resistance since these mutations can result in a constitutively active pathway, even with anti-*EGFR* treatment (Clark & Sanoff, 2024). Consequently, tumors with mutated *KRAS* are unresponsive to anti-*EGFR* therapy. As a result, testing for mutational status as a negative predictive factor for anti-*EGFR* therapy has become part of routine pathological evaluation for CRC. Other mutations in the RAS oncogene (primarily *NRAS*) may also lead to the same phenotype (Frucht & Lucas, 2023). Another gene that may be overexpressed within the *EGFR* pathway is *HER2* (human epidermal growth factor receptor 2). This gene plays a role in activating signal transduction pathways controlling epithelial cell growth. Although *HER2* is more traditionally known as a breast cancer-associated gene, up to five percent of colorectal cancer cases are found to overexpress *HER2* (Clark & Sanoff, 2024).

Another component of the RAS signaling pathway, *BRAF*, has also been found to affect anti-*EGFR* treatment. *BRAF* V600E mutations may also confer a lack of response to anti-*EGFR* treatment even when paired with a wild-type RAS oncogene. Mutations in this region occur in less than 10% of sporadic CRCs, and the mutation at position 600 is the primary polymorphism found in CRC. Non-V600 *BRAF* mutations are rarer (composing about 2.2% of patients with metastatic CRC) and confer a generally better prognosis than their V600 mutated counterparts; a study found non-V600 genotypes to lead to better median overall survival and fewer high-grade tumors (Jones et al., 2017).

Proprietary Testing

Gene expression assays have been commercially produced to predict prognosis of colon cancer. The 12-gene Oncotype DX Colon Cancer Assay (Genomic Health, Inc., Redwood City, CA) is a reverse transcriptase polymerase chain reaction–based assay that provides a Recurrence Score (RS) result (O'Connell et al., 2010). This test assesses the activity level of 12 genes (7 cancer-related genes, 5 reference genes), and this gene expression is scored from 1-100. This test is intended for resected stage II, MMR-P or stage III A/B colon cancer. Low risk is a score under 30, moderate risk is 31-40, and higher risk is ≥41 (Oncotype, 2024a, 2024b).

The ColDx assay (Almac Diagnostics, Craigavon, Northern Ireland) uses microarray technology for assessing the gene expression of 634 genes to stratify patients into low and high recurrence risk groups (Almac Group, 2024). ColDx identified 73 high risk patients with a hazard ratio of 2.62 during cross validation. In an independent validation, the assay identified high-risk patients with a hazard ratio of 2.53 (Kennedy et al., 2011).

ColoPrint (Agendia, Amsterdam, The Netherlands) is a gene expression classifier that uses whole-genome expression data of 18 key genes to distinguish patients with low

versus high risk of disease relapse. In a study using 206 fresh frozen tumor tissue samples from 188 patients with stage I through IV CRC, ColoPrint classified "60% of patients as low risk and 40% as high risk," and was "superior to American Society of Clinical Oncology criteria in assessing the risk of cancer recurrence without prescreening for microsatellite instability" (Salazar et al., 2011). In a study of 416 stage II colon cancer patients, "ColoPrint identified 63% of patients as low risk with a 5-year ROR of 10%, whereas high-risk patients (37%) had a 5-year ROR of 21%." Alternatively, the 2013 NCCN clinical risk factors could not distinguish low and high risk patients (Kopetz et al., 2015).

Analytical Validity

Cenaj et al. (2019) evaluated the correlation between "*ERBB2* amplification by next-generation sequencing (NGS) with HER2 overexpression by immunohistochemistry." NGS was performed on specimens with 20% or more tumor, and 1300 cases of colorectal cancer were included. *ERBB2* amplification was detected in 2% of cases. HER2 amplification was examined in "15 cases with *ERBB2* amplification (six or more copies), 10 with low gain (three to five copies), and 77 copy neutral." *ERBB2* amplification was found to have perfect concordance with HER2 immunochemistry at H-scores of 105 or more. Further, *ERBB2* amplification was found to inversely correlate with RAS/RAF mutations. The authors concluded that "NGS-detected *ERBB2* amplification highly correlates with HER2 overexpression in CRC," which may support authors' original hypothesis that ERBB2 amplification/overexpression may predict response to HER2 inhibitors (Cenaj et al., 2019).

Fan et al. (2021) analyzed the relationship between mismatch repair (MMR) protein, *RAS*, *BRAF,* and *PIK3CA* expression and clinicopathological characteristics in elderly patients with CRC. From 327 patients, the researchers found that "the mutation rates of the *KRAS, NRAS, BRAF* and *PIK3CA* genes in elderly CRC patients were 44.95% (147/327), 2.45% (8/327), 3.36% (11/327) and 2.75% (9/327), respectively." They also identified that "*KRAS* was closely related to tumor morphology ($P = 0.002$) but not to other clinicopathological features ($P > 0.05$), and there were no significant differences between *NRAS* gene mutation and clinicopathological features ($P > 0.05$).
The *BRAF* gene mutation showed a significant difference in pathological type, tumor location, differentiation degree and lymph node metastasis ($P < 0.05$), but was not correlated with sex, tumor size and tumor morphology ($P > 0.05$)" (Fan et al., 2021). This demonstrates the critical nature of mutation analysis for these specific genes to aid in identifying potential therapies that would better patient prognoses especially in such a vulnerable population like the elderly.

Formica et al. (2020) examined tumor tissue (T) mutational analysis in terms of discordance with circulating tumor DNA (ctDNA) obtained by liquid biopsy from plasma (PL) and assessed through real time polymerase chain reaction (PCR). Despite finding concordance for patients with *BRAF* mutations between the tissue and plasma samples, 20% of patients were *RAS* discordant. Mutations identified from ctDNA were able to refine the prognosis determined by tissue samples. "*RAS* wild type in T and mutated in PL had significantly shorter PFS than concordant *RAS* wild type in T and PL: mPFS [median progression free survival] 9.6 vs. 23.3 months, respectively, $p = 0.02$. Patients RAS mutated in T and wild type in PL had longer PFS than concordant RAS mutated in T and PL: 24.4 vs. 7.8 months, respectively, $p = 0.008$." This raises a limitation to using tumor tissue as the mainstay for mutational analysis and considering combining with or replacing tumor tissue genotyping with plasma ctDNA as a measure of prognosis going forward (Formica et al., 2020).

Pinheiro et al. (2022) studied the analytical validity of using ctDNA as a possible strategy to analyze *KRAS* and *NRAS* mutations from patients with metastatic colorectal cancer. The BEAMing Digital PCR (OncoBEAM) and Idylla ctDNA qPCR were compared and the concordance rate was reported. Blood samples from 47 mCRC patients were tested and the overall agreement and concordance rate were noted. "The overall agreement between tumor tissue and ctDNA analyses was 83% and 78.7% using the OncoBEAM and Idylla assays, respectively, with the concordance being 96.2% and 88.5% in naive treatment patients. The overall agreement between OncoBEAM and Idylla ctDNA analyses was 91.7%" (Pinheiro et al., 2022). The authors conclude that Idylla ctDNA qPCR method is a cheaper alternative with equivalent performance in comparison to the OncoBEAM assay. Analysis of ctDNA can be used to detect "RAS mutations in plasma, either at diagnosis or after progression when considering anti-EGFR treatment rechallenge" (Pinheiro et al., 2022).

Clinical Utility and Validity

In a meta-analysis by Xu et al. (2013), a total of 2875 patients were evaluated, with 246 patients having *BRAF* mutations. The objective response rate (ORR) to *EGFR* therapy was 18.4% (40/217) in mutant *BRAF* group and 41.7% (831/1993) in the wild-type *BRAF* group. The overall risk ratio for the ORR of *BRAF* mutations compared to wild-type *BRAF* patients was 0.58. The median progression free survival (hazard ratio 2.98) and overall survival (hazard ratio: 2.85) were significantly shorter of patients with *BRAF* mutations compared to patients with wild-type *BRAF* mutations (Xu et al., 2013).

Douillard et al. (2013) evaluated the effect of panitumumab plus oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) compared to just FOLFOX4 on patients with varying RAS and *BRAF* mutations. A total of 639 patients with metastatic CRC without

mutations in *KRAS* exon 2 had at least one of the following: *KRAS* exon 3 or 4; *NRAS* exon 2, 3, or 4; or *BRAF* exon 15. 228 patients had neither RAS nor *BRAF* mutations, and this group was evaluated to have better survival metrics with panitumumab plus FOLFOX4 than the group with just FOLFOX4 (median of 10.8 months progression-free survival and 28.3 months overall survival for panitumumab group vs 9.2 and 20.9 respectively for the group without). However, 296 patients with either a *RAS* or *BRAF* mutation were treated with panitumumab plus FOLFOX4, and this group's survival metrics were lower than the group only treated with FOLFOX4. The *RAS*/*BRAF* group treated with panitumumab plus FOLFOX4 had a median of only 7.3 months progression-free survival and 15.3 months overall survival vs 8.0 and 18.0 for the 305 patients treated with only FOLFOX4). The authors concluded that additional RAS mutations predicted a lack of response to panitumumab plus FOLFOX4 (Douillard et al., 2013).

Therkildsen et al. (2014) performed a meta-analysis of the clinical impact of anti-*EGFR* treatment on patients with *KRAS*, *NRAS*, and *BRAF* mutations (as well as *PIK3CA* and *PTEN*). A total of 22 studies (2395 participants) were evaluated. Odds ratios for objective response rate (ORR) and hazard ratios (HR) for progression-free survival rate (PFS) and overall survival (OS) were calculated. Mutations in *KRAS* exons 3 and 4 and *BRAF* predicted poor ORR (0.26 and 0.29 respectively), *KRAS*, *NRAS*, and *BRAF* mutations all led to significantly lower progression-free survival (HR = 2.19, 2.30, and 2.95 respectively) and significantly lower overall survival (HR = 1.78, 1.85, and 2.52 respectively) (Therkildsen et al., 2014).

Rebersek et al. (2019) investigated the impact of molecular biomarkers on survival and response to first line therapy in metastatic colorectal cancer patients. The study included 154 patients with 42% harboring *KRAS* mutations and 3% harboring *BRAF* mutations. Median overall survival (OS) was found to be 56.5 months for wild-type *KRAS* patients and 58 months for mutated *KRAS* patients. Median OS for mutated exon 12 patients was 57 months compared to 44 months for mutated exon 13 patients. Wild-type *KRAS* was found to affect the response to first-line systemic therapy, whereas no other parameters were found to affect response (Rebersek et al., 2019).

Sartore-Bianchi et al. (2019) investigated the effect of *HER2* positivity on anti-*EGFR* treatment. A total of 100 patients HER2-positive (of 1485 wild-type *KRAS* exon 2 patients) with metastatic colorectal cancer were included. The authors found that HER2-positive patients had more frequent lung metastases (odds ratio [OR] = 2.04) and higher tumor burden (OR = 1.48). The 79 *HER2*-positive patients given anti-*EGFR* treatment were also found to have poorer clinical outcomes, with lower objective response rate (31.2% compared to 46.9% for all others) and lower progression-free survival (5.7 months vs seven months). The authors concluded that HER2 testing

should be offered because “occurrence of this biomarker is unlikely to be predicted based on main clinicopathological features” (Sartore-Bianchi et al., 2019).

The prognostic benefit was corroborated by Chang et al. (2021), who found that the *BRAF* gene mutation was “associated with cancer thrombosis in blood vessels” and was “negatively correlated with the OS [overall survival] rate of CRC patients” in their patient population (n=410) from Central China. Like Fan et al. (2021), *KRAS* also had the greatest mutation rate at 47.56% in this study, showing more awareness needed for tissue genotyping for mCRC (Chang et al., 2021).

Loree et al. (2021) characterized the clinical prevalence of atypical *KRAS/NRAS* mutations in metastatic colorectal cancer. The authors evaluated tissue and DNA samples from 9,485 patients to characterize atypical *RAS* variants using an in-vitro cell-based assay, studying the signaling changes across mutations. According to the results, "*KRAS* exon 2, extended *RAS*, and atypical *RAS* mutations were noted in 37.8%, 9.5%, and 1.2% of patients, respectively. Among atypical variants, *KRAS* L19F, Q22K, and D33E occurred at prevalence ≥0.1%, whereas no *NRAS* codon 117/146 and only one *NRAS* codon 59 mutation was noted. Atypical *RAS* mutations had worse overall survival than *RAS/BRAF* wild-type mCRC.” Of the 57 atypical *RAS* variants, 18 (31.6%) had signaling below wild-type, 23 (40.4%) had signaling between wild-type and activating control, and 16 (28.1%) were hyperactive beyond the activating control. The authors concluded that "*KRAS* L19F, Q22K, D33E, and T50I are more prevalent than many guideline-included RAS variants and functionally relevant” (Loree et al., 2021).

Benavides et al. (2022) studied how effective liquid biopsy-tailored assays were in identifying guideline-recommended biomarkers, including *RAS* and *BRAF*, in comparison to standard of care tissue genotyping for patients newly diagnosed with mCRC. To quantify the effectivity of liquid biopsy assays for biomarkers, the researchers utilized the Guardant360 for comprehensive ctDNA analysis, and OncoBEAM for targeted RAS and BRAF analysis. Among the 155 patients included in this prospective study, physician discretion standard of care tissue genotyping identified guideline-recommended biomarkers in 52.9% of patients, in comparison to the 56.8% from the comprehensive Guardant360 ctDNA analysis and 44.5% from targeted ctDNA analysis by OncoBEAM. An additional 19.5% more samples were included in the ctDNA assays “by rescuing those without tissue results either due to tissue insufficiency, test failure, or false negatives.” The complete processing of ctDNA assays was faster (10 days versus 27 days on median) and maintained accuracy even 10 days after sample collection (52.0% vs 10.2%). This could allow inclusion of ctDNA genotyping in the care of patients with mCRC and could enable accelerated personalized treatment regimens for patients with the quick turnaround and comparable results to current practices (Benavides et al., 2022).

Several studies have evaluated the impact of the gene expression profiling on clinical decision making in certain colon cancer subgroups. Brenner et al. (2016) assessed the clinical impact of the 12-gene Colon Cancer Recurrence Score Assay in treatment of T3 mismatch repair proficient (MMR-P) stage II colon cancer. Out of 269 patients, 102 patients had their treatment changed because of the assay's results. The authors concluded that testing significantly impacted adjuvant treatment decisions in clinical practice (Brenner et al., 2016).

Cartwright et al. (2014) performed a web-based survey evaluating the impact of the 12-gene Colon Cancer Recurrence Score Assay in stage II colon cancer patients. The authors surveyed 346 oncologists about their use of the Oncotype DX assay; the survey included questions about courses of treatment before and after using the assay and the stage of cancer their patient had. The authors found that 29% of treatment recommendations were changed for patients receiving Recurrence Score testing (Cartwright et al., 2014). Srivastava et al. (2014) conducted a prospective study assessing the impact of recurrence score results on physician recommendations regarding adjuvant chemotherapy in T3 MMR-P stage II colon cancer patients. A total of 141 patients were eligible for analysis, and the study concluded that treatment recommendation changes were made for 63 (45%) of patients (Srivastava et al., 2014).

Chang et al. (2020) reviewed the "entire database" of the OncoType Colon Recurrence Score test to identify any age-related differences in Recurrence Score (RS) and single-gene results. A total of 20478 Stage II and IIIA/B colon cancer patients were included. RS results were categorized into low, medium, and high risk, and single-gene results were organized by median and interquartile ranges. In total, 72.5% of all patients and 72.6% of patients under 40 years old were found to have a low-risk RS. However, there were no significant differences in either RS or single-gene results among the four age groups (<40, 40-54, 55-64, >65). Young-onset cancer was also not found to differ by gene expression in individual RS genes. Overall, most patients in stages II or III colon cancer were found to have low-risk disease per the OncoType assay (Chang et al., 2020).

Allar et al. (2022) evaluated how the OncoType Colon Recurrence Score influences clinical practice. The study included 105 patients with stage IIa colon cancer and investigated the association between the RS and the decision to offer adjuvant chemotherapy after resection. Fifty-two patients underwent RS testing, seven (13%) of whom received adjuvant chemotherapy. The authors found no significant effect or clear association of RS on the odds of undergoing chemotherapy. The authors conclude that "RS was not associated with the decision to start adjuvant chemotherapy" and suggest that "the RS should not be obtained in patients with stage IIa colon cancer" (Allar et al., 2022).

Chaudhari and Issa (2022) conducted a study to compare the cost-effectiveness of various genomic tests used to prognosticate stage II colorectal cancer patients. The researchers compared a 12-gene assay, 18-gene expression assay, 482-gene signature assay, and Immunoscore assay in a hypothetical cohort to investigate recurrence risk and death. Using a Markov model, the authors found that "the cost of the Immunoscore assay strategy in stage II colorectal cancer patients was estimated to be US $23,564 with a gain of 3.903 quality-adjusted life years (QALYs) as compared with the 12-gene assay strategy at US $24,545 and 3.903 QALYs; the 18-gene assay strategy at US $28,374 and 3.623 QALYs; and the 482-gene signature treatment strategy at US $33,315 with 3.704 QALYs." This, along with further analysis, led to the conclusion that the Immunoscore assay may be the "dominant strategy," in that it may reduce costs associated with treatment in long-term, but for the gene expression signature assays alone, the 12-gene assay may generate more cost savings than the 18-gene expression assay, equivalent to $3900 (Chaudhari & Issa, 2022).

Aoki et al. (2023) studied the validity of NGS-based ctDNA genotyping for *RAS* and *BRAF* V600E mutation assessment to guide therapy for metastatic colorectal cancer. The study included 212 mCRC patients. The authors compared NGS-cased ctDNA genotyping results with the results of validated PCR-based tissue testing, specifically looking at the concordance rate, sensitivity, and specificity. For *RAS,* the concordance rate was 92.5%, the sensitivity was 88.7%, and the specificity was 97.2%. For *BRAF* V600E, the concordance rate was 96.2%, the sensitivity was 88.0%, and the specificity was 97.3%. The authors then investigated efficacy of anti-EGFR and BRAF-targeted therapies based on ctDNA results. The progression-free survival pf anti-EGFT therapy was 12.9 months, and the progression-free survival of BRAF-targeted treatment was 3.7 months. The authors concluded that "ctDNA genotyping effectively detected RAS/BRAF mutations" and "clinical outcomes support ctDNA genotyping for determining the use of anti-EGFR and BRAF-targeted therapies in patients with mCRC" (Aoki et al., 2023).

VI. Guidelines and Recommendations

American Society of Clinical Oncology (ASCO)

The ASCO published an endorsement of the College of American Pathologist Guidelines, recommending:

- "For patients with CRC, being considered for immune checkpoint inhibitor therapy, pathologists should use MMR-immunohistochemistry (IHC) and/or microsatellite instability (MSI) by polymerase chain reaction (PCR) for the detection of DNA MMR defects. Although MMR-IHC or MSI by PCR is preferred, pathologists may use a validated MSI by next-generation sequencing (NGS)

assay for the detection of DNA MMR defects. Note: MSI by NGS assay must be validated against MMR-IHC or MSI by PCR and must show equivalency. (Strong recommendation)."

- "For all cancer patients being considered for immune checkpoint inhibitor therapy based on defective MMR, pathologists should not use tumor mutation burden (TMB) as a surrogate for the detection of DNA MMR defects. If a tumor is identified as TMB-high, pathologists may perform IHC and/or MSI by PCR to determine if high TMB is secondary to MMR deficiency. (Strong recommendation)."

- "For cancer patients being considered for immune checkpoint inhibitor therapy, if a MMR deficiency consistent with Lynch syndrome is identified in the tumor, pathologists should communicate this finding to the treating physician. (Strong recommendation)" (Vikas et al., 2023).

American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and the American Society of Clinical Oncology

These joint guidelines focus on "Molecular Biomarkers for the Evaluation of Colorectal Cancer." They list the following recommendations for *KRAS*, *NRAS*, and *BRAF* for CRC:

- "Patients with CRC considered for anti-*EGFR* therapy must receive RAS mutational testing. Mutational analysis should include *KRAS* and *NRAS* codons 12, 13 of exon 2; 59, 61 of exon 3; and 117 and 146 of exon 4 (expanded or extended RAS)."
- "*BRAF* p.V600 (*BRAF* c. 1799 [p.V600]) mutational analysis should be performed in CRC tissue in patients with CRC for prognostic stratification."
- "There is insufficient evidence to recommend *BRAF* c.1799 p.V600 mutational status as a predictive molecular biomarker for response to anti-*EGFR* inhibitors" (Sepulveda et al., 2017).

The joint guidelines state that further research is required to study the clinical validity and utility of gene expression profiling assays in colon cancer patients (Sepulveda et al., 2017).

National Comprehensive Cancer Network (NCCN)

The guidelines version 1.2024 recommend that "all patients with metastatic colorectal cancer should have tumor genotyped for *RAS* (*KRAS* and *NRAS*) and *BRAF* mutations individually or as part of a next-generation sequencing (NGS) panel (preferred). Patients with any known *KRAS* mutation (exons 2, 3, and 4) or *NRAS* mutation (exons 2, 3, and 4) should not be treated with either cetuximab or panitumumab, unless given as

part of a regimen targeting a *KRAS* G12C mutation. *BRAF* V600E mutation makes response to panitumumab or cetuximab highly unlikely unless given with a BRAF inhibitor."

The NCCN guidelines state that testing for *KRAS*, *NRAS* and *BRAF* mutations should be performed only in laboratories that are CLIA-1988 certified as qualified to perform "high complexity clinical laboratory (molecular pathology) testing." Testing can be performed on formalin fixed paraffin embedded tissue (preferred) or blood-based assay.

The NCCN further states that "testing can be performed on the primary CRCs and/or the metastasis, as literature has shown that the *KRAS, NRAS*, and *BRAF* mutations are similar in both specimen types."

BRAF genotyping of tumor tissue is recommended at stage IV disease. Allele-specific polymerase-chain reaction (PCR), NGS, or immunohistochemistry (IHC) may be used to determine *BRAF* status.

The NCCN notes that *HER2* may be overexpressed in *RAS*/*BRAF* wild-type tumors despite being rarely amplified/overexpressed in CRC (~3% overall). HER2-targeted therapies are now recommended in patients with tumors that are *RAS/BRAF* wild-type and with HER2 overexpression. Therefore, the NCCN now recommends testing for HER2 amplifications in patients with metastatic CRC. However, *HER2* testing is not indicated in patients with known *KRAS*/*NRAS* or *BRAF* mutations (NCCN, 2024).

Routine *EGFR* testing is not recommended (NCCN, 2024).

Overall, in patients with suspected or proven mCRC, the NCCN recommends "molecular testing, including: *RAS* and *BRAF* mutations; HER2 amplifications; MMR or MSI status (if not previously done). Testing should be conducted as part of broad molecular profiling, which would identify rare and actionable mutations and fusions such as *POLE/POLD1, RET,* and *NTRK*." (NCCN, 2024).

Regarding the OncoType DX colon cancer assay, the NCCN remarks that clinical validation in patients with stages II or III cancer from the QUASAR and NSABP clinical trials shows that "recurrence scores are prognostic for recurrence, DFS [disease free survival], and OS [overall survival] in stage II and stage III colon cancer but are not predictive of benefit to adjuvant therapy." ColoPrint, an 18-gene classifier for recurrence risk, was also found to independently predict recurrence risk and is currently being validated to predict 3-year relapse rates in patients with stage II colon cancer in a prospective trial. Similarly, ColDx, a microarray based multigene assay, was found to independently predict recurrence risk. However, despite these tests' ability to

further inform risk of recurrence, the panel questions the value added. The panel also noted that "evidence of predictive value in terms of the potential benefit of chemotherapy is lacking" and that "there are insufficient data to recommend the use of multigene assays, Immunoscore, or post-surgical ctDNA to estimate risk of recurrence or determine adjuvant therapy" (NCCN, 2024).

European Society for Medical Oncology (ESMO)

In its 2023 guidelines, ESMO recommends the following for mCRC genetic testing:

- "Determining the *RAS* mutational testing on a tumour biopsy [I, A] (or through a liquid biopsy in case no tumour sample is available [II, B]) is mandatory to guide the best treatment decision.
- Testing for mismatch repair (MMR) status and *KRAS, NRAS* exon 2, 3, and 4 as well as *BRAF* mutations is recommended in all patients at the time of mCRC diagnosis [I, A]
- Identification of human epidermal growth factor receptor (HER2) amplification by immunohistochemistry (IHC) or FISH [fluorescence in-situ hybridization] is recommended in *RAS* wild-type (wt) patients to detect those who may benefit from HER2 blockade [III, B]
- *RAS* testing is mandatory before treatment with anti-EGFR mAbs and can be carried out on either the primary tumour or other metastatic sites [III, A]
- *BRAF* mutation status should be assessed simultaneously with the evaluation of *RAS*, for prognostic assessment [I, B] and for the option of treatment with cetuximab-encorafenib [I, A].
- dMMR [deficient mismatch repair]/MSI testing in mCRC can assist in genetic counselling for Lynch syndrome [II, B].
- dMMR/MSI status is also recommended as the initial molecular work-up in metastatic disease for its predictive value for the use of ICIs [immune checkpoint inhibition] [I, A]" (Cervantes et al., 2023).

With regards to localized colon cancer, ESMO states that "besides MSI status, other genetic markers, e.g. *RAS* and *BRAF* mutations are not recommended for the routine assessment of risk of recurrence in non-metastatic patients, based on their lack of utility in the adjuvant decision-making process" (Argilés et al., 2020).

In their newly released guidelines, ESMO does not provide recommendations for using gene expression profiling assays for prognosticating patients with stage II colon cancer (Cervantes et al., 2023).

Choosing Wisely Canada

Choosing Wisely Canada lists "eleven tests and treatments to question" in their oncology recommendations. In this list, they recommend: "Don't perform routine colonoscopic surveillance every year in patients following their colon cancer surgery; instead, frequency should be based on the findings of the prior colonoscopy and corresponding guidelines" (Choosing Wisely Canada, 2023).

Research Committee and the Guidelines Committee of the European Society of Coloproctology (ESCP)

This systematic review was performed by the committee to assess the consensus levels "in guidelines from member countries of the European Society of Coloproctology, with supporting evidence." This review focuses on follow-up strategies for patients "after treatment with curative intent of nonmetastatic colorectal cancer" (Bastiaenen et al., 2019).

In this review, the committee concluded that "laboratory tests other than CEA [carcinoembryonic antigen] should not be part of follow-up," although it noted that only eight of 21 guidelines reviewed addressed this topic (Bastiaenen et al., 2019).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Cetuximab and panitumumab have FDA marketing approval for treatment of metastatic colorectal cancer in the refractory disease setting, and ongoing studies are investigating the use of these *EGFR* inhibitors as monotherapy and as part of combination therapy in first, second, and subsequent lines of therapy.

On May 23, 2014, the FDA approved therascreen *KRAS* RGQ PCR Kit is a real-time qualitative PCR assay used on the Rotor-Gene Q MDx instrument for the detection of seven somatic mutations in the human *KRAS* oncogene, using DNA extracted from formalin-fixed paraffin-embedded (FFPE), colorectal cancer (CRC) tissue. The therascreen *KRAS* RGQ PCR Kit is intended to aid in the identification of CRC patients

for treatment with Erbitux (cetuximab) and Vectibix (panitumumab) based on a *KRAS* no mutation detected test result (FDA, 2014).

On May 7, 2015, the FDA approved cobas *KRAS* Mutation Test, for use with the cobas® 4800 System. Cobas is a real-time PCR test for the detection of seven somatic mutations in codons 12 and 13 of the *KRAS* gene in DNA derived from formalin-fixed paraffin-embedded human colorectal cancer (CRC) tumor tissue. The test is intended to be used as an aid in the identification of CRC patients for whom treatment with Erbitux (cetuximab) or with Vectibix (panitumumab) may be indicated based on a no mutation detected result (FDA, 2015).

On June 29, 2017, the FDA approved PraxisTM Extended RAS Panel as a qualitative in vitro diagnostic test using targeted high throughput parallel sequencing for the detection of 56 specific mutations in RAS genes [*KRAS* (exons 2, 3, and 4) and *NRAS* (exons 2, 3, and 4)] in DNA extracted from formalin-fixed, paraffin-embedded (FFPE) colorectal cancer (CRC) tissue samples. The Praxis™ Extended RAS Panel is indicated to aid in the identification of patients with colorectal cancer for treatment with Vectibix (panitumumab) based on a no mutation detected test result. The test is intended to be used on the Illumina MiSeqDx instrument (FDA, 2017).

On November 30, 2017, the FDA approved FoundationOne CDx, which is a next generation sequencing oncology panel. From the FDA website: "FoundationOne CDx™ (F1CDx) is a next generation sequencing based in vitro diagnostic device for detection of substitutions, insertion and deletion alterations (indels) and copy number alterations (CNAs) in 324 genes and select gene rearrangements, as well as genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) using DNA isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens. The test is intended as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed Table 1 in accordance with the approved therapeutic product labeling. Additionally, F1CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for cancer patients with solid malignant neoplasms. The F1CDx test is a single-site assay performed at Foundation Medicine, Inc" (FDA, 2017).

In 2021, the ONCO/Reveal Dx Lung & Colon Cancer Assay (O/RDx-LCCA) was approved. O/RDx-LCCA is a highly accurate FDA approved IVD assay for the detection of clinically relevant KRAS variants in CRC and EGFR variants in NSCLC and determination of approved therapy. "The device is a qualitative next generation sequencing based in vitro diagnostic test that uses amplicon-based target enrichment technology for detection of single nucleotide variants (SNVs) and deletions in 2 genes

from DNA isolated from formalin-fixed paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) and colorectal cancer (CRC) tumor tissue specimens. The test is intended as a companion diagnostic to identify patients with NSCLC or CRC who may benefit from treatment with the targeted therapies" (FDA, 2021).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81210	BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer, melanoma), gene analysis, V600 variant(s)
81275	KRAS (Kirsten rat sarcoma viral oncogene homolog) (eg, carcinoma) gene analysis; variants in exon 2 (eg, codons 12 and 13)
81276	KRAS (Kirsten rat sarcoma viral oncogene homolog) (eg, carcinoma) gene analysis; additional variant(s) (eg, codon 61, codon 146)
81311	NRAS (neuroblastoma RAS viral [v-ras] oncogene homolog) (eg, colorectal carcinoma), gene analysis, variants in exon 2 (eg, codons 12 and 13) and exon 3 (eg, codon 61)
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81479	Unlisted molecular pathology procedure
81525	Oncology (colon), mRNA, gene expression profiling by real-time RT-PCR of 12 genes (7 content and 5 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a recurrence score
81599	Unlisted multianalyte assay with algorithmic analysis
0111U	Oncology (colon cancer), targeted KRAS (codons 12, 13, and 61) and NRAS (codons 12, 13, and 61) gene analysis utilizing formalin-fixed paraffin-embedded tissue Proprietary test: Praxis ™ Extended RAS Panel Lab/Manufacturer: Illumina
0471U	Oncology (colorectal cancer), qualitative real-time PCR of 35 variants of KRAS and NRAS genes (exons 2, 3, 4), formalinfixed paraffin-embedded (FFPE), predictive, identification of detected mutations Proprietary test: CRCdx® RAS Mutation Detection Kit

CPT	Code Description
	Lab/Manufacturera: EntroGen, Inc

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

ACS. (2023, January 13). *Key Statistics for Colorectal Cancer*. https://www.cancer.org/cancer/colon-rectal-cancer/about/key-statistics.html

Allar, B. G., Messaris, E., Poylin, V. Y., Schlechter, B. L., & Cataldo, T. E. (2022). Oncotype DX testing does not affect clinical practice in stage IIa colon cancer. *Med Oncol*, *39*(5), 59. https://doi.org/10.1007/s12032-022-01660-9

Allegra, C. J., Jessup, J. M., Somerfield, M. R., Hamilton, S. R., Hammond, E. H., Hayes, D. F., McAllister, P. K., Morton, R. F., & Schilsky, R. L. (2009). American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. *J Clin Oncol*, *27*(12), 2091-2096. https://doi.org/10.1200/jco.2009.21.9170

Allegra, C. J., Rumble, R. B., Hamilton, S. R., Mangu, P. B., Roach, N., Hantel, A., & Schilsky, R. L. (2016). Extended RAS Gene Mutation Testing in Metastatic Colorectal Carcinoma to Predict Response to Anti-Epidermal Growth Factor Receptor Monoclonal Antibody Therapy: American Society of Clinical Oncology Provisional Clinical Opinion Update 2015. *J Clin Oncol*, *34*(2), 179-185. https://doi.org/10.1200/jco.2015.63.9674

Almac Group. (2024). ColDx. https://www.almacgroup.com/diagnostics/portfolio-overview/coldx/

Aoki, Y., Nakamura, Y., Denda, T., Ohta, T., Esaki, T., Shiozawa, M., Yamaguchi, K., Yamazaki, K., Sunakawa, Y., Kato, T., Okano, N., Taniguchi, H., Sato, T., Oki, E., Nishina, T., Komatsu, Y., Matsuhashi, N., Goto, M., Yasui, H., . . . Yoshino, T. (2023). Clinical Validation of Plasma-Based Genotyping for RAS and BRAF V600E Mutation in Metastatic Colorectal Cancer: SCRUM-Japan GOZILA Substudy. *JCO Precis Oncol*, *7*, e2200688. https://doi.org/10.1200/po.22.00688

Argilés, G., Tabernero, J., Labianca, R., Hochhauser, D., Salazar, R., Iveson, T., Laurent-Puig, P., Quirke, P., Yoshino, T., Taieb, J., Martinelli, E., & Arnold, D. (2020). Localised colon cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, *31*(10), 1291-1305. https://doi.org/10.1016/j.annonc.2020.06.022

ASCO. https://www.asco.org/research-guidelines/quality-guidelines/guidelines/gastrointestinal-cancer#/9766

Bardhan, K., & Liu, K. (2013). Epigenetics and colorectal cancer pathogenesis. *Cancers (Basel)*, *5*(2), 676-713. https://doi.org/10.3390/cancers5020676

Bastiaenen, V. P., Hovdenak Jakobsen, I., Labianca, R., Martling, A., Morton, D. G., Primrose, J. N., Tanis, P. J., & Laurberg, S. (2019). Consensus and controversies regarding follow-up after treatment with curative intent of nonmetastatic colorectal cancer: a synopsis of guidelines used in countries represented in the European Society of Coloproctology. *Colorectal Dis*, *21*(4), 392-416. https://doi.org/10.1111/codi.14503

Benavides, M., Alcaide-Garcia, J., Torres, E., Gil-Calle, S., Sevilla, I., Wolman, R., Durán, G., Álvarez, M., Reyna-Fortes, C., Ales, I., Pereda, T., Robles, M., Kushnir, M., Odegaard, J., Faull, I., & Alba, E. (2022). Clinical utility of comprehensive circulating tumor DNA genotyping compared with standard of care tissue testing in patients with newly diagnosed metastatic colorectal cancer. *ESMO Open*, *7*(3), 100481. https://doi.org/10.1016/j.esmoop.2022.100481

Brenner, B., Geva, R., Rothney, M., Beny, A., Dror, Y., Steiner, M., Hubert, A., Idelevich, E., Gluzman, A., Purim, O., Shacham-Shmueli, E., Shulman, K., Mishaeli, M., Man, S., Soussan-Gutman, L., Tezcan, H., Chao, C., Shani, A., & Liebermann, N. (2016). Impact of the 12-Gene Colon Cancer Assay on Clinical Decision Making for Adjuvant Therapy in Stage II Colon Cancer Patients. *Value Health*, *19*(1), 82-87. https://doi.org/10.1016/j.jval.2015.08.013

Cartwright, T., Chao, C., Lee, M., Lopatin, M., Bentley, T., Broder, M., & Chang, E. (2014). Effect of the 12-gene colon cancer assay results on adjuvant treatment recommendations in patients with stage II colon cancer. *Curr Med Res Opin*, *30*(2), 321-328. https://doi.org/10.1185/03007995.2013.855183

Cenaj, O., Ligon, A. H., Hornick, J. L., & Sholl, L. M. (2019). Detection of ERBB2 Amplification by Next-Generation Sequencing Predicts HER2 Expression in Colorectal Carcinoma. *Am J Clin Pathol*, *152*(1), 97-108. https://doi.org/10.1093/ajcp/aqz031

Cervantes, A., Adam, R., Roselló, S., Arnold, D., Normanno, N., Taïeb, J., Seligmann, J., De Baere, T., Osterlund, P., Yoshino, T., & Martinelli, E. (2023). Metastatic colorectal cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol*, *34*(1), 10-32. https://doi.org/10.1016/j.annonc.2022.10.003

Chang, G. J., You, Y. N. Y., Russell, C. A., Tierno, M. B., Turner, M., Bennett, J. P., Lau, A., & Hochster, H. S. (2020). Young-Onset Colon Cancer and Recurrence Risk By Gene Expression. *J Natl Cancer Inst*. https://doi.org/10.1093/jnci/djaa019

Chang, X. N., Shang, F. M., Jiang, H. Y., Chen, C., Zhao, Z. Y., Deng, S. H., Fan, J., Dong, X. C., Yang, M., Li, Y., Cai, K. L., Liu, L., Liu, H. L., & Nie, X. (2021). Clinicopathological Features and Prognostic Value of KRAS/NRAS/BRAF Mutations in Colorectal Cancer Patients of Central China. *Curr Med Sci*, *41*(1), 118-126. https://doi.org/10.1007/s11596-021-2326-1

Chaudhari, V. S., & Issa, A. M. (2022). Cost-effectiveness of precision molecular diagnostic tests for stage II colorectal cancer. *Ann Transl Med*, *10*(23), 1260. https://doi.org/10.21037/atm-2022-77

Chiorean, E. G., Nandakumar, G., Fadelu, T., Temin, S., Alarcon-Rozas, A. E., Bejarano, S., Croitoru, A.-E., Grover, S., Lohar, P. V., Odhiambo, A., Park, S. H., Garcia, E. R., Teh, C., Rose, A., Zaki, B., & Chamberlin, M. D. (2020). Treatment of Patients With Late-Stage Colorectal Cancer: ASCO Resource-Stratified Guideline. *JCO Global Oncology*(6), 414-438. https://doi.org/10.1200/JGO.19.00367

Choosing Wisely Canada. (2023). Eleven Tests and Treatments to Question https://choosingwiselycanada.org/recommendation/oncology/

Clark, J. W., & Sanoff, H. K. (2024, February 28). *Systemic chemotherapy for nonoperable metastatic colorectal cancer: Selecting the initial therapeutic approach*. https://www.uptodate.com/contents/systemic-therapy-for-nonoperable-metastatic-colorectal-cancer-selecting-the-initial-therapeutic-approach

Compton, C. (2023a, 05/01/2023). *Pathology and prognostic determinants of colorectal cancer*. https://www.uptodate.com/contents/pathology-and-prognostic-determinants-of-colorectal-cancer

Compton, C. (2023b). Pathology and prognostic determinants of colorectal cancer - UpToDate. In K. Tanabe (Ed.), *UpToDate*. https://www.uptodate.com/contents/pathology-and-prognostic-determinants-of-colorectal-cancer?source=machineLearning&search=braf%20colorectal&selectedTitle=6~150&sectionRank=2&anchor=H1022797966#H1022797966

De Roock, W., Claes, B., Bernasconi, D., De Schutter, J., Biesmans, B., Fountzilas, G., Kalogeras, K. T., Kotoula, V., Papamichael, D., Laurent-Puig, P., Penault-Llorca, F., Rougier, P., Vincenzi, B., Santini, D., Tonini, G., Cappuzzo, F., Frattini, M., Molinari, F., Saletti, P., . . . Tejpar, S. (2010). Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol*, *11*(8), 753-762. https://doi.org/10.1016/s1470-2045(10)70130-3

Douillard, J. Y., Oliner, K. S., Siena, S., Tabernero, J., Burkes, R., Barugel, M., Humblet, Y., Bodoky, G., Cunningham, D., Jassem, J., Rivera, F., Kocakova, I., Ruff, P., Blasinska-Morawiec, M., Smakal, M., Canon, J. L., Rother, M., Williams, R., Rong, A., . . . Patterson, S. D. (2013). Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med*, *369*(11), 1023-1034. https://doi.org/10.1056/NEJMoa1305275

El-Deiry, W. S., Vijayvergia, N., Xiu, J., Scicchitano, A., Lim, B., Yee, N. S., Harvey, H. A., Gatalica, Z., & Reddy, S. (2015). Molecular profiling of 6,892 colorectal cancer samples suggests different possible treatment options specific to metastatic sites. *Cancer Biol Ther*, *16*(12), 1726-1737. https://doi.org/10.1080/15384047.2015.1113356

Fan, J.-Z., Wang, G.-F., Cheng, X.-B., Dong, Z.-H., Chen, X., Deng, Y.-J., & Song, X. (2021). Relationship between mismatch repair protein, RAS, BRAF, PIK3CA gene expression and clinicopathological characteristics in elderly colorectal cancer patients. *World journal of clinical cases*, *9*(11), 2458-2468. https://doi.org/10.12998/wjcc.v9.i11.2458

FDA. (2014). *SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)*. https://www.accessdata.fda.gov/cdrh_docs/pdf11/P110027B.pdf

FDA. (2015). *SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)*. https://www.accessdata.fda.gov/cdrh_docs/pdf14/P140023B.pdf

FDA. (2017). *SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)*. https://www.accessdata.fda.gov/cdrh_docs/pdf16/P160038B.pdf

FDA. (2021). ONCO/Reveal Dx Lung & Colon Cancer Assay (O/RDx-LCCA). https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm?db=pma&id=454588

Formica, V., Lucchetti, J., Doldo, E., Riondino, S., Morelli, C., Argirò, R., Renzi, N., Nitti, D., Nardecchia, A., Dell'Aquila, E., Ferroni, P., Guadagni, F., Palmieri, G., Orlandi, A., & Roselli, M. (2020). Clinical Utility of Plasma KRAS, NRAS and BRAF Mutational Analysis with Real Time PCR in Metastatic Colorectal Cancer Patients-The Importance of Tissue/Plasma Discordant Cases. *Journal of clinical medicine*, *10*(1), 87. https://doi.org/10.3390/jcm10010087

Frucht, H., & Lucas, A. L. (2023, January 21). *Molecular genetics of colorectal cancer*. https://www.uptodate.com/contents/molecular-genetics-of-colorectal-cancer

Gray, R. G., Quirke, P., Handley, K., Lopatin, M., Magill, L., Baehner, F. L., Beaumont, C., Clark-Langone, K. M., Yoshizawa, C. N., Lee, M., Watson, D., Shak, S., & Kerr, D. J. (2011). Validation study of a quantitative multigene reverse transcriptase-polymerase chain reaction assay for assessment of recurrence risk in patients with stage II colon cancer. *J Clin Oncol*, *29*(35), 4611-4619. https://doi.org/10.1200/jco.2010.32.8732

Gunderson, L. L., Jessup, J. M., Sargent, D. J., Greene, F. L., & Stewart, A. K. (2010). Revised TN categorization for colon cancer based on national survival outcomes data. *J Clin Oncol*, *28*(2), 264-271. https://doi.org/10.1200/jco.2009.24.0952

Harris, E. I., Lewin, D. N., Wang, H. L., Lauwers, G. Y., Srivastava, A., Shyr, Y., Shakhtour, B., Revetta, F., & Washington, M. K. (2008). Lymphovascular invasion in colorectal cancer: an interobserver variability study. *Am J Surg Pathol*, *32*(12), 1816-1821. https://doi.org/10.1097/PAS.0b013e3181816083

Jones, J. C., Renfro, L. A., Al-Shamsi, H. O., Schrock, A. B., Rankin, A., Zhang, B. Y., Kasi, P. M., Voss, J. S., Leal, A. D., Sun, J., Ross, J., Ali, S. M., Hubbard, J. M., Kipp, B. R., McWilliams, R. R., Kopetz, S., Wolff, R. A., & Grothey, A. (2017). (Non-V600) BRAF Mutations Define a Clinically Distinct Molecular Subtype of Metastatic Colorectal Cancer. *J Clin Oncol*, *35*(23), 2624-2630. https://doi.org/10.1200/jco.2016.71.4394

Kennedy, R. D., Bylesjo, M., Kerr, P., Davison, T., Black, J. M., Kay, E. W., Holt, R. J., Proutski, V., Ahdesmaki, M., Farztdinov, V., Goffard, N., Hey, P., McDyer, F., Mulligan,

K., Mussen, J., O'Brien, E., Oliver, G., Walker, S. M., Mulligan, J. M., . . . Harkin, D. P. (2011). Development and independent validation of a prognostic assay for stage II colon cancer using formalin-fixed paraffin-embedded tissue. *J Clin Oncol*, *29*(35), 4620-4626. https://doi.org/10.1200/jco.2011.35.4498

Kopetz, S. (2008). *Adjuvant Chemotherapy for Stage II Colon Cancer*. https://pubmed.ncbi.nlm.nih.gov/18494354/

Kopetz, S., Tabernero, J., Rosenberg, R., Jiang, Z. Q., Moreno, V., Bachleitner-Hofmann, T., Lanza, G., Stork-Sloots, L., Maru, D., Simon, I., Capellà, G., & Salazar, R. (2015). Genomic classifier ColoPrint predicts recurrence in stage II colorectal cancer patients more accurately than clinical factors. *Oncologist*, *20*(2), 127-133. https://doi.org/10.1634/theoncologist.2014-0325

Loree, J. M., Wang, Y., Syed, M. A., Sorokin, A. V., Coker, O., Xiu, J., Weinberg, B. A., Vanderwalde, A. M., Tesfaye, A., Raymond, V. M., Miron, B., Tarcic, G., Zelichov, O., Broaddus, R. R., Ng, P. K. S., Jeong, K. J., Tsang, Y. H., Mills, G. B., Overman, M. J., . . . Kopetz, S. (2021). Clinical and Functional Characterization of Atypical KRAS/NRAS Mutations in Metastatic Colorectal Cancer. *Clinical Cancer Research*, *27*(16), 4587-4598. https://doi.org/10.1158/1078-0432.Ccr-21-0180

NCCN. (2024, March 29). *NCCN Clinical Practice Guidelines in Oncology; Colon Cancer v1.2023*. https://www.nccn.org/professionals/physician_gls/pdf/colon.pdf

O'Connell, M. J., Lavery, I., Yothers, G., Paik, S., Clark-Langone, K. M., Lopatin, M., Watson, D., Baehner, F. L., Shak, S., Baker, J., Cowens, J. W., & Wolmark, N. (2010). Relationship between tumor gene expression and recurrence in four independent studies of patients with stage II/III colon cancer treated with surgery alone or surgery plus adjuvant fluorouracil plus leucovorin. *J Clin Oncol*, *28*(25), 3937-3944. https://doi.org/10.1200/jco.2010.28.9538

Oncotype. (2024a). *About the Oncotype DX Colon Recurrence Score Test*. https://www.oncotypeiq.com/en-US/colon-cancer/healthcare-professionals/oncotype-dx-colon-recurrence-score/about-the-test

Oncotype. (2024b). *Interpreting the Results*. https://www.oncotypeiq.com/en-US/colon-cancer/healthcare-professionals/oncotype-dx-colon-recurrence-score/interpreting-the-results

Pinheiro, M., Peixoto, A., Rocha, P., Veiga, I., Pinto, C., Santos, C., Pinto, P., Guerra, J., Escudeiro, C., Barbosa, A., Silva, J., & Teixeira, M. R. (2022). KRAS and NRAS mutational analysis in plasma ctDNA from patients with metastatic colorectal cancer by real-time PCR and digital PCR. *International Journal of Colorectal Disease*, *37*(4), 895-905. https://doi.org/10.1007/s00384-022-04126-6

Rebersek, M., Mesti, T., Boc, M., & Ocvirk, J. (2019). Molecular biomarkers and histological parameters impact on survival and response to first- line systemic therapy of metastatic colorectal cancer patients. *Radiol Oncol*, *53*(1), 85-95. https://doi.org/10.2478/raon-2019-0013

Ribic, C. M., Sargent, D. J., Moore, M. J., Thibodeau, S. N., French, A. J., Goldberg, R. M., Hamilton, S. R., Laurent-Puig, P., Gryfe, R., Shepherd, L. E., Tu, D., Redston, M., & Gallinger, S. (2003). Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med*, *349*(3), 247-257. https://doi.org/10.1056/NEJMoa022289

Salazar, R., Roepman, P., Capella, G., Moreno, V., Simon, I., Dreezen, C., Lopez-Doriga, A., Santos, C., Marijnen, C., Westerga, J., Bruin, S., Kerr, D., Kuppen, P., van de Velde, C., Morreau, H., Van Velthuysen, L., Glas, A. M., Van't Veer, L. J., & Tollenaar, R. (2011). Gene expression signature to improve prognosis prediction of stage II and III colorectal cancer. *J Clin Oncol*, *29*(1), 17-24. https://doi.org/10.1200/jco.2010.30.1077

Sargent, D. J., Marsoni, S., Monges, G., Thibodeau, S. N., Labianca, R., Hamilton, S. R., French, A. J., Kabat, B., Foster, N. R., Torri, V., Ribic, C., Grothey, A., Moore, M., Zaniboni, A., Seitz, J. F., Sinicrope, F., & Gallinger, S. (2010). Defective mismatch repair as a predictive marker for lack of efficacy of fluorouracil-based adjuvant therapy in colon cancer. *J Clin Oncol*, *28*(20), 3219-3226. https://doi.org/10.1200/jco.2009.27.1825

Sartore-Bianchi, A., Amatu, A., Porcu, L., Ghezzi, S., Lonardi, S., Leone, F., Bergamo, F., Fenocchio, E., Martinelli, E., Borelli, B., Tosi, F., Racca, P., Valtorta, E., Bonoldi, E., Martino, C., Vaghi, C., Marrapese, G., Ciardiello, F., Zagonel, V., . . . Siena, S. (2019). HER2 Positivity Predicts Unresponsiveness to EGFR-Targeted Treatment in Metastatic Colorectal Cancer. *Oncologist*, *24*(10), 1395-1402. https://doi.org/10.1634/theoncologist.2018-0785

Sepulveda, A. R., Hamilton, S. R., Allegra, C. J., Grody, W., Cushman-Vokoun, A. M., Funkhouser, W. K., Kopetz, S. E., Lieu, C., Lindor, N. M., Minsky, B. D., Monzon, F. A., Sargent, D. J., Singh, V. M., Willis, J., Clark, J., Colasacco, C., Rumble, R. B., Temple-Smolkin, R., Ventura, C. B., & Nowak, J. A. (2017). Molecular Biomarkers for the Evaluation of Colorectal Cancer: Guideline From the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology. *J Mol Diagn*, *19*(2), 187-225. https://doi.org/10.1016/j.jmoldx.2016.11.001

Srivastava, G., Renfro, L. A., Behrens, R. J., Lopatin, M., Chao, C., Soori, G. S., Dakhil, S. R., Mowat, R. B., Kuebler, J. P., Kim, G., Mazurczak, M., Lee, M., & Alberts, S. R. (2014). Prospective multicenter study of the impact of oncotype DX colon cancer assay results on treatment recommendations in stage II colon cancer patients. *Oncologist*, *19*(5), 492-497. https://doi.org/10.1634/theoncologist.2013-0401

Therkildsen, C., Bergmann, T. K., Henrichsen-Schnack, T., Ladelund, S., & Nilbert, M. (2014). The predictive value of KRAS, NRAS, BRAF, PIK3CA and PTEN for anti-EGFR treatment in metastatic colorectal cancer: A systematic review and meta-analysis [review-article]. https://doi.org/10.3109/0284186X.2014.895036

Venook, A. P., Niedzwiecki, D., Lopatin, M., Ye, X., Lee, M., Friedman, P. N., Frankel, W., Clark-Langone, K., Millward, C., Shak, S., Goldberg, R. M., Mahmoud, N. N., Warren, R. S., Schilsky, R. L., & Bertagnolli, M. M. (2013). Biologic determinants of tumor recurrence in stage II colon cancer: validation study of the 12-gene recurrence score in cancer and leukemia group B (CALGB) 9581. *J Clin Oncol*, *31*(14), 1775-1781. https://doi.org/10.1200/jco.2012.45.1096

Vikas, P., Messersmith, H., Compton, C., Sholl, L., Broaddus, R. R., Davis, A., Estevez-Diz, M., Garje, R., Konstantinopoulos, P. A., Leiser, A., Mills, A. M., Norquist, B., Overman, M. J., Sohal, D., Turkington, R. C., & Johnson, T. (2023). Mismatch Repair and Microsatellite Instability Testing for Immune Checkpoint Inhibitor Therapy: ASCO Endorsement of College of American Pathologists Guideline. *J Clin Oncol*, *41*(10), 1943-1948. https://doi.org/10.1200/jco.22.02462

Wang, J., Li, S., Liu, Y., Zhang, C., Li, H., & Lai, B. (2020). Metastatic patterns and survival outcomes in patients with stage IV colon cancer: A population-based analysis. *Cancer Med*, *9*(1), 361-373. https://doi.org/10.1002/cam4.2673

Xu, Q., Xu, A. T., Zhu, M. M., Tong, J. L., Xu, X. T., & Ran, Z. H. (2013). Predictive and prognostic roles of BRAF mutation in patients with metastatic colorectal cancer treated with anti-epidermal growth factor receptor monoclonal antibodies: a meta-analysis. *J Dig Dis*, *14*(8), 409-416. https://doi.org/10.1111/1751-2980.12063

Testing for Targeted Therapy of Non-Small-Cell Lung Cancer

Policy Number: AHS – M2030 – Testing for Targeted Therapy of Non-Small-Cell Lung Cancer	Prior Policy Name and Number, as applicable:
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Non-small cell lung cancer (NSCLC) is a heterogeneous group of cancers encompassing any type of epithelial lung cancer, other than small cell lung cancer (SCLC), which arise from the epithelial cells of the lung and include squamous cell carcinoma, large cell carcinoma, and adenocarcinoma (Thomas et al., 2023). Recently, oncogenesis in NSCLC has been associated with mutations in the epidermal growth factor receptor (*EGFR*), or rearrangements of the anaplastic lymphoma kinase (*ALK*) gene or *ROS1* gene (Sequist & Neal, 2024).

For guidance concerning the use of circulating tumor cells in NSCLC, please refer to AHS-G2054-Liquid Biopsy. For guidance concerning Tumor Mutational Burden Testing (TMB) and/or Microsatellite instability (MSI) analysis, please refer to AHS-M2178-Microsatellite Instability and Tumor Mutational Burden Testing policy.

II. Related Policies

Policy Number	Policy Title
AHS-G2054	Liquid Biopsy
AHS-M2029	Molecular Testing for Cutaneous Melanoma
AHS-M2078	Genetic Testing for Germline Mutations of the RET Proto-Oncogene
AHS-M2160	Molecular Testing for Pulmonary Disease
AHS-M2178	Microsatellite Instability and Tumor Mutational Burden Testing

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals with non-small cell lung cancer (NSCLC), molecular profiling to identify established actionable driver mutations (*ALK*, *BRAF*, *EGFR*, *ERBB2(HER2), KRAS*, *METex14* skipping, *NTRK 1/2/3*, *RET*, *ROS1)* **MEETS COVERAGE CRITERIA**.

2) To direct therapy in patients with NSCLC, analysis of PD-L1 expression by immunohistochemistry **MEETS COVERAGE CRITERIA**.

3) As a routine stand-alone assay and as a sole determinant of targeted therapy, *KRAS* molecular testing **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

4) To direct targeted therapy for individuals with NSCLC, analysis for genetic alterations in genes not mentioned above **DOES NOT MEET COVERAGE CRITERIA.**

NOTES:

Note: For 2 or more gene tests being run on the same platform, please refer to AHS-R2162 Reimbursement Policy.

IV. Table of Terminology

Term	Definition
AKT1	*AKT serine/threonine kinase 1 gene*
ALK	*Anaplastic lymphoma kinase*
AMP	Association for Molecular Pathology
ARMS	Amplification Refractory Mutation System
ASCO	American Society of Clinical Oncology
BRAF	*B-Raf proto-oncogene serin/threonine kinase gene*
CAP	College Of American Pathologists
CD74	*Cluster of differentiation 74*
cfDNA	Cell-free deoxyribose nucleic acid
CGP	Comprehensive genomic profiling
CLIA	Clinical Laboratory Improvement Amendment Of 1988

CMS	The Centers for Medicare and Medicaid
CNS	Central nervous system
DCB	Durable clinical benefit
DNA	Deoxyribose nucleic acid
EGFR	*Epidermal Growth Factor Receptor gene*
ERBB2	*Erythroblastic oncogene B*
ERBB2(HER2)	*Erythroblastic oncogene B (Human epidermal growth factor receptor 2)*
ESMO	European Society for Medical Oncology
FBXW7	*F-Box and WD repeat domain containing 7*
FDA	Food and Drug Administration
FIG	fluoroethyl-L-tyrosine (FET) in glioblastoma
FISH	Fluorescence in situ hybridization
HER2	*Human epidermal growth factor receptor 2*
HGF	Hepatocyte growth factor
IASLC	International Association for the Study of Lung Cancer
ICI	Immune checkpoint inhibitor
IHC	Immunohistochemistry
KRAS	*Kirsten rat sarcoma viral oncogene homolog*
LDTs	Laboratory-developed tests
MAPK	Mitogen-activated protein kinase
MET	*MET Proto-Oncogene, Receptor Tyrosine Kinase*
MSI	Microsatellite instability
NCCN	National Comprehensive Cancer Network
NDB	No durable benefit
NGS	Next-generation sequencing
NICE	National Institute for Health and Care Excellence
NOS	Not otherwise specified
NSCLC	Non-small cell lung cancer
NTRK	*Neurotrophic tyrosine receptor kinase gene*
OH	Ontario Health
PBRM1	*Protein Polybromo-1 gene*
PCR	Polymerase chain reaction
PD-L1	Programmed death-ligand 1
PFS	Progression-free survival
PI3K	Phosphatidyl 3-kinase (Pi3K)
PIK3CA	*Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha gene*
PTEN	*Phosphatase and TENsin homolog deleted on chromosome 10 gene*
pts	Patients

RAS	*Rat sarcoma virus gene*
RET	*Rearranged during transfection gene*
ROS1	*ROS proto-oncogene 1*
RT-PCR	Real-time polymerase chain reaction
SCLC	Small-cell lung cancer
SETD2	*SET Domain Containing 2, Histone Lysine Methyltransferase*
STK11	*Serine/threonine kinase 11 gene*
TMB	Tumor mutational burden
TP53	*Tumor protein p53 gene*
TSC2	*TSC complex subunit 2 gene*

V. Scientific Background

Primary lung cancer is one of the most common malignancies. In the United States, approximately 235,000 individuals are diagnosed and more than 130,000 deaths occur annually. Approximately 95% of lung cancers are either non-small cell or small cell, and 80%-85% are non-small cell lung cancers (NSCLC) (ACS, 2024; Midthun, 2022).

Specific molecular treatments for patients based on certain genetic mutations have been developed. Currently, *EGFR-, ALK-, ROS1-, BRAF-*, and *NTRK*-positive cases of NSCLC have FDA-approved targeted therapies (i.e., specific treatments for specific mutations), whereas *HER2-, MET-*, and *RET*-positive cases are treated with off-label therapies. Therapies for other mutations such as *RAS, PTEN, AKT1*, and *PIK3CA* mutations are currently in development. Still, other genetic biomarkers, such as PD-L1 expression and microsatellite instability (MSI) testing may contribute to the management of NSCLC cases (Sequist & Neal, 2024).

EGFR tyrosine kinase mutations are observed in approximately 15% of NSCLC adenocarcinoma cases in the United States and occur more frequently in nonsmokers. The presence of an *EGFR* mutation usually confers a better prognosis and may be treated by EGFR tyrosine kinase inhibitors (TKIs) such as erlotinib (Sequist & Neal, 2024).

ALK tyrosine kinase translocations are present in approximately 4% of NSCLC adenocarcinoma cases in the United States and occur more frequently in nonsmokers and younger patients. In advanced-stage NSCLC, the presence of an ALK translocation may be treated by ALK TKIs such as crizotinib. Other effective TKIs include ceritinib, alectinib, brigatinib, and lorlatinib (Sequist & Neal, 2024). Studies have indicated that treatment with these therapeutic TKIs significantly prolongs progression-free survival.

ROS1 is a receptor tyrosine kinase that acts as a driver oncogene in 1 to 2% of NSCLC cases by a translocation between *ROS1* and other genes such as CD74. *ROS1* translocations are usually associated with younger patients and individuals who have never smoked tobacco. Since the ALK and ROS tyrosine kinases are significantly homologous, the ROS1 tyrosine kinase is treatable by ALK TKIs such as crizotinib (Sequist & Neal, 2024).

HER2 (ERBB2) is an EGFR family receptor tyrosine kinase. Mutations in *HER2* have been detected in approximately 1 to 3% of NSCLC tumors. These mutations are most frequent in exon 20, resulting primarily in adenocarcinomas and these mutations are more prevalent among individuals who have never smoked tobacco (Sequist & Neal, 2024).

BRAF is a downstream signaling mediator of KRAS that activates the mitogen-activated protein kinase (MAPK) pathway. Activating *BRAF* mutations have been observed in 1 to 3% of NSCLC cases and are usually associated with smokers. BRAF inhibition with oral small-molecule TKIs has been used to treat this version of NSCLC (Sequist & Neal, 2024).

MET is a tyrosine kinase receptor for hepatocyte growth factor (HGF). *MET* mutations include *MET* exon 14 skipping, *MET* gene amplification, and *MET* and *EGFR* co-mutations. Tepotinib has shown evidence of promise in treating *MET*-exon 14 skipping cases. Crizotinib, an ALK/ROS inhibitor, has also been used to treat *MET*-exon 14 skipping cases of NSCLC. Other MET-specific therapies are under investigation such as glesatinib and savolitinib. For those with *MET* amplification, capmatinib or crizotinib are suggested lines of treatment, but are not yet approved for this indication by the FDA and continue to be a line of active research (Sequist & Neal, 2024).

The *RET* gene encodes a cell surface tyrosine kinase receptor that may be translocated in adenocarcinomas. These mutations occur more frequently in younger patients and in individuals who have never smoked tobacco. Off-label RET inhibitors, such as alectinib, have been used to treat *RET*-positive cases of NSCLC. In addition, the FDA has approved selpercatinib and pralsetinib for advanced NSCLC in adult patients (Sequist & Neal, 2024).

RAS mutations, in either *KRAS* or *NRAS*, are associated with NSCLC. Activating *KRAS* mutations have been observed in approximately 20 to 25% of lung adenocarcinomas in the United States and are generally associated with smoking. The presence of a *KRAS* mutation has a limited effect on overall survival in patients with early-stage NSCLC. *NRAS* is homologous to *KRAS* and associated with smoking as well; however, *NRAS* mutations comprise only 1% of NSCLC cases. The clinical significance of *NRAS*

mutations is unclear, and no effective targeted therapies exist at this time (Sequist & Neal, 2024).

PIK3CA, AKT1, and *PTEN* are three genes involved in the same pathway. *PIK3CA* encodes the catalytic subunit of phosphatidyl 3-kinase (PI3K), AKT1 acts immediately downstream of PI3K, and phosphatase and tensin homolog (PTEN) inhibits AKT by dephosphorylation. Oncogenic alterations in this pathway include gain-of-function mutations in *PIK3CA* and *AKT1*, and loss of PTEN function. *PIK3CA* mutations may also cause resistance to EGFR TKIs in *EGFR*-mutated NSCLC. Small-molecule inhibitors of PI3K and AKT are being developed, but clinical efficacy of these agents against specific molecular alterations is unknown (Sequist & Neal, 2024).

Other genetic biomarkers include PD-L1 assessment and microsatellite instability (MSI) testing. Programmed death-1 ligand (PD-L1) expression testing via immunohistochemistry (IHC) is used to guide therapy for patients with NSCLC. Tumor cells present PD-L1 to T-cells to inhibit the immune response by downregulating cytokine production and T-cell proliferation, thereby allowing these tumor cells to evade immune system activity. Monoclonal antibody therapy (immune therapy) has been developed to inhibit this pathway and overcome this mechanism of immune system evasion (Teixidó et al., 2018). Tumor PD-L1 protein expression through immunohistochemistry can be ordered to pinpoint first-line treatment options for NSCLC outside of chemotherapy (Sequist & Neal, 2024).

Microsatellites are short tandem repeat sequences located throughout the genome. However, these sequences are subject to instability caused by faulty mismatch repair genes. This has historically been reported in other cancers, such as Lynch syndrome, and has been reported in NSCLC. MSI testing may be used to evaluate NSCLC cases (Fong et al., 1995).

Precision oncology is now the evidence-based standard of care for the management of many advanced NSCLCs. Expert consensus guidelines have defined minimum requirements for routine testing and identification of *EGFR* and *ALK* mutations in advanced lung adenocarcinomas. Targeted use of TKIs based on certain genetic mutations has consistently led to more favorable outcomes compared with traditional cytotoxic agents (Shea et al., 2016). The concept of targeted testing has been approved by the FDA, as package inserts for drugs such as erlotinib specify used for *EGFR* mutations and other drugs such as pembrolizumab have gained approval for specific types of tumors (in this case, high-MSI tumors) (Boyiadzis et al., 2018; FDA, 2004; Lemery et al., 2017). Proprietary tests are available for identification of relevant mutations, including larger genetic panels. FoundationOne's 324-gene panel and

Oncomine's 23-gene panel are both FDA-approved as companion diagnostics for non-small cell lung cancer targeted therapies (FDA, 2023). Recently, Guardant 360 CDx was FDA-approved for use as a companion diagnostic to identify NSCLC patients with *EGFR* mutations who may benefit from Tagrisso (osimertinib) (Guardant, 2021). The company Cobas has a diagnostic approved to identify patients with metastatic NSCLC who might benefit from Tarceva® (erlotinib) based on formalin-fixed tissue preparation to identify EGFR mutation; a Cobas assay was also FDA approved as a companion diagnostic using liquid biopsy and circulating-free tumor DNA (FDA, 2016).

Clinical Utility and Validity

Lin et al. (2017) evaluated the association between EGFR and EGFR-TKI efficacy in stage IV NSCLC patients. In this study, 94 patients were assessed. The authors calculated a 74.5% objective response rate and a 97.9% disease control rate for EGFR-TKI treatment. The authors concluded that EGFR-TKI therapy resulted in survival benefits for EGFR-mutant patients regardless of "gender, smoking history, pathologic type, type of EGFR mutations, brain metastasis and timing of targeted therapy" (Lin et al., 2017).

Li et al. (2017) examined the effect of number of *EGFR* mutations on the efficacy of EGFR TKIs. In this study, 201 patients with *EGFR* mutations were evaluated. These patients were quantitatively separated into "low" and "high" groups based on "amplification refractory mutation system (ARMS) method optimized with competitive blockers and specific mutation quantitation (ARMS+)." The cutoff value was determined by a receiving operating characteristic analysis in a training group and further validated in another group. The investigators found the median progression-free survival (PFS) to be 15 months in the high group compared to two months in the low group. Similar results were reported in the validation group. The authors concluded that the abundance of *EGFR* mutations was significantly associated with objective response to EGFR TKIs. However, they also noted the abundance of *EGFR T790M* mutation may adversely affect PFS rather than objective response rate (Li et al., 2017).

Wang et al. (2017) investigated the effect of *ALK* rearrangements on NSCLC patients. The authors reviewed 15 studies including 4981 patients. The study found that *ALK* positive (ALK+) patients faced better prognoses (hazard ratio 0.81 of ALK negative patients) except in the non-smoking population (hazard ratio 1.65). *ALK*+ patients also experienced a significantly higher objective response rate in pemetrexed-based chemotherapy, but not with EGFR-TKI treatment (Wang et al., 2017).

Gainor et al. (2016) performed a study evaluating the efficacy of PD-L1 blockades on EGFR and ALK positive patients. The study evaluated 58 patients; 28 had an *EGFR* or *ALK* mutation whereas 30 were wild-type. The investigators found only one of the 28

patients (3.6%) with either mutation had an objective response whereas seven of the 30 (23.3%) wild-type patients had an objective response (Gainor et al., 2016).

Planchard et al. (2016) evaluated the efficacy of the FDA-approved combination of daBRAFenib plus trametinib on previously treated BRAF-mutant metastatic NSCLC. The study included 57 patients; 36 of these patients achieved an overall response. However, serious adverse events were reported in 32 of these patients. The authors concluded that this combination may represent a robust therapy with a management safety profile in BRAF-positive NSCLC patients (Planchard et al., 2016).

A 2019 comprehensive study by Singal et al. (2019) examined the electronic health records (EHR) of 4064 individuals with NSCLC from 275 different oncology practices to explore "associations between tumor genomics and patient characteristics with clinical outcomes…." They note that 21.4% of these individuals had a mutation in *EGFR*, *ALK*, or *ROS1*, and that patients with driver mutations who had targeted therapies had significantly improved overall survival times than individuals who did not have targeted therapies (median of 18.6 versus 11.4 months, respectively); moreover, a tumor mutational burden (TMB) of 20 or higher was associated with improved overall survival for patients on PD-L1-targeted therapy than those patients with a TMB less than 20. The authors concluded that they replicated similar associations from previous research "between clinical and genomic characteristics, between driver mutations and response to targeted therapy, and between TMB and response to immunotherapy" (Singal et al., 2019).

Siena et al. (2019) reported integrated data from three clinical trials focusing on entrectinib. Patients had either *ROS1*-driven or *NTRK*-driven cases of NSCLC. Out of 53 patients with *ROS1* mutations, approximately 80% responded to entrectinib. Out of 54 patients with *NTRK* mutations, approximately 60% responded. The authors considered entrectinib to be "tolerable with a manageable safety profile", and concluded that "entrectinib induced clinically meaningful durable responses in [patients] with ROS1+ NSCLC or NTRK+ solid tumors with or without CNS disease" (Siena et al., 2019).

Volckmar et al. (2019) assessed the "feasibility and clinical utility of comprehensive, NGS-based genetic profiling for routine workup of advanced NSCLC." The authors based their study on the first 3000 patients seen in their facility. Of the patients tested, the authors identified 807 patients eligible for "currently approved, EGFR-/BRAF-/ALK- and ROS1-directed therapies," while 218 additional cases with *MET*, *ERBB2* (HER2) and *RET* alterations could "potentially benefit from experimental targeted compounds." Other co-mutations such as *TP53* and *STK11* were also frequently identified, which may be potentially useful predictive and prognostic tools. The authors also noted logistical successes, such as reliability, low dropout rate, fast turnaround times, and minimal

tissue requirements. Overall, the authors concluded that this diagnostic approach demonstrated "practicability in order to support individualized decisions in routine patient care, enrollment in molecularly stratified clinical trials, as well as translational research" (Volckmar et al., 2019).

Signorovitch et al. (2019) aimed to evaluate the "budget impact of increased use of CGP [comprehensive genomic profiling] using a 324-gene panel (FoundationOne) vs non-CGP (represented by a mix of conventional molecular diagnostic testing and smaller NGS hotspot panels) and the number needed to test with CGP to gain 1 life year." The authors developed a decision analytic model to assess the financial impact of increased CGP in advanced non-small cell lung cancer (NSCLC). The study included two million covered lives, of which 532 had advanced NSCLC. Of these patients, 266 underwent molecular diagnostic testing. An increased in CGP among those tested (from 2%-10%) was associated with a $0.02 per member per month budget impact, of which $0.013 "was attributable to costs of prolonged drug treatment and survival and $0.005 to testing cost." Overall, the addition of one life year was met with 12 patients tested. The authors concluded that a 2%-10% increase in CGP use was associated with a "modest budget impact, most of which was attributed to increased use of more effective treatment and prolonged survival" (Signorovitch et al., 2019).

In a prospective study, Peled et al. (2020) investigated the clinical utility of early cell-free deoxyribose nucleic acid (cfDNA) analysis using Guardant 360 CDx in treatment-naive NSCLC patients. Ten patients were studied and the median time from blood draw to receiving the cfDNA results was nine days. Actionable biomarkers were identified in four of the ten patients by both biopsy analysis and cfDNA analysis. Overall, three patients received biomarker-based treatment (one osimertinib, one alectinib, and one crizotinib). The authors concluded that "cfDNA analysis should be ordered by the pulmonologists early in the evaluation of patients with NSCLC, which might complement the tumor biopsy" (Peled et al., 2020).

Al-Ahmadi et al. (2021) studied the overall impact and racial differences of NGS testing in NSCLC patients. The study tested 295 patients with Stage IV NSCLC using the FoundationOne assay and genomic differences were compared by race. "Patients undergoing NGS testing had significantly longer survival of 25.3 months versus those who did not undergo sequencing with a median survival of 14.6 months (P=.002) irrespective if they received targeted therapy or not." In addition, there was no difference in NGS results based on race. However, African American patients had a higher rate of mutations in *PBRM1*, *SETD2*, *TSC2*, and *FBXW7*. Overall, there is no racial difference in NGS utilization and testing does increase survival (Al-Ahmadi et al., 2021).

Boeckx et al. (2020) convened a small study of 46 never-smoking, non-small cell lung cancer (NSCLC) patients to investigate genomic alterations in non-smoking individuals. There are few genomic studies focused primarily on this subgroup of patients with NSCLC who have never smoked. Whole exome and low-coverage whole genome sequencing was performed on tumors and matched germline DNA. Fewer somatic mutations, genomic breakpoints, and a smaller percentage of the genome with chromosomal instability in lung tumors were observed in non-smokers compared to smokers. In addition, *TSC22D1* was noted as a potential driver gene of NSCLC. The frequency of mutation of *TP53*, which is associated with negative long-term outcomes, was lower in those who were never-smokers than in smokers. That said, they found driver genes such as *EGFR* and *ERBB2*, as well as amplifications in *MET* were higher in never-smokers. The authors concluded there was a "more favorable prognosis for never smokers with NSCLC" (Boeckx et al., 2020).

VI. Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

In the version 4.2023 update for NSCLC, NCCN states that "Numerous gene alterations have been identified that impact therapy selection. Testing of lung cancer specimens for these alterations is important for identification of potentially efficacious targeted therapies, as well as avoidance of therapies unlikely to provide clinical benefit" (NCCN, 2024). NCCN then expounds on their stance, providing a set of "several methodologies are generally considerations for use" that is delineated below.

- "Next-generation sequencing (NGS) is used in clinical laboratories. Not all types of alterations are detected by individual NGS assays and it is important to be familiar with the types of alterations identifiable in individual assays or combination(s) of assays."
- "It is recommended at this time that when feasible, testing be performed via a broad, panel-based approach, most typically performed by next generation sequencing (NGS). For patients who, in broad panel testing don't have identifiable driver oncogenes (especially in never smokers), consider RNA-based NGS if not already performed, to maximize detection of fusion event."
- "Real-time polymerase chain reaction (PCR) can be used in a highly targeted fashion (specific mutations targeted). When this technology is deployed, only those specific alterations that are targeted by the assay are assessed."
- "Sanger sequencing requires the greatest degree of tumor enrichment. Unmodified Sanger sequencing is not appropriate for detection of mutations in tumor samples with less than 25% to 30% tumor after enrichment and is not appropriate for assays in which identification of subclonal events (eg, resistance

mutations) is important. If Sanger sequencing is utilized, tumor enrichment methodologies are nearly always recommended."

- "Any method that interrogates sequences other than a subset of highly specific alterations (eg, NGS, Sanger) has the potential to identify variants of uncertain significance (VUS). Any variant classified as a VUS, even if in a gene in which other variants are clinically actionable, should not be considered as a basis for therapy selection."
- "Other methodologies may be utilized, including multiplex approaches not listed above (ie, SNaPshot, MassARRAY)."
- "Fluorescence in situ hybridization (FISH) analysis is utilized for many assays examining copy number, amplification, and structural alterations such as gene rearrangements. FISH may have better sensitivity for gene amplification events in some circumstances" (NCCN, 2024).

In order "To minimize tissue use and potential wastage, the NCCN NSCLC Panel recommends that broad molecular profiling be done as part of biomarker testing using a validated test(s) that assesses a minimum of the following potential genetic variants: *ALK* rearrangements, *BRAF* mutations, *EGFR* mutations, *KRAS* mutations, *METex14* skipping mutations, *NTRK* 1/2/3 gene fusions, *RET* rearrangements, and *ROS1* rearrangements. Both FDA and laboratory-developed test platforms are available that address the need to evaluate these and other analytes. Broad molecular profiling is also recommended to identify rare driver mutations for which effective therapy may be available, such as high-level *MET* amplifications and *ERBB2* mutations" (NCCN, 2024).

The NCCN also states that, "First-line targeted therapy options are recommended for eligible patients with metastatic NSCLC and positive test results for actionable driver mutations such as, *ALK*, *BRAF p.V600E*, *EGFR*, *MET*ex14 skipping, *NTRK 1/2/3*, *RET*, and *ROS1*. Second-line targeted therapy is recommended for patients with metastatic NSCLC and positive test results for *EGFR exon 20* insertions or *KRAS p. G12C mutations*" (NCCN, 2024).

In the 2022 NCCN update, the NCCN clarified that "any variant that is classified as VUS should not be used to select targeted therapy even if the VUS occurs in a gene in which other variants are clinically actionable" (NCCN, 2024).

The NCCN Panel added important information about general standards for biomarker testing in eligible patients with NSCLC. They noted that broad molecular profiling is molecular testing that "identifies all of the classic actionable driver mutations described in the algorithm [eg. *ALK*, *BRAF*, *EGFR*, *KRAS*, *MET*ex14 skipping, *NTRK 1/2/3*, *RET*, *ROS1*]–using either a single assay or a combination of a limited number of assays—and optimally also identifies the emerging actionable molecular biomarkers,

including high-level *MET* amplification and *ERBB2* mutations. Tiered testing approaches, based on the low prevalence of co-occurring biomarkers, are acceptable" (NCCN, 2024).

EGFR mutations

EGFR mutations are most often assessed using RT-PCR, Sanger sequencing, and NGS. *EGFR* mutation status is important for determining use of tyrosine kinase inhibitor (TKI) therapies. *EGFR* mutations include, but are not limited to, exon 19 deletions, p.L858R point mutation, p.L861Q, p.G719X, p.S768I0, exon 20 insertion variants, and p.T790M. As a category 1 recommendation, *EGFR* mutation testing is recommended for advanced or metastatic disease, including adenocarcinoma, large cell, and NSCLC NOS. As a category 2A recommendation, it is recommended to consider it for individuals with squamous cell carcinoma who have never been smokers, small biopsy specimens, or mixed histology (NCCN, 2024).

ALK gene rearrangements

ALK gene rearrangements are most often assessed using FISH, but IHC can also be effective. The NCCN states that NGS can detect *ALK* fusions, but PCR is less likely to detect any ALF fusion with a novel partner(s). The most common fusion partner for *ALK* is EML4; however, other partners have been isolated and identified. Like *EGFR*, *ALK* status is used in determining whether TKI therapies are appropriate. As a category 1 recommendation, *ALK* testing is recommended for advanced or metastatic disease, including adenocarcinoma, large cell, and NSCLC NOS. As a category 2A recommendation, it is recommended to consider it for individuals with squamous cell carcinoma who have never been smokers, small biopsy specimens, or mixed histology (NCCN, 2024).

ROS1 rearrangements

In NSCLC, *ROS1* rearrangements can result in inappropriate *ROS1* kinase signaling. Similar to *ALK*, FISH break-apart testing is often used, but this methodology "may under-detect the FIG-*ROS1* variant" (NCCN, 2024). IHC requires confirmatory testing because it has a low specificity for *ROS1*. PCR, if used, can be unlikely to detect novel fusion partners. The use of NGS can detect *ROS1* fusions, but DNA-based NGS is prone to under-detection of *ROS1* fusions. *ROS1* status is important for responsiveness to oral *ROS1* TKIs. As category 2A recommendations, *ROS1* testing should be performed for advanced or metastatic disease, including adenocarcinoma, large cell, and NSCLC NOS; it should be considered in individuals with squamous cell carcinoma with small biopsy specimens or mixed histology. Entrectinib has been noted as a preferred treatment option for *ROS1* rearrangements in advanced or metastatic NSCLC by the

NCCN since 2019. However, it should be noted that "Targeted real-time PCR assays are utilized in some settings, although they are unlikely to detect fusions with novel partners" (NCCN, 2024).

BRAF point mutations

Sequencing methods, especially NGS and Sanger (ideally paired with tumor enrichment), and rtPCR are most often used for detecting *BRAF* point mutations. *BRAF* V600 mutations are associated with responsiveness to certain combination therapies. Many *BRAF* mutations have been identified in NSCLC, but the impact of these mutations is not well-understood as of date. As category 2A recommendations, *BRAF* testing should be performed for advanced or metastatic disease, including adenocarcinoma, large cell, and NSCLC NOS; it should be considered in individuals with squamous cell carcinoma with small biopsy specimens or mixed histology (NCCN, 2024).

KRAS point mutations

Like *BRAF*, sequencing methods are used in the identification of point mutations within the *KRAS* gene. For NSCLC, the most common *KRAS* mutations are located in codon 12 even though other point mutations may occur elsewhere. *KRAS* mutations have been linked as a prognostic indicator of poor survival and can impact *EGFR* TKI therapy. The NCCN states, "*EGFR, KRAS, ROS1*, and *ALK* genetic alterations do not usually overlap; thus, testing for *KRAS* mutations may identify patients who will not benefit from further molecular testing." A newly designed oral KRAS G12C inhibitor was found to be effective in use against the *KRAS* p.G12C mutation, but this class of inhibitor has not been evaluated for any other mutations (NCCN, 2024).

MET exon 14 skipping variants

NGS-based testing, particularly RNA-based as it provides improved detection, is used to detect *METex14* skipping events. Immunohistochemistry is not used. Oral TKI therapy is used to address a *METex14* skipping mutation when detected. The NCCN states that "NGS-based testing is the primary method for detection of *METex14* skipping events; RNA-based NGS may have improved detection. IHC is not a method for detection of *METex14* skipping" (NCCN, 2024).

RET

FISH break-apart probe methodology is one appropriate method used to detect a RET mutation, though it may under-detect some fusions. NCCN also states that "Targeted real-time reverse-transcriptase PCR assays are utilized in some settings, although they are unlikely to detect fusions with novel partners. NGS-based methodology has a high

specificity, and RNA-based NGS is preferable to DNA-based NGS for fusion detection." Sequencing methods such as NGS and rtPCR are effective but rtPCR has difficulty detecting fusions with novel partners. RNA-based NGS has better fusion detection capability than DNA-based NGS. Regardless of fusion partner, *RET* mutations are responsive to oral *RET* TKI therapies (NCCN, 2024).

PD-L1

PD-L1 is expressed on tumor cells; its presence is used to determine possible pembrolizumab therapy. The FDA has approved IHC use for assessing PD-L1. The FDA-approved companion diagnostic for PD-L1 guides utilization of pembrolizumab in patients with NSCLC and is based on the tumor proportion score. As a category 1 recommendation, PD-L1 testing is recommended for all cases of advanced or metastatic disease, including adenocarcinoma, large cell, NSCLC NOS, and squamous cell carcinoma. NCCN states that, in comparison to TMB, "PD-L1 expression level is a more useful immune biomarker than TMB for deciding how to use immunotherapy, because test results are obtained more quickly, less tissue is needed for testing, and data demonstrate relative reproducibility across platforms and individuals." However, "While some clones for PD-L1 IHC are FDA-approved for specific indications, use of multiple IHC tests is not necessary, provided any individual IHC test has been internally validated for comparability for categorical results against the FDA-approved clone" (NCCN, 2024).

NTRK gene fusion

The NCCN has an *NTRK* gene fusion positive algorithm where larotrectinib is to be used as a first-line therapy if the gene fusion was discovered prior to first-line systemic therapy. If the *NTRK* gene fusion was discovered during a different first-line systemic therapy, then they recommend completing the planned systemic therapy, including maintenance therapy, and then follow this first-line therapy up with larotrectinib. As a category 2A recommendation, the NCCN recommends *NTRK* gene fusion testing to be included as part of molecular profiling for all forms of advanced or metastatic disease, including adenocarcinoma, large cell, NSCLC NOS, and squamous cell carcinoma. The NCCN NSCLC Panel recommends larotrectinib and entrectinib (both are category 2A) as either first-line or subsequent therapy options for patients with *NTRK* gene fusion-positive metastatic NSCLC based on data and the FDA approvals. As of the v3 2020 update, both agents are considered "preferred" first-line therapies for patients with *NTRK* gene fusion-positive metastatic disease (NCCN, 2024).

Tumor Mutational Burden (TMB)

NCCN reports that "In 2020, the NCCN Panel deleted tumor mutational burden (TMB)

as an emerging immune biomarker based on clinical trial data and other issues". Preliminary data from PFS from CHECKMATE 227, a phase 3 randomized trial with a complex design, had suggested that TMB might be a useful immune biomarker for deciding whether to use immunotherapy in patients with metastatic NSCLC, but updated data indicated that "overall survival was improved with nivolumab plus ipilimumab regardless of TMB or PD-L1 expression levels". Furthermore, "Several trials have shown that high TMB levels do not correlate with PD-L1 expression levels in patients with NSCLC". This lack of clinical data, coupled with technical problems with measuring TMB—including ": 1) lack of agreement on the definition of a cut off for designating high TMB levels; and 2) lack of standardization of TMB measurements across laboratories"—drives the NCCN Guidelines to "not recommend measurement of TMB levels before deciding whether to use nivolumab plus ipilimumab regimens or to use other ICIs, such as pembrolizumab" (NCCN, 2024).

Emerging biomarkers to identify novel therapies

The NCCN version 2.2022 also lists the following emerging biomarkers to identify novel therapies for patients with metastatic NSCLC.

Genetic Alteration (ie, Driver event)	**Available Targeted Agents with Activity Against Driver Event in Lung Cancer**
High-level *MET* amplification	Capmatinib Tepotinib Crizotinib
ERBB2 (HER2) mutations (Subsequent therapy)	Fam-trastuzumab deruxtecan-nxki Ado-trastuzumab emtansine

College of American Pathologists, the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology

The CAP/IASLC/AMP joint guidelines indicate that "*EGFR* molecular testing should be used to select patients for *EGFR*-targeted TKI [tyrosine kinase inhibitor] therapy" (Evidence Grade: A) (Lindeman et al, 2013). Testing is recommended for adenocarcinomas and mixed lung cancers "regardless of histologic grade." However, in the setting of fully excised lung cancer specimens, *EGFR* testing is not recommended for lung cancer without any adenocarcinoma component (Evidence Grade: A). In the setting of more limited lung cancer specimens where an adenocarcinoma component cannot be completely excluded, *EGFR* testing is recommended "in cases showing squamous or small cell histology but clinical criteria (eg, young age, lack of smoking

history) may be useful in selecting a subset of these samples for testing" (Evidence Grade: A)" (Lindeman et al, 2013). The 2018 CAP guidelines reaffirmed the 2013 guideline recommendations of universal testing of lung cancer patients with advanced-stage cancers with an adenocarcinoma component, using molecular diagnosis for activating ''hot-spot'' mutations in *EGFR* exons 18 to 21 with at least 1% prevalence (ie, codons 709 and 719, exon 19 deletion 768, and exon 20 insertions 790, 858, and 861) (Lindeman et al., 2018).

CAP also added the recommendation that: "In lung adenocarcinoma patients who harbor sensitizing *EGFR* mutations and have progressed after treatment with an *EGFR*-targeted tyrosine kinase inhibitor, physicians must use *EGFR* T790M mutational testing when selecting patients for third-generation *EGFR*-targeted therapy. Laboratories testing for *EGFR* T790M mutation in patients with secondary clinical resistance to *EGFR* targeted kinase inhibitors should deploy assays capable of detecting *EGFR* T790M mutations in as little as 5% of viable cells" (Lindeman et al., 2018).

The CAP recommendations were updated to include "3 categories into which genes should be placed. One set of genes must be offered by all laboratories that test lung cancers, as an absolute minimum: *EGFR*, *ALK*, and *ROS1*. A second group of genes should be included in any expanded panel that is offered for lung cancer patients: *BRAF*, *MET, RET, ERBB2* (*HER2*), and *KRAS*, if adequate material is available...All other genes are considered investigational at the time of publication." They elaborate to recommend: "In this context, institutions providing care for lung cancer patients have a choice: (1) offer a comprehensive cancer panel that includes all of the genes in the first 2 categories (*EGFR*, *ALK*, *ROS1*, *BRAF*, *MET, ERBB2* [*HER2*], *KRAS*, *RET*) for all appropriate patients, or (2) offer targeted testing for the genes in the must-test category (*EGFR*, *ALK*, *ROS1*) for all appropriate patients and offer as a second test an expanded panel containing the second-category genes (*BRAF*, *MET, ERBB2* [*HER2*], and RET) for patients who are suitable candidates for clinical trials, possibly after performing a single-gene *KRAS* test to exclude patients with *KRAS*-mutant cancers from expanded panel testing" (Lindeman et al., 2018). However, the CAP states that "*KRAS* molecular testing is not indicated as a routine stand-alone assay as a sole determinant of targeted therapy. It is appropriate to include *KRAS* as part of larger testing panels performed either initially or when routine *EGFR*, *ALK*, and *ROS1* testing are negative" and that "[*RET, MET*, *KRAS*, and *ERBB* (*HER2*)] molecular testing is not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include [*RET, MET, KRAS*, and

ERBB (*HER2*)] as part of larger testing panels performed either initially or when routine *EGFR*, *ALK*, and *ROS1* testing are negative" (Lindeman et al., 2018).

The guidelines indicate that "*ALK* molecular testing should be used to select patients for *ALK*-targeted TKI therapy" (Evidence Grade: B) (Lindeman et al., 2013). Testing is recommended for adenocarcinomas and mixed lung cancers "regardless of histologic grade." However, in the setting of fully excised lung cancer specimens, *ALK* testing is not recommended for lung cancer without any adenocarcinoma component (Evidence Grade: A). In the setting of more limited lung cancer specimens where an adenocarcinoma component cannot be completely excluded, *ALK* testing is recommended "in cases showing squamous or small cell histology but clinical criteria (eg, young age, lack of smoking history) may be useful in selecting a subset of these samples for testing" (Evidence Grade: A) (Lindeman et al., 2013).

The CAP recommends that "Multiplexed genetic sequencing panels are preferred over multiple single-gene tests to identify other treatment options beyond *EGFR*, *ALK*, and *ROS1*." They found that "NGS enables the simultaneous assessment of all 3 of the ''must-test'' genes in lung cancer—*EGFR*, *ALK*, *ROS1*—as well as each of the genes suggested for inclusion in larger panels—*BRAF*, *RET, ERBB2* (*HER2*), *KRAS*, *MET*—and hundreds to thousands of other genes that may have potential roles in cancer development. In addition to small mutations, NGS assays can detect fusions/rearrangements and copy number changes in the targeted genes, if designed with these alterations in mind. Numerous studies have demonstrated the excellent sensitivity of NGS methods relative to single-gene targeted assays, particularly for single-nucleotide–substitution mutations. Next-generation sequencing methods typically require less input DNA and can accommodate smaller samples with lower concentrations of malignant cells, and, although typically slower than 1 single-gene assay, can often be performed more rapidly than sequential multiple single-gene assays. A reduced need for repeat biopsy is an additional benefit of panel testing" (Lindeman et al., 2018).

When asked about new genes that should be tested for in lung cancer patients, CAP strongly recommends that "ROS1 testing must be performed on all lung adenocarcinoma patients, irrespective of clinical characteristics" (Lindeman et al., 2018).

Other guidance was backed by expert consensus opinion, and these recommendations include the following:

- "ROS1 IHC may be used as a screening test in lung adenocarcinoma patients; however, positive ROS1 IHC results should be confirmed by a molecular or cytogenetic method."
- "*BRAF* molecular testing is currently not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include *BRAF* as part of larger testing panels performed either initially or when routine *EGFR*, *ALK*, and *ROS1* testing are negative."
- ""RET molecular testing is not recommended as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include RET as part of larger testing panels performed either initially or when routine EGFR, ALK, and ROS1 testing are negative."
- "ERBB2 (HER2) molecular testing is not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include ERBB2 (HER2) mutation analysis as part of a larger testing panel performed either initially or when routine EGFR, ALK, and ROS1 testing are negative."
- "KRAS molecular testing is not indicated as a routine stand-alone assay as a sole determinant of targeted therapy. It is appropriate to include KRAS as part of larger testing panels performed either initially or when routine EGFR, ALK, and ROS1 testing are negative."
- "MET molecular testing is not indicated as a routine stand-alone assay outside the context of a clinical trial. It is appropriate to include MET as part of larger testing panels performed either initially or when routine EGFR, ALK, and ROS1 testing are negative" (Lindeman et al., 2018).

In 2018, CAP added the recommendation that "IHC is an equivalent alternative to FISH for *ALK* testing", and that "although at the time of writing RT-PCR and NGS are not approved by the FDA in the United States as first-line methods for determining *ALK* status in selection of patients for *ALK* inhibitor therapy, these approaches have shown comparable performance with IHC when designed to detect the majority of fusions, and are standard practice in many other countries. These methods are highly specific for most fusions, and patients with positive results should be treated with an *ALK* inhibitor, although patients with negative results may benefit from a more sensitive method to exclude the possibility of a variant fusion. Similarly, amplicon-based NGS assays of DNA may likewise fail to detect all fusion variants, and therefore a capture-based DNA or RNA approach is preferred for NGS testing for *ALK* fusions. Current data are still too limited to develop a specific recommendation either for or against the use of NGS for *ALK* fusions as a sole determinant of *ALK* TKI therapy" (Lindeman et al., 2018).

When performing molecular testing, CAP suggests that "Multiplexed genetic sequencing panels are preferred over multiple single-gene tests to identify other treatment options beyond *EGFR, ALK*, and *ROS1*" and that "Laboratories should ensure test results that are unexpected, discordant, equivocal, or otherwise of low confidence are confirmed or resolved using an alternative method or sample" (Lindeman et al., 2018).

Testing is indicated for patients with targetable mutations who have relapsed on targeted therapy. The CAP notes that

- "In lung adenocarcinoma patients who harbor sensitizing EGFR mutations and have progressed after treatment with an EGFR-targeted tyrosine kinase inhibitor, physicians must use EGFR T790M mutational testing when selecting patients for third-generation EGFR-targeted therapy" (strong recommendation); and
- "Laboratories testing for EGFR T790M mutation in patients with secondary clinical resistance to EGFR-targeted kinase inhibitors should deploy assays capable of detecting EGFR T790M mutations in as little as 5% of viable cells" (recommendation).

However, CAP also finds that "There is currently insufficient evidence to support a recommendation for or against routine testing for *ALK* mutational status for lung adenocarcinoma patients with sensitizing *ALK* mutations who have progressed after treatment with an *ALK*-targeted tyrosine kinase inhibitor" (Lindeman et al., 2018).

Regarding the use of circulating cell-free DNA, CAP claims that "There is currently insufficient evidence to support the use of circulating cell-free plasma DNA molecular methods for the diagnosis of primary lung adenocarcinoma." Moreover, "There is currently insufficient evidence to support the use of circulating tumor cell molecular analysis for the diagnosis of primary lung adenocarcinoma, the identification of EGFR or other mutations, or the identification of EGFR T790M mutations at the time of EGFR TKI resistance." However, CAP concedes that "In some clinical settings in which tissue is limited and/or insufficient for molecular testing, physicians may use a cell-free plasma DNA assay to identify EGFR mutations" and that "Physicians may use cell-free plasma DNA methods to identify EGFR T790M mutations in lung adenocarcinoma patients with progression or secondary clinical resistance to EGFR-targeted tyrosine kinase inhibitors; testing of the tumor sample is recommended if the plasma result is negative" (Lindeman et al., 2018).

American Society of Clinical Oncology (ASCO)

The ASCO published a joint update on "Therapy for Stage IV Non–Small-Cell Lung Cancer Without Driver Alterations" with Ontario Health (OH). These guidelines are intended for patients without alterations in *EGFR* or *ALK*. These recommendations divide PD-L1 expression into three categories: negative (0%), low positive (1-49%) and high (>50%). Pembrolizumab, carboplatin, pemetrexed, atezolizumab, paclitaxel, and bevacizumab are all listed as potential treatments, some of which may stand alone and some which are to be used in combination (Hanna et al., 2021).

Another joint update with Cancer Care Ontario remarked that "Mutations in *KRAS* are not predictive for benefit from adjuvant chemotherapy" (Kris et al., 2017).

ASCO published an endorsement of the joint guidelines from the CAP/IASLC/AMP with minor modifications. Relevant differences from the joint guidelines include:

- *BRAF* testing should be performed on all patients with advanced lung adenocarcinoma, irrespective of clinical characteristics.
- Physicians may use molecular biomarker testing in tumors with an adenocarcinoma component or nonsquamous non–small-cell histology (in addition to "any non–small-cell histology when clinical features indicate a higher probability of an oncogenic driver (eg, young age [50 years]; light or absent tobacco exposure)" (Kalemkerian et al., 2018).

European Society for Medical Oncology (ESMO)

According to ESMO, genetic alterations, which are key oncogenic events (driver mutations), have been identified in NSCLC, with two of these—*EGFR* mutations and the anaplastic lymphoma kinase (*ALK*) rearrangements—determining approved, selective pathway-directed systemic therapy. The ESMO guidelines do not specifically mention *KRAS* mutation testing. NGS is also mentioned for *ALK*, *RET*, *ROS1*, *MET, HER2*, and *BRAF* mutations (Novello et al., 2016).

ESMO remarks that the role of targeted agents in stages I-III NSCLC have not been evaluated properly. Therefore, they state that "there is no role for targeted agents in stage III NSCLC outside clinical trials" (Postmus et al., 2017).

ESMO published a guideline regarding metastatic NSCLC in 2020. In it, they note *EGFR, ALK, ROS1, BRAF*, and PD-L1 expression as usable biomarkers for "personalised medicine." *HER2, MET*, *NTRK*, and *RET* are considered "evolving targets/biomarkers". ESMO's specific recommendations are listed below.

- "*EGFR* mutation status should be systematically analysed in advanced NSCC [non-

small cell lung cancer] [level of evidence "I", strength of recommendation "A"]. Test methodology should have adequate coverage of mutations in exons 18–21, including those associated with resistance to some therapies [III, B]. At a minimum, when resources or material are limited, the most common activating mutations (exon 19 deletion, exon 21 L858R point mutation) should be determined"

- "The availability of TKIs effective against T790M-mutant recurrent disease makes T790M testing on disease relapse mandatory [I, A]"
- "Testing for *ALK* rearrangement should be systematically carried out in advanced non-squamous NSCLC [I, A]"
- "Testing for *ROS1* rearrangement should be systematically carried out in advanced NSCLC [III, A]. Detection of the *ROS1* translocation by FISH remains a standard; IHC may be used as a screening approach [IV, A]"
- "*BRAF* V600 mutation status should be systematically analysed in advanced NSCLC for the prescription of *BRAF*/MEK inhibitors"
- "Molecular *EGFR* and *ALK* testing are not recommended in patients with a confident diagnosis of SCC, except in unusual cases, e.g. never/former light smokers or long-time ex-smokers"
- "If available, multiplex platforms (NGS) for molecular testing are preferable [III, A]."
- "PD-L1 IHC should be systematically determined in advanced NSCLC [I, A]"
- "Testing is required for pembrolizumab therapy but may also be informative when nivolumab or atezolizumab are used" (Planchard et al., 2020).

In 2023, ESMO issued clinical practice guidelines focusing on oncogene-addicted metastatic non-small-cell lung cancer. These guidelines include

- "Adequate tissue material for histological diagnosis and molecular testing should be obtained to allow for individual treatment decisions [IV, A]."
- "Pathological diagnosis should be made according to the 2021 World Health Organization classification of lung tumours [IV, A].
- "Specific subtyping of all NSCLCs is necessary for therapeutic decision making and should be carried out wherever possible. IHC stains should be used to reduce the NSCLC-not otherwise specified rate to fewer than 10% of cases diagnosed [IV, A].
- The molecular tests below are recommended in patients with advanced non-squamous-cell carcinoma, and not recommended in patients with a confident diagnosis of squamous-cell carcinoma, except in unusual cases, e.g. young (<50 years) patients, never (<100 cigarettes in a lifetime)/former light smokers (≤15 pack-years, all kinds of tobacco) or long-time ex-smokers (quit smoking >15 years ago, all kinds of tobacco) [IV, A].

- *EGFR* mutation status should be determined [I, A]. Test methodology should have adequate coverage of mutations in exons 18-21, including those associated with resistance to some therapies [III, A]. At a minimum, when resources or material are limited, the most common activating mutations (exon 19 deletion, exon 21 L858R point mutation) should be determined [I, A].
- The availability of TKIs effective against T790M-mutated recurrent disease makes T790M testing on disease relapse on first- or second-generation EGFR TKIs mandatory [I, A].
- Testing for *ALK* rearrangements should be carried out [I, A].
- Detection of the *ALK* translocation by FISH remains a standard, but IHC with high-performance ALK antibodies and validated assays may be used for screening [III, A] and have been accepted as an equivalent alternative to FISH for ALK testing.
- Testing for *ROS1* rearrangements should be carried out [II, A]. Detection of the *ROS1* translocation by FISH remains a standard; IHC may be used as a screening approach [IV, A].
- *BRAF V600* mutation status testing should be carried out [II, A].
- Testing for *NTRK* rearrangements should be carried out [II, A]. Screening for NTRK rearrangements may use IHC or NGS, with appropriate testing follow-up to validate a positive result [II, A].
- Testing for *MET* exon 14 skipping mutations, MET amplifications, *RET* rearrangements, *KRAS* G12C mutations and *HER2* mutations should be carried out [II, A].
- If available, multiplex platforms (NGS) for molecular testing are preferable [III, A].
- RNA-based NGS is preferred for identifying an expanding range of fusion genes [III, B]. Whichever testing modality is used, it is mandatory that adequate internal validation and quality control measures are in place and that laboratories participate in, and perform adequately in, external quality assurance schemes for each biomarker test [III, A].
- cfDNA (liquid biopsy) can be used to test for oncogenic drivers as well as resistance mutations, but all patients with a negative cfDNA blood test still require tissue biopsy [II, A]" (Hendriks et al., 2023).

National Institute for Health and Care Excellence (NICE)

NICE has provided guidance for EGFR-TK mutation testing in adults with locally advanced or metastatic non-small-cell lung cancer.

"1.1 The tests and test strategies listed below are recommended as options for detecting epidermal growth factor receptor tyrosine kinase (EGFR-TK) mutations in the tumours of adults with previously untreated, locally advanced

or metastatic non-small-cell lung cancer (NSCLC), when used in accredited laboratories participating in an external quality assurance scheme. The laboratory-developed tests should be designed to detect the mutations that can be detected by one of the CE-marked tests as a minimum.

- therascreen EGFR RGQ PCR Kit (CE-marked, Qiagen)
- cobas EGFR Mutation Test (CE-marked, Roche Molecular Systems)
- Sanger sequencing of samples with more than 30% tumour cells and therascreen EGFR RGQ PCR Kit for samples with lower tumour cell contents
- Sanger sequencing of samples with more than 30% tumour cells and cobas EGFR Mutation Test for samples with lower tumour cell contents
- Sanger sequencing followed by fragment length analysis and polymerase chain reaction (PCR) of negative samples.

1.2 There was insufficient evidence for the Committee to make recommendations on the following methods:

- high-resolution melt analysis
- pyrosequencing combined with fragment length analysis
- single-strand conformation polymorphism analysis
- next-generation sequencing
- therascreen EGFR Pyro Kit (CE-marked, Qiagen)" (NICE, 2013).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81194	NTRK (neurotrophic receptor tyrosine kinase 1, 2, and 3) (eg, solid tumors) translocation analysis
81210	BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer, melanoma), gene analysis, V600 variant(s)
81235	EGFR (epidermal growth factor receptor) (eg, non-small cell lung cancer) gene analysis, common variants (eg, exon 19 LREA deletion, L858R, T790M, G719A, G719S, L861Q)
81275	KRAS (Kirsten rat sarcoma viral oncogene homolog) (eg, carcinoma) gene analysis; variants in exon 2 (eg, codons 12 and 13)
81276	KRAS (Kirsten rat sarcoma viral oncogene homolog) (eg, carcinoma) gene analysis; additional variant(s) (eg, codon 61, codon 146)
81404	Molecular pathology procedure, Level 5 (eg, analysis of 2-5 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 6-10 exons, or characterization of a dynamic mutation disorder/triplet repeat by Southern blot analysis)
81405	Molecular pathology procedure, Level 6 (eg, analysis of 6-10 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 11-25 exons, regionally targeted cytogenomic array analysis)
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons)
81479	Unlisted molecular pathology procedure
88342	Immunohistochemistry or immunocytochemistry, per specimen; initial single antibody stain procedure
88360	Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, per specimen, each single antibody stain procedure; manual
88361	Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, per specimen, each single antibody stain procedure; using computer-assisted technology
0414U	Oncology (lung), augmentative algorithmic analysis of digitized whole slide imaging for 8 genes (ALK, BRAF, EGFR, ERBB2, MET, NTRK1-3, RET, ROS1), and KRAS G12C and PD-L1, if performed, formalin-fixed paraffin-embedded (FFPE) tissue, reported as positive or negative for each biomarker

	Proprietary test: LungOI Lab/Manufacturer: Imagene
0448U	Oncology (lung and colon cancer), dna, qualitative, nextgeneration sequencing detection of single-nucleotide variants and deletions in egfr and kras genes, formalin-fixed paraffinembedded (ffpe) solid tumor samples, reported as presence or absence of targeted mutation(s), with recommended therapeutic options Proprietary test: oncoRevealTM DX Lung and Colon Cancer Assay Lab/Manufacturer: Pillar® Biosciences

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

ACS. (2024). *What Is Non-Small Cell Lung Cancer?* https://www.cancer.org/cancer/types/lung-cancer/about/what-is.html

Al-Ahmadi, A., Ardeshir-Larijani, F., Fu, P., Cao, S., Lipka, M. B., Dowlati, A., & Bruno, D. S. (2021). Next Generation Sequencing of Advanced Non–Small Cell Lung Cancer: Utilization Based on Race and Impact on Survival. *Clinical Lung Cancer*, *22*(1), 16-22.e11. https://doi.org/10.1016/j.cllc.2020.08.004

Boeckx, B., Shahi, R. B., Smeets, D., De Brakeleer, S., Decoster, L., Van Brussel, T., Galdermans, D., Vercauter, P., Decoster, L., Alexander, P., Berchem, G., Ocak, S., Vuylsteke, P., Deschepper, K., Lambrechts, M., Cappoen, N., Teugels, E., Lambrechts, D., & De Greve, J. (2020). The genomic landscape of nonsmall cell lung carcinoma in never smokers. *Int J Cancer*, *146*(11), 3207-3218. https://doi.org/10.1002/ijc.32797

Boyiadzis, M. M., Kirkwood, J. M., Marshall, J. L., Pritchard, C. C., Azad, N. S., & Gulley, J. L. (2018). Significance and implications of FDA approval of pembrolizumab for biomarker-defined disease. *Journal for ImmunoTherapy of Cancer*, *6*(1), 35. https://doi.org/10.1186/s40425-018-0342-x

FDA. (2004). HIGHLIGHTS OF PRESCRIBING INFORMATION. https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021743s14s16lbl.pdf

FDA. (2016). *cobas EGFR Mutation Test v2*. https://www.fda.gov/drugs/resources-information-approved-drugs/cobas-egfr-mutation-test-v2

FDA. (2023). List of Cleared or Approved Companion Diagnostic Devices (In Vitro and Imaging Tools). https://www.fda.gov/medical-devices/vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-vitro-and-imaging-tools

Fong, K. M., Zimmerman, P. V., & Smith, P. J. (1995). Microsatellite instability and other molecular abnormalities in non-small cell lung cancer. *Cancer Res*, *55*(1), 28-30. https://pubmed.ncbi.nlm.nih.gov/7805035/

Gainor, J. F., Shaw, A. T., Sequist, L. V., Fu, X., Azzoli, C. G., Piotrowska, Z., Huynh, T. G., Zhao, L., Fulton, L., Schultz, K. R., Howe, E., Farago, A. F., Sullivan, R. J., Stone, J. R., Digumarthy, S., Moran, T., Hata, A. N., Yagi, Y., Yeap, B. Y., . . . Mino-Kenudson, M. (2016). EGFR Mutations and ALK Rearrangements Are Associated with Low Response Rates to PD-1 Pathway Blockade in Non-Small Cell Lung Cancer: A Retrospective Analysis. *Clin Cancer Res*, *22*(18), 4585-4593. https://doi.org/10.1158/1078-0432.Ccr-15-3101

Guardant. (2021). Guardant360® CDx liquid biopsy is helping to bring the promise of precision oncology to more advanced cancer patients. https://guardanthealth.com/wp-content/uploads/Guardant360_CDx_Backgrounder_Document.pdf#:~:text=Guardant360%20CDx%20is%20now%20FDA%20approved%20for%20tumor,from%20Tagrisso%C2%AE%20%28osimertinib%29%2C%20RYBREVANT%E2%84%A2%20%28amivantamab-vmjw%29%2C%20and%20LUMAKRAS%E2%84%A2%20%28sotorasib%29.

Hanna, N. H., Robinson, A. G., Temin, S., Jr, S. B., Brahmer, J. R., Ellis, P. M., Gaspar, L. E., Haddad, R. Y., Hesketh, P. J., Jain, D., Jaiyesimi, I., Johnson, D. H., Leighl, N. B., Moffitt, P. R., Phillips, T., Riely, G. J., Rosell, R., Schiller, J. H., Schneider, B. J., . . . Masters, G. (2021). Therapy for Stage IV Non–Small-Cell Lung Cancer With Driver Alterations: ASCO and OH (CCO) Joint Guideline Update. *Journal of Clinical Oncology*, *39*(9), 1040-1091. https://doi.org/10.1200/jco.20.03570

Hendriks, L. E., Kerr, K. M., Menis, J., Mok, T. S., Nestle, U., Passaro, A., Peters, S., Planchard, D., Smit, E. F., Solomon, B. J., Veronesi, G., & Reck, M. (2023). Oncogene-addicted metastatic non-small-cell lung cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol*. https://doi.org/10.1016/j.annonc.2022.12.009

Kalemkerian, G. P., Narula, N., Kennedy, E. B., Biermann, W. A., Donington, J., Leighl, N. B., Lew, M., Pantelas, J., Ramalingam, S. S., Reck, M., Saqi, A., Simoff, M., Singh, N., & Sundaram, B. (2018). Molecular Testing Guideline for the Selection of Patients With Lung Cancer for Treatment With Targeted Tyrosine Kinase Inhibitors: American Society of Clinical Oncology Endorsement of the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology Clinical Practice Guideline Update. *Journal of Clinical Oncology*, *36*(9), 911-919. https://doi.org/10.1200/JCO.2017.76.7293

Kris, M. G., Gaspar, L. E., Chaft, J. E., Kennedy, E. B., Azzoli, C. G., Ellis, P. M., Lin, S. H., Pass, H. I., Seth, R., Shepherd, F. A., Spigel, D. R., Strawn, J. R., Ung, Y. C., & Weyant, M. (2017). Adjuvant Systemic Therapy and Adjuvant Radiation Therapy for Stage I to IIIA Completely Resected Non-Small-Cell Lung Cancers: American Society of Clinical Oncology/Cancer Care Ontario Clinical Practice Guideline Update. *J Clin Oncol*, *35*(25), 2960-2974. https://doi.org/10.1200/jco.2017.72.4401

Lemery, S., Keegan, P., & Pazdur, R. (2017). First FDA Approval Agnostic of Cancer Site - When a Biomarker Defines the Indication. *N Engl J Med*, *377*(15), 1409-1412. https://doi.org/10.1056/NEJMp1709968

Li, X., Cai, W., Yang, G., Su, C., Ren, S., Zhao, C., Hu, R., Chen, X., Gao, G., Guo, Z., Li, W., Zhou, C., & Hirsch, F. R. (2017). Comprehensive Analysis of EGFR-Mutant Abundance and Its Effect on Efficacy of EGFR TKIs in Advanced NSCLC with EGFR Mutations. *J Thorac Oncol*, *12*(9), 1388-1397. https://doi.org/10.1016/j.jtho.2017.06.006

Lin, J. H., Lin, D., Xu, L., Wang, Q., Hu, H. H., Xu, H. P., & He, Z. Y. (2017). The association between clinical prognostic factors and epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) efficacy in advanced non-small-cell lung cancer patients: a retrospective assessment of 94 cases with EGFR mutations. *Oncotarget*, *8*(2), 3412-3421. https://doi.org/10.18632/oncotarget.13787

Lindeman, N. I., Cagle, P. T., Aisner, D. L., Arcila, M. E., Beasley, M. B., Bernicker, E. H., Colasacco, C., Dacic, S., Hirsch, F. R., Kerr, K., Kwiatkowski, D. J., Ladanyi, M., Nowak, J. A., Sholl, L., Temple-Smolkin, R., Solomon, B., Souter, L. H., Thunnissen, E., Tsao, M. S., . . . Yatabe, Y. (2018). Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors: Guideline From the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. *J Mol Diagn*, *20*(2), 129-159. https://doi.org/10.1016/j.jmoldx.2017.11.004

Lindeman, N. I., Cagle, P. T., Beasley, M. B., Chitale, D. A., Dacic, S., Giaccone, G., Jenkins, R. B., Kwiatkowski, D. J., Saldivar, J. S., Squire, J., Thunnissen, E., & Ladanyi, M. (2013). Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. *Arch Pathol Lab Med*, *137*(6), 828-860. https://doi.org/10.5858/arpa.2012-0720-OA

Midthun, D. (2022, 04/28/2022). *Clinical manifestations of lung cancer*. https://www.uptodate.com/contents/clinical-manifestations-of-lung-cancer

NCCN. (2024). *NCCN Clinical Practice Guidelines in Oncology; Non-Small Cell Lung Cancer v4.2024* https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf

NICE. (2013, August 14, 2013). *EGFR-TK mutation testing in adults with locally advanced or metastatic non-small-cell lung cancer*. https://www.nice.org.uk/guidance/dg9/chapter/1-Recommendations

Novello, S., Barlesi, F., Califano, R., Cufer, T., Ekman, S., Levra, M. G., Kerr, K., Popat, S., Reck, M., Senan, S., Simo, G. V., Vansteenkiste, J., & Peters, S. (2016). Metastatic non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. https://doi.org/10.1093/annonc/mdw326

Peled, M., Bar, J., Avni, L., Chatterji, S., Somech, D., Dvir, A., Soussan-Gutman, L., & Onn, A. (2020). Early Blood-based Liquid Biopsy in Patients with Treatment-naïve

Metastatic Adenocarcinoma of the Lung: A Case Series. *The Israel Medical Association journal : IMAJ*, *22*(12), 784-787. http://europepmc.org/abstract/MED/33381953

Planchard, D., Besse, B., Groen, H. J. M., Souquet, P. J., Quoix, E., Baik, C. S., Barlesi, F., Kim, T. M., Mazieres, J., Novello, S., Rigas, J. R., Upalawanna, A., D'Amelio, A. M., Jr., Zhang, P., Mookerjee, B., & Johnson, B. E. (2016). Dabrafenib plus trametinib in patients with previously treated BRAF(V600E)-mutant metastatic non-small cell lung cancer: an open-label, multicentre phase 2 trial. *Lancet Oncol*, *17*(7), 984-993. https://doi.org/10.1016/s1470-2045(16)30146-2

Planchard, D., Popat, S., Kerr, K., Novello, S., Smit, E. F., Faivre-Finn, C., Mok, T. S., Reck, M., Van Schil, P. E., Hellmann, M. D., & Peters, S. (2020). Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, *29*(Suppl 4), iv192-iv237. https://doi.org/10.1093/annonc/mdy275

Postmus, P. E., Kerr, K. M., Oudkerk, M., Senan, S., Waller, D. A., Vansteenkiste, J., Escriu, C., & Peters, S. (2017). Early and locally advanced non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, *28*(suppl_4), iv1-iv21. https://doi.org/10.1093/annonc/mdx222

Sequist, L. V., & Neal, J. W. (2024, February 1, 2024). *Personalized, genotype-directed therapy for advanced non-small cell lung cancer*. https://www.uptodate.com/contents/personalized-genotype-directed-therapy-for-advanced-non-small-cell-lung-cancer

Shea, M., Costa, D. B., & Rangachari, D. (2016). Management of advanced non-small cell lung cancers with known mutations or rearrangements: latest evidence and treatment approaches. *Ther Adv Respir Dis*, *10*(2), 113-129. https://doi.org/10.1177/1753465815617871

Siena, S., Doebele, R. C., Shaw, A. T., Karapetis, C. S., Tan, D. S.-W., Cho, B. C., Kim, D.-W., Ahn, M.-J., Krebs, M., Goto, K., Garrido Lopez, P., Farago, A. F., Loong, H. H. F., Tosi, D., Barve, M. A., Simmons, B. P., Ye, C., Eng, S., & Drilon, A. E. (2019). Efficacy of entrectinib in patients (pts) with solid tumors and central nervous system (CNS) metastases: Integrated analysis from three clinical trials. *Journal of Clinical Oncology*, *37*(15_suppl), 3017-3017. https://doi.org/10.1200/JCO.2019.37.15_suppl.3017

Signorovitch, J., Zhou, Z., Ryan, J., Anhorn, R., & Chawla, A. (2019). Budget impact analysis of comprehensive genomic profiling in patients with advanced non-small cell lung cancer. *J Med Econ*, *22*(2), 140-150. https://doi.org/10.1080/13696998.2018.1549056

Singal, G., Miller, P. G., Agarwala, V., Li, G., Kaushik, G., Backenroth, D., Gossai, A., Frampton, G. M., Torres, A. Z., Lehnert, E. M., Bourque, D., O'Connell, C., Bowser, B., Caron, T., Baydur, E., Seidl-Rathkopf, K., Ivanov, I., Alpha-Cobb, G., Guria, A., . . . Miller, V. A. (2019). Association of Patient Characteristics and Tumor Genomics With Clinical Outcomes Among Patients With Non-Small Cell Lung Cancer Using a Clinicogenomic Database. *Jama*, *321*(14), 1391-1399. https://doi.org/10.1001/jama.2019.3241

Teixidó, C., Vilariño, N., Reyes, R., & Reguart, N. (2018). PD-L1 expression testing in non-small cell lung cancer. *Ther Adv Med Oncol*, *10*, 1758835918763493. https://doi.org/10.1177/1758835918763493

Thomas, K. W., Gould, M. K., & Naegar, D. (2023, September 6, 2023). *Overview of the initial evaluation, diagnosis, and staging of patients with suspected lung cancer*. https://www.uptodate.com/contents/overview-of-the-initial-evaluation-diagnosis-and-staging-of-patients-with-suspected-lung-cancer

Volckmar, A. L., Leichsenring, J., Kirchner, M., Christopoulos, P., Neumann, O., Budczies, J., Morais de Oliveira, C. M., Rempel, E., Buchhalter, I., Brandt, R., Allgauer, M., Talla, S. B., von Winterfeld, M., Herpel, E., Goeppert, B., Lier, A., Winter, H., Brummer, T., Frohling, S., . . . Stenzinger, A. (2019). Combined targeted DNA and RNA sequencing of advanced NSCLC in routine molecular diagnostics: Analysis of the first 3,000 Heidelberg cases. *Int J Cancer*, *145*(3), 649-661. https://doi.org/10.1002/ijc.32133

Wang, Z., Yang, H., Luo, S., Liu, B., Zhang, N., Li, L., Zhou, S., Shen, R., & Xie, X. (2017). Anaplastic lymphoma kinase gene rearrangement predicts better prognosis in NSCLC patients: A meta-analysis. *Lung Cancer*, *112*, 1-9. https://doi.org/10.1016/j.lungcan.2017.07.029

Testing of Homocysteine Metabolism-Related Conditions

Policy Number: AHS – M2141 – Testing of Homocysteine Metabolism-Related Conditions	Initial Policy Effective Date: 01/01/2026

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Homocystinuria is a metabolic condition in which the body is unable to properly process certain amino acids, resulting in an abnormal accumulation of homocysteine and its metabolites in the blood and urine (NIH, 2023). Homocystinuria is primarily due to genetic causes; however, homocystinuria can also be due to nongenetic causes, including severe deficiency of vitamin B12, also known as cobalamin (Mudd et al., 2000).

Genetic counseling is strongly recommended for individuals pursuing genetic testing for homocystinuria.

II. Related Policies

Policy Number	Policy Title
AHS-G2014	Vitamin B12 And Methylmalonic Acid Testing
AHS-G2035	Prenatal Screening (Nongenetic)
AHS-G2050	Cardiovascular Disease Risk Assessment
AHS-M2041	Venous and Arterial Thrombosis Risk Testing
AHS-M2180	Genetic Markers for Assessing Risk of Cardiovascular Disease

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" of this policy document.

1) For individuals with signs and symptoms consistent with classic homocystinuria, genetic testing of *CBS* **MEETS COVERAGE CRITERIA**.

2) Genetic testing for variants of *MTHFR* known to cause homocystinuria **MEETS COVERAGE CRITERIA** for **either** of the following conditions**:**

   a) For symptomatic individuals that have tested negative for classic homocystinuria due to CBS deficiency.

   b) For individuals with a first-degree relative (see Note 1) positive for known variants of *MTHFR* that cause homocystinuria.

3) Newborn screening for homocysteine-related conditions **MEETS COVERAGE CRITERIA** in **any** of the following situations:

   a) Screening for classic homocystinuria due to CBS deficiency by performing quantitative plasma amino acids analysis and/or plasma or urine total homocysteine analysis.

   b) Screening for homocystinuria in dried blood spots.

   c) Screening for hypermethioninemia in dried blood spots.

4) When the initial screening test result exceeds the cut-off level of methionine, a repeat dried blood specimen submitted to the newborn screening program, **or** a quantitative plasma amino acid analysis and analysis of plasma total homocysteine **MEETS COVERAGE CRITERIA**.

5) For the diagnosis of phenotype variants of classic homocystinuria due to CBS deficiency, the pyridoxine (B6) challenge test **MEETS COVERAGE CRITERIA**.

6) For individuals over 18 years of age with homocystinuria suspected to be caused by CBS deficiency **and** for monitoring therapy in those with confirmed CBS deficiency, total homocysteine testing in plasma **MEETS COVERAGE CRITERIA**.

7) Plasma free homocysteine testing **DOES NOT MEET COVERAGE CRITERIA.**

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

8) Genetic testing for *MTR*, *MTRR*, and *MMADHC* genes **DOES NOT MEET COVERAGE CRITERIA.**

NOTES:

Note 1: First-degree relatives include parents, full siblings, and children of the individual.

IV. Table of Terminology

Term	Definition
3-MST	3-mercaptopyruvate
ACMG	American College of Medical Genetics
cblC	Homocystinuria type C
cblD	Cobalamin D
cblD-Hcy	Cobalamin D homocysteine
CblE	Methylcobalamin type E
cblF	Cobalamin F
cblG	Methylcobalamin type G
cblJ	Methylcobalamin type J
CBS	*Cystathionine β-synthase*
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMS	Centers for Medicare and Medicaid
CT	Computed tomography
HT	Heterozygous state
CTH	Cystathionine
DNA	Deoxyribonucleic acid
EDTA	Chelating agentin deoxyribose nucleic acid extraction
E-HOD	European Network and Registry for Homocystinuria and Methylation Defects
FDA	Food and Drug Administration
FPIA	Fluorescence polarization immunoassay
GCLC	Glutamylcysteine
GC-MS	Gas chromatography–mass spectrometry
Hcy	Homocysteine
HHS	The U.S. Department of Health and Human Services
HPLC	High performance liquid chromatography
LC-MS	Liquid chromatography–mass spectrometry
LC-MS-MS	Liquid chromatography tandem mass spectrometry
LDTs	Laboratory-developed tests
MENA	Middle East and North Africa
Met	Methionine
MMA	Methylmalonic acid
MMADHC	Methylmalonic aciduria and homocystinuria type D protein

MRI	Magnetic resonance imaging
MTHFR	Methylene tetrahydrofolate reductase
MTR	Methionine synthase
MTRR	Methionine synthase reductase
NIH	National Institutes of Health
Phe	Phenylalanine
SAM	S-adenosyl methionine
tHcy	Total homocysteine
TT	Homozygous state
UPLC-MS/MS	Ultra performance liquid chromatography-tandem mass spectrometry

V. Scientific Background

Homocysteine (Hcy), a naturally occurring intermediary amino acid, is involved in multiple metabolic pathways, including the transsulfuration pathway as well as methionine (Met) metabolism. Classic homocystinuria, which results in an accumulation of Hcy and its metabolites in the blood and urine, is due to genetic mutations in *cystathionine-β-synthase* (*CBS*). CBS is the enzyme responsible for the rate-limiting step of the transsulfuration pathway and is dependent on pyridoxine (vitamin B6) (Zhu et al., 2018). If this enzyme is blocked, the transsulfuration of Hcy and the accumulation of both Hcy and Met will be limited, as Met concentration is enhanced by remethylation. The disruption of the Met metabolic pathway, as shown in Figure 1 below (Zhu et al., 2018), prevents Hcy from being used properly; this creates in a buildup of Hcy and toxic by-products in the blood, with excess Hcy excreted in urine (Mazaheri et al., 2017).

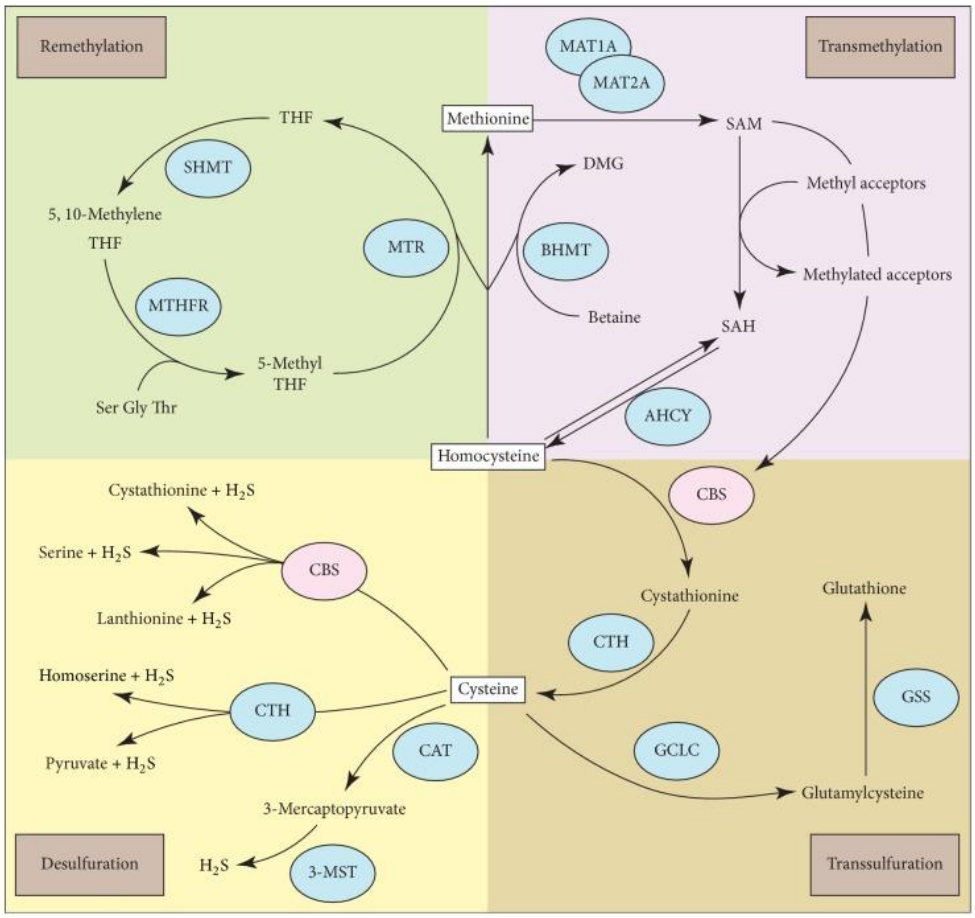


Figure 1: Homocysteine is a common metabolite linked to multiple metabolic pathways, including methionine/S-adenosyl methionine (SAM) metabolism, transsulfuration, and desulfuration. Figure taken from (Zhu et al., 2018).

Homocystinuria due to CBS deficiency can cause eye problems, skeletal abnormalities, an increased risk for blood clots, and developmental delay. Homocystinuria may also generate white matter abnormalities in the brain, potentially mimicking other disorders such as leukoencephalopathy when imaged with computed tomography (CT) and magnetic resonance imaging (MRI) scans (Ismayilova et al., 2019; Li et al., 2018).

The exact incidence of homocystinuria due to CBS deficiency is unknown. In 1985, the incidence was estimated to be around 1:344,000 worldwide (Mudd et al., 1985). However, the National Institutes of Health (NIH) is now estimating these rates to be much higher, around 1:150,000 worldwide, 1:200,000-300,000 in the United States, and 1:1,800 in Qatar (NIH, 2023). In European populations, incidence rates have been predicted by molecular epidemiological studies to be between 1:6,400 and 1:20,500 (Gaustadnes et al., 1999; Janosík et al., 2009). Higher prevalence in the MENA (Middle East and North Africa) region could be attributed to high consanguinity in those

communities (Al-Sadeq & Nasrallah, 2020). Infants with homocystinuria due to CBS deficiency are asymptomatic at birth, with symptoms slowly developing if left untreated. However, these symptoms are highly variable. Some affected patients may exhibit mild symptoms of the disorder while others may develop potentially life-threatening complications. Depending on the population affected and type of *CBS* gene mutation, symptoms can be as severe as ectopia lentis, Marfanoid features, mental retardation, idiopathic infertility, osteoporosis, and severe premature atherosclerosis (Al-Sadeq & Nasrallah, 2020; Rosenson et al., 2024). The phenotype of these patients mainly relates to pyridoxine-responsiveness: pyridoxine treatment responders exhibit a milder phenotype and a later onset than pyridoxine treatment nonresponders (Abbott et al., 1987; Mudd et al., 1985). Early detection and treatment is important in preventing or reducing the severity of the disorder. Screening for homocystinuria is frequently incorporated into state newborn screening programs (Rose & Dolan, 2012). While a newborn blood spot specimen for hypermethioninemia will detect homocystinuria due to CBS deficiency in some, not all affected individuals will be detected by this test (Sacharow et al., 2004).

According to Sacharow et al. (2004), the biochemical features of homocystinuria include:

- Markedly increased concentrations of total homocysteine, plasma homocysteine, homocysteine-cysteine mixed disulfide, and methionine
- Increased concentration of homocysteine in urine
- Reduced CBS enzyme activity

Classical biochemical findings establishing the diagnosis are summarized in the following table titled: Cardinal Biochemical Findings that Establish the Diagnosis of Homocystinuria (Sacharow et al., 2004).

Analyte	**Specimen**	**Expected Findings**		
		Neonate with homocystinuria	**Untreated older individual with homocystinuria**	**Control**
Total homocysteine (tHcy)	Plasma	50 to >100 µmol/L	>100 µmol/L	<15 µmol/L
Methionine (on amino acid analysis)	Plasma	200-1500 µmol/L (3-23 mg/dL)	>50 µmol/L (>0.7 mg/dL)	10-40 µmol/L (0.2-0.6 mg/dL)

Homocystinuria due to genetic causes is inherited in an autosomal recessive pattern. Many different forms of homocystinuria can occur and signs and symptoms vary depending on the gene mutation. *CBS* gene mutations cause the most common form of homocystinuria. This mutation is referred to as "classic" homocystinuria or CBS deficiency. Other gene mutations that can result in homocystinuria include *MTHFR*, *MTR*, *MTRR*, and *MMADHC*. The *MTHFR*, *MTR*, and *MTRR* genes all revolve around the remethylation pathway of Hcy, while the *MMADHC* gene plays a role in Vitamin B12 metabolism (Froese et al., 2015; Wang et al., 2016).

Homocystinuria may also be associated with a diagnosis of methylmalonic acidemia, when the body cannot efficiently break down specific fats or proteins, leading to a methylmalonic acid buildup in the blood. Methylmalonic aciduria and homocystinuria type C (cblC) is characterized by a vitamin B12 disorder initiated by a mutation in the *MMACHC* gene; symptoms of this disorder fall into several categories, including thromboembolic and neurological issues such as cognitive and psychiatric episodes (Collison et al., 2015).

Analytical Validity

This concentration of total homocysteine (tHcy) in blood plasma is the primary clinical analyte measured to diagnose homocystinuria. A study using liquid chromatography–mass spectrometry (LC-MS) calculated limits of detection (0.06 µmol/L) and quantification (0.6 µmol/Lu) of tHcy (Nelson et al., 2003). Another study using gas chromatography–mass spectrometry (GC-MS) found a detection limit of 0.4 µmol/L as well as intra- and inter-run variations of five and eight percent, respectively. Furthermore, this method was found to compare well with the LC-MS-MS method; the GC-MS method had a mean difference of -0.4 µmol compared to the LC-MS-MS method (Belkhiria et al., 2007). Fluorescence polarization immunoassay (FPIA) was found to compare favorably to the high performance liquid chromatography (HPLC) and MS approaches as well (five percent imprecision with negative two percent to three percent bias) so it is a practical option if the more precise approaches are not available; unfortunately, this study only measured levels up to 45 µmol/L, whereas severe homocystinuria can exceed 100 µmol/L (Nexo et al., 2000).

More recently, Concepción-Alvarez et al. (2016) have validated a method to quantify Hcy in plasma samples via HPLC. Hcy levels were measured in a total of 46 patients and the authors found that HPLC was able to "identify and quantify Hcy without interferences" and that the identified detection limit was 3.12 µM and quantification limit 6.25 µM (Concepción-Alvarez et al., 2016). This research has provided further

validation for Hcy plasma testing in ailments such as homocystinuria where this amino acid is increased.

For the detection of Hcy-related conditions, methylmalonic acid and tHcy are commonly measured in both plasma samples and dried blood spots. Using ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), de Sain-van der Velden et al. (2015) recently compared methylmalonic acid and tHcy levels collected either from a dried blood spot or from plasma concentration testing methods to determine which is the more efficient and accurate method. The authors note that the plasma assay performed better than the dried blood spot testing method in most areas, but that dried blood spot testing was superior for tHcy stability. Furthermore, a strong correlation of tHcy was found in both testing methods, ($y=0.46\pm1.12$ ($r(2)=0.91$)), leading to the authors suggestion that tHcy testing in plasma can be replaced by tHcy in dried blood spots (de Sain-van der Velden et al., 2015).

Clinical Utility and Validity

A diagnosis of classic homocystinuria (caused by CBS deficiency) is established by measurement of tHcy. The normal level is <15 µMol/L, whereas a newborn with homocystinuria is expected to measure at >50 µMol/L and an older, untreated individual will likely measure at >100 µMol/L (Sacharow et al., 2004). A measurement of Met in plasma can corroborate a diagnosis, as the metabolic pathway involves a buildup of Met in addition to the buildup of Hcy (Sacharow et al., 2004). While free Hcy composes about 15-25% of tHcy levels, separate free Hcy testing is unnecessary: tHcy measurement already includes all forms of Hcy (Rosenson et al., 2024).

The detection of biallelic pathogenic variants in CBS can substantiate a diagnosis of classic homocystinuria (Sacharow et al., 2004). There are two phenotypic variants in homocystinuria, both caused by *CBS*: B6-responsive and B6-non-responsive homocystinuria. The pyridoxine (B6) challenge test is performed to determine the variant and if vitamin B6 therapy will be beneficial (Sacharow et al., 2004). Testing for homocystinuria usually involves biochemical testing in urine and/or genetic testing for known mutations. Genetic testing can be done using a single gene or multi-gene panel which may include sequence analysis, deletion/duplication analysis, and/or other non-sequencing-based tests. Homocystinuria typically involves CBS deficiency and while the activity of the CBS enzyme could be performed in cultured fibroblasts when genetic tests are inconclusive, enzymatic testing for CBS deficiency is no longer available in USA (Sacharow et al., 2004).

Methylene tetrahydrofolate reductase (*MTHFR*) mutations are of interest in homocystinuria. The MTHFR enzyme catalyzes the reduction of 5,10-

methylenetetrahydrofolate to 5,10-methyltetrahydrofolate, the methyl donor for the conversion of Hcy to Met. Failure of this enzyme (<20% of normal levels) leads to increased Hcy and Met, as well as the production of other symptoms associated with homocystinuria (Long & Goldblatt, 2016). The two most common mutations in the *MTHFR* gene are 677T (changed from a C nucleotide) and 1298C (changed from an A). Both mutations can be heterozygotic or homozygotic, and both can lead to loss of enzymatic function (Gonzales et al., 2017). The 677T mutation is more severe, as in the homozygous state (TT) it results in up to 70% loss of enzymatic function, compared to only a 35% loss of function in the heterozygous state (CT) (Frosst et al., 1995). The 1298C mutation results in a loss of enzymatic function; 30% and 15% for its homozygous and heterozygous forms, respectively (Weisberg et al., 1998).

However, it is possible that dietary factors (notably low levels of folate or Vitamin B12) influence tHcy levels more than genetic factors. A study covering 452 young adults found tHcy variance to have a nine percent total genetic contribution (i.e. genetic polymorphisms) compared to a 35% contribution from dietary factors. The only polymorphism found to have a significant effect on tHcy levels was the 677T mutation, which interacted with low folate levels to produce a high tHcy phenotype. Compared to the authors earlier studies of genetic influence on tHcy levels, the younger cohort's genetic contribution on tHcy levels was measured out to be higher than the older cohort's (nine percent compared to seven percent for the older cohort). Furthermore, the authors suggest that genetic influence on tHcy levels are more pronounced during early life and environmental factors are more influential as time passes (Gaughan et al., 2001; Harmon et al., 1999; Kluijtmans et al., 2003).

Another study conducted by Gales et al. (2018) found that focal epilepsy presentation in the context of adult or adolescent onset could result from a mutation leading to *MTHFR* deficiency. It is critical that this mutation found in homocystinuria be detected early for treatment, as the neuropsychiatric syndrome could be easily treated with a combination of vitamin B9, vitamin B12, and betaine (Gales et al., 2018).

A novel newborn screening method has been developed by researchers: a two-tier algorithm using a methionine (Met) to phenylalanine (Phe) ratio. Data from 125,047 neonates was utilized to determine this accuracy of this method (Okun et al., 2017). It was reported that "Met to Phe ratio was found to be more effective for first sieve than Met, sorting out nearly 90% of normal samples. Only 10% of the samples would have to be processed by second-tier measurement of Hcy in dried blood spots" (Okun et al., 2017). This novel testing method resulted in 100% sensitivity and specificity for classic homocystinuria newborn screening (Okun et al., 2017).

Guo et al. (2022) investigated the effects of maternal Hcy concentrations, *MTHFR*, and *MTRR* gene presence on the occurrence of fetal aneuploidy through a retrospective case-control study. From the differences in the maternal *MTHFR* 677C>T, *MTHFR* 1298A>C and *MTRR* 66A>G genetic polymorphisms and maternal Hcy concentrations between aneuploidy and control mothers, they found that the mutations were associated with multiple trisomies, including Down syndrome (Trisomy 21), trisomy 15, and Turner syndrome. The 677C>T polymorphism was associated with the most trisomies and transfer genes. Lastly, there was an increased concentration of Hcy among mothers of fetuses with trisomies 22, 21, 18, 16, and 15 and Turner syndrome in comparison to the control mothers. Due to the identified associations, it becomes even more crucial to study effects of combining maternal genetic factors and body systemic factors like homocysteine concentration on spontaneous fetal aneuploidy formation that were not previously understood (Guo et al., 2022).

VI. Guidelines and Recommendations

The United States Department of Health and Human Services

The Secretary of the U.S. Department of HHS has developed a recommended uniform screening panel for every universal newborn screening program; the amino acid disorder homocystinuria is recommended as a core condition for newborn screening, and the organic acid condition methylmalonic acidemia with homocystinuria is recommended as a secondary screening condition. Methylmalonic acidemia due to methylmalonyl-CoA mutase or cobalamin disorders is included as a core condition as well (HHS, 2024).

American College of Medical Genetics (ACMG)

The ACMG recommends quantitative testing of plasma amino acids to determine increased levels of Hcy and Met; classic homocystinuria is characterized by increases in both Hcy and Met, while increased Met may be indicative of other disorders (ACMG, 2021b). Also, plasma Hcy analysis will show increased Hcy in classic homocystinuria and normal or only slightly increased Hcy in the other disorders. Urine Hcy will be significantly increased in classic homocystinuria (ACMG, 2021b).

In the Confirmatory Algorithms for Met, ACMG indicates that increased Met and increased tHcy are indicative of homocystinuria due to CBS deficiency (ACMG, 2021a).

European Network and Registry for Homocystinuria and Methylation Defects (E-HOD)

In 2015, a project by the E-HOD released the *Newborn Screening for Homocystinurias and Methylation Disorders: Systematic Review and Proposed Guidelines*. In this guideline, authors recommend newborn screening for CBS deficiency by detecting elevated Met, methionine-to-phenylalanine ratio, and/or tHcy in dried blood spots. Specificity is increased by analyzing tHcy as a second-tier marker and calculating Met/tHcy ratio is also suggested (Huemer et al., 2015)

Newborn screening for the cblD-Hcy, CblE, and cblG defects, and for *MTHFR* deficiency could be possible by measuring Met and methionine-to-phenylalanine ratio in dried blood spots followed by analysis of tHcy as a second-tier marker. However, it is stated that the efficacy and feasibility of screening for these disorders is largely unknown (Huemer et al., 2015).

As a part of E-HOD, the *Guidelines for Diagnosis and Management of The Cobalamin-Related Remethylation Disorders cblC, cblD, cblE, cblF, cblG, cblJ and MTHFR Deficiency* were released in 2017. Huemer et al. (2017) "strongly recommend measuring plasma total homocysteine in any patient presenting with the combination of neurological and/or visual and/or haematological symptoms, subacute spinal cord degeneration, atypical haemolytic uraemic syndrome or unexplained vascular thrombosis." For a "valid, timely laboratory diagnosis," the authors also add:

- "We strongly recommend that investigations in patients with a suspected remethylation disorder should start with the measurement of total homocysteine in blood. We recommend the blood sample for tHcy to be centrifuged within an hour and kept at four degrees or frozen until analysis. Immunoassays or chromatographic methods are suitable for tHcy measurement. (Quality of the evidence: moderate)
- We strongly recommend against measuring free homocysteine instead of total homocysteine. (Quality of the evidence: moderate)
- We strongly recommend that in the case of high total homocysteine, plasma and urine samples for determination of MMA, methionine, folate and vitamin B12 are to be obtained before treatment is started. (Quality of the evidence: moderate)" (Huemer et al., 2017).

Another guideline written as a part of E-HOD provides practical guides to recognition, diagnosis and management of CBS deficiency. The guideline presented 41 separate recommendations based on a literature review by the Guideline Development Group and the authors admitted that the quality of the identified data was poor and many of their recommendations were grade D; however, the highest recommendation was given to measuring the plasma total homocysteine concentrations in any patient whose signs and symptoms strongly suggest the diagnosis (Morris et al., 2017).

For the biochemical diagnosis, a tHcy test is recommended as "the frontline test" for the diagnosis of CBS deficiency. Plasma free Hcy is only detectable at tHcy concentrations above 50-60 µmol/L; its measurement is not particularly sensitive or even reproducible and is, therefore, not recommended. Untreated patients with a CBS deficiency typically have tHcy concentrations above 100 µmol/L and a diagnosis is likely if an elevated tHcy is found along with high or borderline high plasma Met concentrations. Further information such as low plasma cystathionine concentration or increased Met:Cystathionine ratio can support a diagnosis. Finally, tHcy measurement using dried blood spots can be done if plasma processing is not possible.

The European Network and Registry for Homocystinuria and Methylation Defects recommends confirming CBS deficiency by measuring cystathionine synthase activity in fibroblasts or plasma and/or by mutation analysis of CBS gene. The gold standard for confirming CBS deficiency is determination of cystathionine production of Hcy and serine in cultured fibroblasts. Either the enzyme or DNA can be analyzed and if one method does not confirm a diagnosis, the other method should be done. The grade of this recommendation is B-C.

Despite technical pitfalls of DNA testing, E-HOD recommends a molecular genetic analysis of the CBS gene for the confirmation of CBS deficiency and for carrier and prenatal testing (grade B). For the prenatal diagnosis, the molecular analysis is a preferred technique during the first trimester of pregnancy. If the mutations are known in the family, enzyme analysis can be performed in cultured amniocytes, but not in chorionic villi. Preimplantation analysis could also be done (grade C-D).

For newborn screening, it is recommended to increase specificity of Met testing by using tHcy as a second marker and calculating Met/tHcy ratio (grade C). Several other metabolic disorders can cause an increased Met concentration and the exact sensitivity of detecting Met in newborns with a CBS deficiency is unknown. Although the median Met concentration of CBS deficient patients is far greater than the median of a healthy neonate (103 µmol/L compared to 20 µmol/L), individual Met values may still vary.

Screening for family members at risk is recommended by measuring tHcy but molecular genetic testing may also be utilized in exceptional cases (grade D).

Monitoring of tHcy, amino acids, folate and vitamin B12 is recommended in all patients during therapy. The frequency of the monitoring is variable on a case-by-case basis (due to severity, treatment plan, age, etc). The targeted concentration ranges for total plasma homocysteine are proposed to be <50 ▪ mol/L in pyridoxine-responsive patients and at <11 ▪ mol/L free homocysteine (about 120 ▪ mol/L total homocysteine) in pyridoxine-unresponsive patients (Morris et al., 2017).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

On May 13, 2011, the FDA approved the Invader *MTHFR* 677 created by Hologic, Inc. The Invader *MTHFR* 677 is an in vitro diagnostic test intended for the detection and genotyping of a single point mutation (C to T at position 677) of the human 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene in isolated genomic DNA obtained from whole blood potassium EDTA samples from patients with suspected thrombophilia (FDA, 2011a).

On April 25, 2011, the FDA approved the Invader *MTHFR* 1298 created by Hologic, Inc. The Invader *MTHFR* 1298 test is an in vitro diagnostic test intended for the detection and genotyping of a single point mutation (A to C at position 1298) of the human 5,10-methylenetetrahydrofo late reductase (*MTHFR*) gene in isolated genomic DNA obtained from whole blood potassium EDTA samples from patients with suspected thrombophilia (FDA, 2011b).

On April 22, 2010, the FDA approved the eSensor Thrombophilia Risk Test on XT-8 System created by Osmetech Molecular Diagnostics. The *MTHFR*-specific portion is as follows: The eSensor *MTHFR* Genotyping Test is an in vitro diagnostic for the detection and genotyping of point mutations (C to T at position 677) and (A to C at position 1298) of the human 5,10-methylenetetrahydrofo late reductase (*MTHFR*) gene in isolated genomic DNA obtained from whole blood samples. The test is intended to be used on the eSensor XT-8 System (FDA, 2010).

On October 11, 2007, the FDA approved the Verigene System created by Nanosphere Inc. The *MTHFR*-specific portion is as follows: The Verigene *MTHFR* Nucleic Acid Test is an in vitro diagnostic for the detection and genotyping of a single point mutation (C to T at position 677) of the human 5,10-methylenetetrahydrofolate reductase gene (*MTHFR*) in patients with suspected thrombophilia, from isolated genomic DNA obtained from whole blood samples. The test is intended to be used on the Verigene System (FDA, 2007).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81291	MTHFR (5,10-methylenetetrahydrofolate reductase) (eg, hereditary hypercoagulability) gene analysis, common variants (eg, 677T, 1298C)
81401	Molecular pathology procedure, Level 2 (eg, 2-10 SNPs, 1 methylated variant, or 1 somatic variant [typically using nonsequencing target variant analysis], or detection of a dynamic mutation disorder/triplet repeat)
81406	Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons, cytogenomic array analysis for neoplasia)Molecular pathology procedure, Level 7 (eg, analysis of 11-25 exons by DNA sequence analysis, mutation scanning or duplication/deletion variants of 26-50 exons, cytogenomic array analysis for neoplasia)
81479	Unlisted molecular pathology procedure
82136	Amino acids, 2 to 5 amino acids, quantitative, each specimen
82139	Amino acids, 6 or more amino acids, quantitative, each specimen
82615	Cystine and homocysteine, urine, qualitative
83090	Homocysteine
83921	Organic acid, single, quantitative
84207	Pyridoxal phosphate (Vitamin B-6)

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Abbott, M. H., Folstein, S. E., Abbey, H., & Pyeritz, R. E. (1987). Psychiatric manifestations of homocystinuria due to cystathionine beta-synthase deficiency: prevalence, natural history, and relationship to neurologic impairment and vitamin B6-responsiveness. *Am J Med Genet*, *26*(4), 959-969. https://doi.org/10.1002/ajmg.1320260427

ACMG. (2021a, December). *Methionine Elevated or Decreased*. American College of Medical Genetics and Genomics. http://www.acmg.net/PDFLibrary/Methionine-Algorithm.pdf
ACMG. (2021b). Newborn Screening ACT Sheet [Increased Methionine] Homocystinuria (CBS Deficiency) file:///C:/Users/AHCS8503/Downloads/Methionine.pdf
Al-Sadeq, D. W., & Nasrallah, G. K. (2020). The Spectrum of Mutations of Homocystinuria in the MENA Region. *Genes (Basel)*, *11*(3). https://doi.org/10.3390/genes11030330
Belkhiria, M. N., Ducros, V., Harzallah, K., Jarraya, F., Cordonnier, D., Favier, A., & Achour, A. (2007). [Evaluation of plasmatic homocysteine determination by gas chromatography-mass spectrometry]. *Ann Biol Clin (Paris)*, *65*(4), 393-398. https://pubmed.ncbi.nlm.nih.gov/17627920/ (Evaluation d'un transfert de methode : dosage de l'homocysteine plasmatique par chromatographie en phase gazeuse couplee a la spectrometrie de masse.)
Collison, F. T., Xie, Y. A., Gambin, T., Jhangiani, S., Muzny, D., Gibbs, R., Lupski, J. R., Fishman, G. A., & Allikmets, R. (2015). Whole Exome Sequencing Identifies an Adult-Onset Case of Methylmalonic Aciduria and Homocystinuria Type C (cblC) with Non-Syndromic Bull's Eye Maculopathy. *Ophthalmic Genet*, *36*(3), 270-275. https://doi.org/10.3109/13816810.2015.1010736
Concepción-Alvarez, A., Camayd-Viera, I., & Nuevas-Paz, L. (2016). Validation of an HPLC method for total homocysteine quantification in plasma. *Revista del Laboratorio Clinico*, *9*(2), 40-47. https://doi.org/10.1016/j.labcli.2016.02.003
de Sain-van der Velden, M. G. M., van der Ham, M., Jans, J. J., Visser, G., van Hasselt, P. M., Prinsen, H., & Verhoeven-Duif, N. M. (2015). Suitability of methylmalonic acid and total homocysteine analysis in dried bloodspots. *Anal Chim Acta*, *853*, 435-441. https://doi.org/10.1016/j.aca.2014.10.043
FDA. (2007, October 11, 2007). *Nanosphere 510(k) Summary*. Retrieved October 21, 2021 from https://www.accessdata.fda.gov/cdrh_docs/pdf7/K070597.pdf
FDA. (2010, March 24, 2010). *eSensor Thrombophila Risk Test on XT-8 System*. Retrieved October 21, 2021 from https://www.accessdata.fda.gov/cdrh_docs/pdf9/K093974.pdf
FDA. (2011a, May 13, 2011). *Invader MTHFR 677 510(k) Summary*. Retrieved October 21, 2021 from https://www.accessdata.fda.gov/cdrh_docs/pdf10/K100987.pdf
FDA. (2011b, April 25, 2011). *Invader MTHFR 1298 510(k) Summary*. Retrieved October 21, 2021 from https://www.accessdata.fda.gov/cdrh_docs/pdf10/K100496.pdf
Froese, D. S., Kopec, J., Fitzpatrick, F., Schuller, M., McCorvie, T. J., Chalk, R., Plessl, T., Fettelschoss, V., Fowler, B., Baumgartner, M. R., & Yue, W. W. (2015). Structural Insights into the MMACHC-MMADHC Protein Complex Involved in Vitamin B12 Trafficking. *J Biol Chem*, *290*(49), 29167-29177. https://doi.org/10.1074/jbc.M115.683268
Frosst, P., Blom, H. J., Milos, R., Goyette, P., Sheppard, C. A., Matthews, R. G., Boers, G. J., den Heijer, M., Kluijtmans, L. A., van den Heuvel, L. P., & et al. (1995). A candidate

genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet*, *10*(1), 111-113. https://doi.org/10.1038/ng0595-111

Gales, A., Masingue, M., Millecamps, S., Giraudier, S., Grosliere, L., Adam, C., Salim, C., Navarro, V., & Nadjar, Y. (2018). Adolescence/adult onset MTHFR deficiency may manifest as isolated and treatable distinct neuro-psychiatric syndromes. *Orphanet J Rare Dis*, *13*(1), 29. https://doi.org/10.1186/s13023-018-0767-9

Gaughan, D. J., Kluijtmans, L. A., Barbaux, S., McMaster, D., Young, I. S., Yarnell, J. W., Evans, A., & Whitehead, A. S. (2001). The methionine synthase reductase (MTRR) A66G polymorphism is a novel genetic determinant of plasma homocysteine concentrations. *Atherosclerosis*, *157*(2), 451-456. https://doi.org/10.1016/s0021-9150(00)00739-5

Gaustadnes, M., Ingerslev, J., & Rütiger, N. (1999). Prevalence of Congenital Homocystinuria in Denmark. *New England Journal of Medicine*, *340*(19), 1513-1513. https://doi.org/10.1056/NEJM199905133401915

Gonzales, M. C., Yu, P., & Shiao, S. P. K. (2017). MTHFR Gene Polymorphism-Mutations and Air Pollution as Risk Factors for Breast Cancer: A Metaprediction Study. *Nursing research*, *66*(2), 152-163. https://doi.org/10.1097/NNR.0000000000000206

Guo, Q.-n., Wang, L., Liu, Z.-y., Wang, H.-d., Wang, L., Long, J.-g., & Liao, S.-x. (2022). Different effects of maternal homocysteine concentration, MTHFR and MTRR genetic polymorphisms on the occurrence of fetal aneuploidy. *Reproductive BioMedicine Online*, *45*(6), 1207-1215. https://doi.org/10.1016/j.rbmo.2022.06.024

Harmon, D. L., Shields, D. C., Woodside, J. V., McMaster, D., Yarnell, J. W., Young, I. S., Peng, K., Shane, B., Evans, A. E., & Whitehead, A. S. (1999). Methionine synthase D919G polymorphism is a significant but modest determinant of circulating homocysteine concentrations. *Genet Epidemiol*, *17*(4), 298-309. https://doi.org/10.1002/(SICI)1098-2272(199911)17:4%3C298::AID-GEPI5%3E3.0.CO;2-V

HHS. (2024). *Recommended Uniform Screening Panel*. https://www.hrsa.gov/advisory-committees/heritable-disorders/rusp/index.html

Huemer, M., Diodato, D., Schwahn, B., Schiff, M., Bandeira, A., Benoist, J. F., Burlina, A., Cerone, R., Couce, M. L., Garcia-Cazorla, A., la Marca, G., Pasquini, E., Vilarinho, L., Weisfeld-Adams, J. D., Kožich, V., Blom, H., Baumgartner, M. R., & Dionisi-Vici, C. (2017). Guidelines for diagnosis and management of the cobalamin-related remethylation disorders cblC, cblD, cblE, cblF, cblG, cblJ and MTHFR deficiency. *J Inherit Metab Dis*, *40*(1), 21-48. https://doi.org/10.1007/s10545-016-9991-4

Huemer, M., Kožich, V., Rinaldo, P., Baumgartner, M. R., Merinero, B., Pasquini, E., Ribes, A., & Blom, H. J. (2015). Newborn screening for homocystinurias and methylation disorders: systematic review and proposed guidelines. *Journal of inherited metabolic disease*, *38*(6), 1007-1019. https://doi.org/10.1007/s10545-015-9830-z

Ismayilova, N., MacKinnon, A. D., Mundy, H., & Fallon, P. (2019). Reversible Cerebral White Matter Abnormalities in Homocystinuria. *JIMD Rep*, *44*, 115-119. https://doi.org/10.1007/8904_2018_135

Janosík, M., Sokolová, J., Janosíková, B., Krijt, J., Klatovská, V., & Kozich, V. (2009). Birth prevalence of homocystinuria in Central Europe: frequency and pathogenicity of mutation c.1105C>T (p.R369C) in the cystathionine beta-synthase gene. *The Journal of pediatrics*, *154*(3), 431-437. https://doi.org/10.1016/j.jpeds.2008.09.015

Kluijtmans, L. A., Young, I. S., Boreham, C. A., Murray, L., McMaster, D., McNulty, H., Strain, J. J., McPartlin, J., Scott, J. M., & Whitehead, A. S. (2003). Genetic and nutritional factors contributing to hyperhomocysteinemia in young adults. *Blood*, *101*(7), 2483-2488. https://doi.org/10.1182/blood.V101.7.2483

Li, Barshop, Feigenbaum, & Khanna. (2018). Brain Magnetic Resonance Imaging Findings in Poorly Controlled Homocystinuria. *J Radiol Case Rep*. https://doi.org/10.3941/jrcr.v12i1.3207

Long, S., & Goldblatt, J. (2016). MTHFR genetic testing: Controversy and clinical implications. *Australian Family Physician*, *45*, 237-240. http://www.racgp.org.au/afp/2016/april/mthfr-genetic-testing-controversy-and-clinical-implications/

Mazaheri, Mostofizadeh, & Hashemipour. (2017). Homocystinuria with Stroke and Positive Familial History. *Adv Biomed Res*. https://doi.org/10.4103/2277-9175.217215

Morris, A. A. M., Kožich, V., Santra, S., Andria, G., Ben-Omran, T. I. M., Chakrapani, A. B., Crushell, E., Henderson, M. J., Hochuli, M., Huemer, M., Janssen, M. C. H., Maillot, F., Mayne, P. D., McNulty, J., Morrison, T. M., Ogier, H., O'Sullivan, S., Pavlíková, M., de Almeida, I. T., . . . Chapman, K. A. J. J. o. I. M. D. (2017). Guidelines for the diagnosis and management of cystathionine beta-synthase deficiency [journal article]. *40*(1), 49-74. https://doi.org/10.1007/s10545-016-9979-0

Mudd, S. H., Finkelstein, J. D., Refsum, H., Ueland, P. M., Malinow, M. R., Lentz, S. R., Jacobsen, D. W., Brattstrom, L., Wilcken, B., Wilcken, D. E., Blom, H. J., Stabler, S. P., Allen, R. H., Selhub, J., & Rosenberg, I. H. (2000). Homocysteine and its disulfide derivatives: a suggested consensus terminology. *Arterioscler Thromb Vasc Biol*, *20*(7), 1704-1706. https://doi.org/10.1161/01.atv.20.7.1704

Mudd, S. H., Skovby, F., Levy, H. L., Pettigrew, K. D., Wilcken, B., Pyeritz, R. E., Andria, G., Boers, G. H., Bromberg, I. L., Cerone, R., & et al. (1985). The natural history of homocystinuria due to cystathionine beta-synthase deficiency. *Am J Hum Genet*, *37*(1), 1-31. https://pubmed.ncbi.nlm.nih.gov/3872065/

Nelson, B. C., Pfeiffer, C. M., Sniegoski, L. T., & Satterfield, M. B. (2003). Development and evaluation of an isotope dilution LC/MS method for the determination of total homocysteine in human plasma. *Anal Chem*, *75*(4), 775-784. https://doi.org/10.1021/ac0204799

Nexo, E., Engbaek, F., Ueland, P. M., Westby, C., O'Gorman, P., Johnston, C., Kase, B. F., Guttormsen, A. B., Alfheim, I., McPartlin, J., Smith, D., Møller, J., Rasmussen, K., Clarke, R., Scott, J. M., & Refsum, H. (2000). Evaluation of Novel Assays in Clinical Chemistry: Quantification of Plasma Total Homocysteine. *Clinical Chemistry*, *46*(8), 1150. https://doi.org/10.1093/clinchem/46.8.1150

NIH. (2023). *Homocystinuria due to CBS deficiency*. https://rarediseases.info.nih.gov/diseases/6667/homocystinuria-due-to-cbs-deficiency

Okun, J. G., Gan-Schreier, H., Ben-Omran, T., Schmidt, K. V., Fang-Hoffmann, J., Gramer, G., Abdoh, G., Shahbeck, N., Al Rifai, H., Al Khal, A. L., Haege, G., Chiang, C. C., Kasper, D. C., Wilcken, B., Burgard, P., & Hoffmann, G. F. (2017). Newborn Screening for Vitamin B6 Non-responsive Classical Homocystinuria: Systematical Evaluation of a Two-Tier Strategy. *JIMD Rep*, *32*, 87-94. https://doi.org/10.1007/8904_2016_556

Rose, N. C., & Dolan, S. M. (2012). Newborn screening and the obstetrician. *Obstetrics and gynecology*, *120*(4), 908-917. https://doi.org/10.1097/AOG.0b013e31826b2f03

Rosenson, R. S., Smith, C. C., & Bauer, K. A. (2024, Aug 29, 2024). *Overview of homocysteine*. https://www.uptodate.com/contents/overview-of-homocysteine

Sacharow, S. J., Picker, J. D., & Levy, H. L. (2004). Homocystinuria Caused by Cystathionine Beta-Synthase Deficiency. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews((R))*. University of Washington, Seattle. https://www.ncbi.nlm.nih.gov/pubmed/20301697

Wang, W., Jiao, X. H., Wang, X. P., Sun, X. Y., & Dong, C. (2016). MTR, MTRR, and MTHFR Gene Polymorphisms and Susceptibility to Nonsyndromic Cleft Lip With or Without Cleft Palate. *Genet Test Mol Biomarkers*, *20*(6), 297-303. https://doi.org/10.1089/gtmb.2015.0186

Weisberg, I., Tran, P., Christensen, B., Sibani, S., & Rozen, R. (1998). A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Mol Genet Metab*, *64*(3), 169-172. https://doi.org/10.1006/mgme.1998.2714

Zhu, H., Blake, S., Chan, K. T., Pearson, R. B., & Kang, J. (2018). Cystathionine beta-Synthase in Physiology and Cancer. *Biomed Res Int*, *2018*, 3205125. https://doi.org/10.1155/2018/3205125

Transplant Rejection Testing

Policy Number: AHS – M2091 – Transplant Rejection Testing	Prior Policy Name and Number, as applicable: • AHS – M2091 – Heart Transplant Rejection
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Transplant rejection involves an immune response to a transplanted organ. The recipient's immune system recognizes the donated organ as "foreign," thereby initiating an immune response as if the transplanted organ was a foreign antigen. This response may cause the transplanted organ to fail (Vella, 2024). Gene expression profiling tests and serum cell-free DNA evaluation are possible ways to monitor organ transplant rejection (Carey et al., 2018; Crespo-Leiro et al., 2016; Gielis et al., 2015).

II. Related Policies

Policy Number	Policy Title
AHS-G2098	Immune Cell Function Assay

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For heart transplant recipients who are 15 years of age or older and who are 55 or more days post-transplant, the use of AlloMap® **MEETS COVERAGE CRITERIA** at the following frequency:
   a) Every month for individuals who are 2-12 months post-transplant.
   b) Every 3 months for individuals who are 12-36 months post-transplant.
   c) Every 6 months for individuals who are greater than 36 months post-transplant.

2) For heart transplant recipients who are 28 or more days post-transplant, the use of donor-derived cell-free DNA tests (e.g., AlloSure Heart, Prospera™ Heart) **MEETS COVERAGE CRITERIA** at the following frequency:
   a) Every month for individuals who are 1-12 months post-transplant.
   b) Every 3 months for individuals who are 12-36 months post-transplant.
   c) Every 6 months for individuals who are greater than 36 months post-transplant.

3) For single-kidney transplant recipients who are 18 years of age or older and who are at least 14 days post-transplant, the use of donor-derived cell-free DNA tests (e.g., AlloSure Kidney) to assess the probability of allograft rejection **MEETS COVERAGE CRITERIA** at the following frequency:
   a) Once per month for individuals who are 1-4 months post-transplant.
   b) Every 3 months for individuals who are greater than 6 months post-transplant.

4) For kidney transplant recipients with stable renal function, the use of peripheral blood microarray-based genomic tests that analyze gene expression profiles to rule out kidney transplant rejection (e.g., TruGraf) **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

5) For heart transplant recipients, testing for heart transplant rejection with simultaneous gene expression testing and donor-derived cell-free DNA testing (e.g., HeartCare®) **DOES NOT MEET COVERAGE CRITERIA.**

6) For any other organ transplant not listed above (e.g., lungs, liver), the use of donor-derived cell-free DNA tests **DOES NOT MEET COVERAGE CRITERIA**.

7) For any other organ transplant not listed above (e.g., kidneys, lungs, liver), the use of peripheral blood gene expression profiling tests **DOES NOT MEET COVERAGE CRITERIA**.

8) To assess for rejection and injury in transplanted organs, mRNA expression profiling of biopsied tissue from a transplanted organ (e.g., MMDx Heart, MMDx Kidney) **DOES NOT MEET COVERAGE CRITERIA.**

9) To assist in the detection of moderate grade 2R/grade 3 heart transplant rejection, the measurement of volatile organic compounds **DOES NOT MEET COVERAGE CRITERIA**.

IV. Table of Terminology

Term	Definition
AASLD	American Association for the Study of Liver Diseases
ACR	Acute cellular rejection
ALAD	Acute lung allograft dysfunction
AMR	Antibody-mediated rejection
AR	Active rejection
AST	American Society of Transplantation
ASTS	American Society of Transplant Surgeons
AUC	Area under curve
BAL	Bronchoalveolar lavage
BL	Borderline rejection
CARGO	Cardiac Allograft Rejection Gene Expression Observational
cfDNA	Cell-free deoxyribonucleic acid
CLAD	Chronic lung allograft dysfunction
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare and Medicaid
dd-cfDNA	Donor-derived cell-free deoxyribonucleic acid
DNA	Deoxyribonucleic acid
DSA	Donor-specific antibodies
EAU	European Association of Urology
EMA	European Medicines Agency
EMB	Endomyocardial biopsy
ESC	European Society of Cardiology
ESOT	European Society of Organ Transplantation
FCXM	Flow crossmatch
FDA	Food and Drug Administration
FEV1	Forced expiratory volume in one second
GEP	Gene expression profiling
HLA	Human leukocyte antigen
INTERCOMEX	International Collaborative Microarray Study Extension
IFN-γ ELISPOT	Interferon-γ enzyme-linked immunospot
ISHLT	International Society of Heart and Lung Transplantation
IVDMIA	In vitro diagnostic multivariate index assay
KDIGO	Kidney Disease: Improving Global Outcomes
LDT	Laboratory-developed test
LFT	Liver function test

MFI	Mean fluorescence intensity
MHC	Major histocompatibility complex
NGS	Next-generation sequencing
NPV	Negative predictive value
OI	Other injury
PBMC	Peripheral blood mononuclear cells
PPV	Positive predictive value
qRT-PCR	Quantitative real-time polymerase chain reaction methodology
RA	Renal Association
RNA	Ribonucleic acid
rsEMB	Routine surveillance endomyocardial biopsy
SAB	Single-antigen bead
STA	Stable
subAR	Subclinical acute rejection
TCMR	T cell-mediated rejection
TLC	Total lung capacity
VOCs	Volatile organic compounds

V. Scientific Background

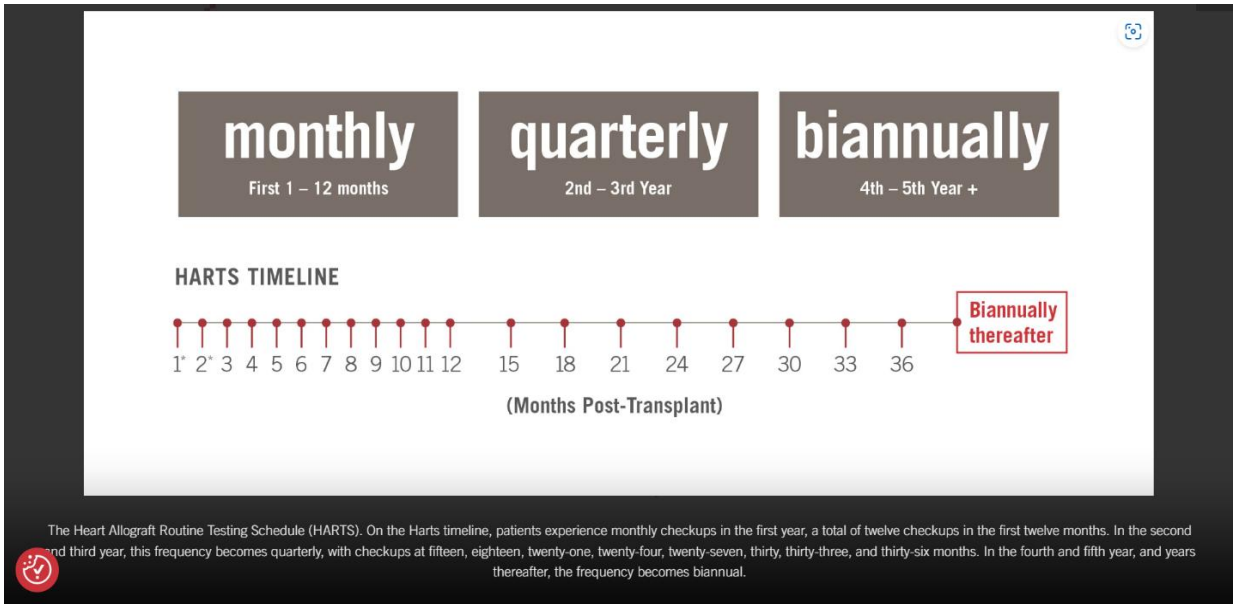
Solid organ transplant is a delicate process, requiring much oversight and evaluation of every party involved. Rejection, or failure of the transplant, is a potential outcome of any transplant case. At the molecular level, rejection is primarily caused by a component of the adaptive immune system, the major histocompatibility complex (MHC) proteins. These proteins must match between donor and recipient, or the transplant can fail (Vella, 2024).

The primary function of MHC proteins is acting as the platform on which T cells identify antigens. Typically, these MHC proteins bind foreign antigens, which are then recognized as such by T cells. From there, the T cells can generate an immune response to handle the antigen. However, the MHC protein products must be identified as "self" by these T cells as well. If an organ donor's MHC protein does not match the recipient's, the recipient's T cells may identify the MHC of the donated organ as "foreign" and subsequently implement an immune response. This eventually starts the cascade of events that causes the transplant to fail (Vella, 2024).

Numerous methods mitigate this immune response. Immunosuppressants, which cause desensitization of the immune response, and more have been proposed as methods to circumvent this immune response (Vella, 2024).

Proprietary Tests – Gene Expression

AlloMap®



The AlloMap test is based on quantitative real-time polymerase chain reaction methodology (qRT-PCR) using RNA purified from peripheral blood mononuclear cells (PBMC). It is a gene expression test (11 informative genes, 9 control genes) that proposes to "aid in the identification of heart transplant recipients with stable allograft function who have a low probability of moderate/severe acute cellular rejection (ACR) at the time of testing in conjunction with standard clinical assessment" (FDA, 2008). Its FDA approval states that it is intended for heart transplant recipients, 15 years or older, and ≥55 days post-transplant (FDA, 2008). From CareDX, Inc., the makers of AlloMap: "The Heart Allograft Routine Testing Schedule (HARTS). On the Harts Timeline, patients experience monthly checkups the first year, a total of twelve checkups in the first year. In the second and third year, this frequency becomes quarterly, with checkups at fifteen, eighteen, twenty-one, twenty-four, twenty-seven, thirty, thirty-three, and thirty-six months. In the fourth and fifth year, and years after, the frequency becomes biannual" (CareDX, 2024d).

MMDx®

Gene expression profiles available through One Lambda for assessment of transplant rejection include MMDx Heart (1283 genes for heart transplants) and MMDx Kidney (1494 genes for kidney transplants). Whereas other tests are performed on a peripheral blood sample, MMDx Heart and MMDx Kidney requires a biopsy sample. The mRNA levels of a set number of genes (depending on the organ) are assessed in the biopsied

tissue and are then compared to the mRNA levels from a reference set of biopsies (One Lambda, 2024).

TruGraf® (Kidney and Liver)

The TruGraf assay is available for kidney and liver transplant recipients. TruGraf gene expression panels are intended for transplant patients and are based on microarray analysis of peripheral blood. TruGraf identifies if a patient is "immune activating" (potentially rejecting) or "immune quiescent" (stable), allowing a clinician to evaluate potential pre-symptomatic kidney damage without use of a biopsy (Eurofins Viracor, 2024; Peddi et al., 2020). According to Eurofins, "120 genes with the highest discriminatory power between the non-TX ["inadequately immunosuppressed"] and TX ["adequately immunosuppressed"] phenotypes are the IP incorporated in TruGraf" (Eurofins, 2023).

Proprietary Tests – Donor-derived Cell-free DNA & Combination

Tissue gene expression is not the only medium tested for rejection. The use of cell-free DNA has shown much promise as a minimally invasive detection method for allograft rejection, and may be used to complement or, ultimately, replace tissue biopsies in the future (Pattar & Greenway, 2020).

Viracor TRAC®

The Viracor TRAC® (Transplant Rejection Allograft Check) donor-derived cell-free DNA (dd-cfDNA) assay uses a non-invasive liquid biopsy to monitor the percentage of dd-cfDNA in transplant recipient plasma post-transplant using next generation sequencing (NGS). The test uses "a bioinformatics pipeline that analyzes NGS and genome-wide recipient genotype data to determine the percentage of dd-cfDNA present, which strongly correlates with allograft injury due to rejection. The licensed algorithm accurately quantifies the donor-derived fraction of cell-free DNA without need for donor genotype information, even when the donor is closely related (e.g., sibling kidney donors). The design of this test also enables the determination of dd-cfDNA concentrations over a much wider dynamic range than currently possible with other methods" (eurofins, 2024). As of 2023, this technology has not been approved by the FDA. Originally TRAC® was also offered as part of a dual-marker rejection panel by the name of OmniGraf; however, this test is no longer commercially available as of April 1, 2023.

AlloSure® Kidney

AlloSure Kidney, a test offered by the same parent company as AlloMap, evaluates cell-free DNA (cfDNA) in the blood for kidney transplant patients. The test states that when

graft injury occurs, donor-derived cell-free DNA is released into the blood where it can be measured as a marker of kidney transplant surveillance. Bloom et al. (2017) evaluated AlloSure Kidney with 102 kidney recipients. They concluded that a donor-derived cell-free DNA (dd-cfDNA) level of >1% indicated active rejection of the graft (Bloom et al., 2017). According to the manufacturer, AlloSure Kidney is validated for use in stable and high-risk patients, patients with allograft rejection, repeat kidney transplant patients, and pediatric patients (CareDX, 2024a).

A 2021 study from Pai et al. examined the clinical rationale behind a routine testing schedule utilizing dd-cfDNA. In this study, they found that dd-cfDNA surveillance was effective at months one, two, three, four, six, nine, and 12 during the first year following kidney transplantation and quarterly thereafter. They noted that these time points "coincide with major immunologic transition points after transplantation" and that these times points provide clinicians with "molecular information to help inform decision making" (Pai et al., 2021).

AlloSure® Heart

AlloSure Heart detects graft injury via a blood test; the test detects injury and rejection by measuring the amount of dd-cfDNA in the blood. Allosure Heart is covered by Medicare after 55 days when ordered in conjunction with AlloMap and with clinical suspicion of rejection. Heartcare by CareDx is another proprietary combination test that offers AlloMap gene expression profiling combined with next-generation sequencing to quantify dd-cfDNA in cardiac transplant recipients. This combination test includes an AlloMap score, the variability of the AlloMap score, and the percent of dd-cfDNA (using Allosure Heart) in one comprehensive single assessment of heart health (CareDx, 2024c).

AlloSure® Lung

AlloSure® Lung is another donor-derived cell-free DNA (dd-cfDNA) test used for transplant recipients that is now commercially available (CareDx, 2024b).

As evidence of its prowess, AlloSure cites what they describe as "the first and only multi-center study to clinically validate the use of dd-cfDNA in lung transplantation"(CareDx, 2024b). In a multicenter, retrospective cohort study, Keller et al. (2022) collected data from lung transplant recipients within three years of transplant and found that "dd-cfDNA was higher in patients with ALAD [acute lung allograft dysfunction] than stable patients" and that the estimated sensitivity of dd-cfDNA ≥1% was 73.9%, specificity of 87.7%, positive predictive value of 43.4% and negative predictive value of 96.5%." Consequently, the authors concluded that "dd-cfDNA identified acute lung allograft dysfunction in asymptomatic lung transplant patients

that may not have been identified by using a clinically indicated biopsy strategy alone," and as such may "support its use as a potential noninvasive marker for surveillance monitoring" (Keller et al., 2022).

Prospera™

Natera, a genetic testing company, has developed the Prospera test, which uses dd-cfDNA to assess kidney, heart, and lung transplant rejection. This blood test is now covered by Medicare for all kidney transplant recipients (Natera, 2024).

Proprietary Tests – Volatile Organic Compounds

Heartsbreath

Another medium used for assessment of rejection is breath. Heartsbreath is an FDA-approved test that purports to predict the probability of grade 3 rejection in heart transplant patients. The test detects "volatile organic compounds" (FDA, 2004a). The FDA notes that this test does not replace biopsy and is only intended as an adjunct to biopsies. The breath markers are considered to be markers of "oxidative stress" (FDA, 2004b).

Clinical Utility and Validity

Pham et al. (2010) conducted a randomized study comparing gene expression profiling and endomyocardial biopsies for monitoring heart transplant patients. A total of 602 patients who had undergone cardiac transplantation six months to five years previously were included. Both groups were found to have similar rates of primary outcomes, hazard ratios, and two year all-causes of mortality. Patients monitored with gene expression profiling underwent fewer biopsies. The researchers concluded that "Among selected patients who had received a cardiac transplant more than 6 months previously and who were at a low risk for rejection, a strategy of monitoring for rejection that involved gene-expression profiling, as compared with routine biopsies, was not associated with an increased risk of serious adverse outcomes and resulted in the performance of significantly fewer biopsies" (Pham et al., 2010).

Deng et al. (2014) evaluated the variability of a heart recipient's gene expression profiling test (AlloMap) scores. Variability was defined as the "the standard deviation of an individual's cumulative test scores." A total of 369 patients from the Invasive Monitoring Attenuation by Gene Expression Profiling (IMAGE) study were included, and "gene expression profiling score variability, but not ordinal scores or scores over threshold, was independently associated with future clinical events." The hazard ratio for a one unit increase in variability was found to be 1.76 (Deng et al., 2014).

Kobashigawa et al. (2015) conducted a single-center randomized controlled trial to evaluate gene expression profiling (GEP) versus endomyocardial biopsy (Moayedi et al.) starting at 55 days post-transplant. Sixty heart transplant patients meeting inclusion criteria were randomized beginning at 55 days post-transplant to either GEP or EMB arms. A positive GEP ≥30 between two and six months, or ≥34 after six months, prompted a follow-up biopsy. The primary end point included a composite of death/retransplant, rejection with hemodynamic compromise or graft dysfunction at 18 months post-transplant. The researchers concluded that "GEP starting at 55 days post-transplant seems comparable with EMB for rejection surveillance in selected heart transplant patients and does not result in increased adverse outcomes. GEP also seems useful to guide corticosteroid weaning" (Kobashigawa et al., 2015).

M. G. Crespo-Leiro et al. (2015) assessed the "prognostic utility of within-patient variability of GEP scores in predicting future significant clinical events, the negative predictive value (NPV) and the positive predictive value (PPV) of GEP score variability in predicting future significant clinical events." A total of 737 patients from the Cardiac Allograft Rejection Gene Expression Observational (CARGO) II trial were included. Estimated prevalence of events was found to be 17%, and events occurred at a median of 391 days after the final GEP test. The authors found that "the GEP variability area under the receiver operator characteristics curve for the prediction of a composite event was 0.72. The NPV for GEP score variability of 0.6 was 97% and the PPV for GEP score variability of 1.5 was 35.4%." The authors concluded that "The GEP score variability may be used in estimating the likelihood of events of death, re-transplantation or graft dysfunction occurring in patients beyond 315 days post-transplant" (M. G. Crespo-Leiro et al., 2015).

Furthermore, Crespo-Leiro et al. (2016) validated the clinical performance of the gene-expression profiling technology in an independent patient population from the CARGO II study. A total of 399 patients were included. The GEP score ranged from 0-39, and the authors identified the optimal cut-off to be 34. At this score (at ≥6 months after transplant), "95.5% (381/399) of GEP tests were true negatives, 4.5% (18/399) were false negatives, 10.2% (6/59) were true positives, and 89.8% (53/59) were false positives." Based on 938 paired biopsies, the area under the curve for distinguishing ≥3A rejection was found to be 0.70 and 0.69 for 2-6 months and ≥6 months, respectively. The authors concluded, "[T]he choice of threshold score for practical use of GEP testing should consider overall clinical assessment of the patient's baseline risk for rejection" (Crespo-Leiro et al., 2016).

Fujita et al. (2017) followed up on the CARGO study by investigating the long-term mortality of 46 patients. They found that 23 patients had an increased AlloMap score 6-9 months after heart transplant whereas the remaining 23 patients had a decreased

score. After a median follow-up time of 8.1 years, all-cause mortality was significantly elevated in patients with an AlloMap increase compared with patients with a decreased score. The authors concluded, "Dynamic changes of the AlloMap score between 6 and 9 months after HT [heart transplant] were strongly related to all-cause long-term survival after HT. These results suggest that AlloMap potentially displays a useful tool to estimate the patients' risk for long-term mortality" (Fujita et al., 2017).

Carey et al. (2018) analyzed 18 months of follow-up in a national cohort of 27 dual organ recipients (18 heart-kidney, eight heart-liver, one heart-lung) matched to 54 heart-only recipients for gender, age, and time to first GEP (AlloMap) test. They found that "during the first 90 days post-transplant, the mean GEP score for dual organ recipients was 25.2 ± 9.1, vs. 23.5 ± 7.7 for heart-only recipients (P = 0.48), with final GEP scores being 29.1 ± 6.1 and 32.3 ± 3.4, respectively (P = 0.34). GEP scores increased over time at a similar rate (P = 0.33) for both groups. During follow-up, mean GEP score among patients with cytomegalovirus infection was 32.3 (n = 14), compared to 26.7 in patients without cytomegalovirus. Only four (2%) of 233 biopsies were positive for mild antibody-mediated rejection; all occurring in two heart-only recipients (GEP scores = 18-33)" (Carey et al., 2018).

Bakir et al. (2018) analyzed time-dependent phenomapping of clinical and molecular data sets from 94 heart transplant patients (1557 clinical encounters) to determine its accuracy in guiding clinical management. Phenomapping's associations were analyzed with "immunosuppression therapy, biomarkers, and the combined clinical end point of death, allograft loss, retransplantation, and rejection," and these findings were further correlated with "clinical parameters, human leucocyte (*sic*) antigen antibody titers, and peripheral blood mononuclear cell gene expression of the AlloMap test genes" (Bakir et al., 2018). The authors found that patients in the group with higher event rates had "increased human leukocyte antigen class I and II antibody titers, higher expression of the *FLT3* AlloMap gene, and lower expression of the *MARCH8* and *WDR40A* AlloMap genes." The authors concluded that "time-dependent precision phenotyping is a mechanistically insightful, data-driven approach to characterize patterns of clinical care and identify ways to improve clinical management and outcomes" (Bakir et al., 2018).

Phillips et al. (2004) evaluated another novel marker of heart transplant rejection: volatile organic compounds (VOCs). A total of 1061 samples were taken from 539 patients prior to endomyocardial biopsy. The combination of nine VOCs in the algorithm "identified Grade 3 rejection (sensitivity 78.6%, specificity 62.4%, cross-validated sensitivity 59.5%, cross-validated specificity 58.8%, positive predictive value 5.6%, negative predictive value 97.2%). Site pathologists identified the same cases with sensitivity of 42.4%, specificity 97.0%, positive predictive value 45.2% and negative predictive value 96.7%" (Phillips et al., 2004). The authors concluded that "a breath test

for markers of oxidative stress was more sensitive and less specific for Grade 3 heart transplant rejection than a biopsy reading by a site pathologist, but the negative predictive values of the 2 tests were similar" (Phillips et al., 2004). However, the Centers for Medicare and Medicaid (CMS) determined that the evidence does not adequately define the technical characteristics of the test nor demonstrate that Heartsbreath testing to predict heart transplant rejection improves health outcomes (CMS, 2009).

Agbor-Enoh et al. (2019) assessed the donor-derived cell-free DNA (ddcfDNA or dd-cfDNA) levels in 106 lung transplant patients and monitored them for development of allograft failure ("defined as severe chronic lung allograft dysfunction [CLAD], retransplantation, and/or death from respiratory failure"). The average level of donor-derived cell-free DNA (%ddcfDNA) was measured and correlated with allograft failure. The authors separated the patients into three tertiles, with median values of 3.6% in the highest tertile, 1.6% in the middle, and 0.7% in the lowest. The highest tertile was calculated to have a 6.6-fold higher risk of allograft failure compared to the lowest and middle tertiles. The researchers concluded, "lung transplant patients with early unresolving allograft injury measured via %ddcfDNA are at risk of subsequent allograft injury, which is often clinically silent, and progresses to allograft failure" (Agbor-Enoh et al., 2019).

A 14-center post-transplant longitudinal study by Bromberg et al. (2017) published in *The Journal of Applied Laboratory Medicine* measured the dd-cfDNA at one, two, three, four, six, nine, and 12 months post-transplant. A total of 380 blood samples were taken during the study, and the median dd-cfDNA value was 0.21%. A value of 1.20% is at the 97.5th percentile. The authors conclude, "In a renal transplant recipient, a dd-cfDNA level above 1.2% is out of range and potentially abnormal" (Bromberg et al., 2017).

Huang et al. (2019) evaluated the ability of cell-free DNA to detect rejection in kidney transplant patients. A total of 63 kidney transplant patients with suspicion of rejection were included. Twenty-seven of these had donor-specific antibodies, and 34 were considered to have rejection by biopsy. The percentage of donor-specific cell-free DNA (dd-cfDNA) was higher in patients with antibody-mediated rejection (AMR) compared to those with no rejection and cell-mediated rejection (AMR: 1.35%, no rejection: 0.38%, cell-mediated rejection: 0.27%). A dd-cfDNA percentage of 0.74% was found to yield a sensitivity of 100%, a specificity of 71.8%, a positive predictive value of 68.6%, and a negative predictive value of 100%. The authors concluded that "the dd-cfDNA test did not discriminate CMR from no rejection among kidney transplant recipients, although performance characteristics were stronger for the discrimination of [AMR]" (Huang et al., 2019).

Schutz et al. (2017) evaluated graft-derived cell-free DNA (GcfDNA)'s ability as a marker for liver transplant rejection. A total of 115 patients were included, and 17 patients contributed samples (n = 31) during a biopsy-proven rejection episode; the remaining 88 contributed samples (n = 282) during stable periods. The samples from the rejection cohort were found to have a higher percentage of GcfDNA than the stable cohort (29.3% vs 3.3%). Liver function tests (LFTs) had low correlation rates with GcfDNA, and the area under the curve was 97.1% for GcfDNA. Overall, the authors concluded that "in this study, determination of GcfDNA allowed for earlier and more sensitive discrimination of acute rejection in liver transplant patients as compared with conventional LFTs" (Schutz et al., 2017).

A meta-analysis focusing on donor-derived cell-free DNA as a diagnostic tool in transplantation conducted by Oellerich et al. (2022) reported that the clinical validity of dd-cfDNA in kidney transplantation has been documented in over 20 studies, and that the exponential decline to a baseline level two weeks after transplantation can be used to discriminate graft injury. Moreover, "Although different concepts have been used to establish threshold values for detection of rejection median dd-cfDNA values in reference populations were very similar ranging from 0.21% to 0.40%," as such consistency is imputed to be evidence of its validity (Oellerich et al., 2022). Elevations of dd-cfDNA have also been observed in patients with TCMR and AMBR, and as such subclinical detection may enable earlier therapeutic interventions and improved outcomes (Halloran et al., 2022; Knight et al., 2019; Oellerich et al., 2022; Oellerich et al., 2021).

Grskovic et al. (2016) performed a validation of AlloSure. The authors included 1117 samples, and AlloSure was used to quantify the fraction of donor-derived cell-free DNA (dd-cfDNA) in both related and unrelated donor-recipient pairs. The quantifiable range was found to be linear from 0.2% to 16%, and the across-runs coefficient of variation was found to be 6.8%. The limit of blank was found to be 0.10%, limit of detection was 0.16%, and limit of quantification was 0.20%. The authors concluded that "application of the assay to clinical samples from heart transplant recipients demonstrated increased levels of dd-cfDNA in patients with biopsy-confirmed rejection and decreased levels of dd-cfDNA after successful rejection treatment" (Grskovic et al., 2016).

M. Crespo-Leiro et al. (2015) compared the levels of dd-cfDNA in heart transplant recipients with biopsy-confirmed rejection to recipients without rejection. A total of 151 plasma samples from 63 patients were evaluated, and 132 of these samples were biopsied. An AlloMap score was also taken. The dd-cfDNA levels were found to be higher in patients with rejection (1.7% vs 0.99%), and an area under curve (Halloran et al.) was measured to be 0.68. The mean AlloMap score was found to be 24.3 in non-

rejection patients and 28.3 for rejection patients. The authors found that the dd-cfDNA levels and AlloMap score were not significantly correlated, proposing that these tests may be complementary. Combining the AlloMap and plasma dd-cfDNA levels yielded an AUC of 0.78 (M. Crespo-Leiro et al., 2015).

Jordan et al. (2018) investigated the use of dd-cfDNA alongside donor-specific antibodies (DSA) testing in identifying antibody-mediated rejection (AMR) of renal allograft recipients (n = 87 patients). They note that the median level of dd-cfDNA was 2.9% in DSA+ patients who have active AMR whereas the dd-cfDNA was significantly lower in both DSA+ patients without AMR (0.34%) or DSA- patients (0.29%). "The positive predictive value of dd-cfDNA (at 1%) to detect active ABMR in DSA+ patients was 81%, whereas the negative predictive value was 83%. The positive predictive value for DSA+ alone was 48%... The combined use of dd-cfDNA and DSA testing may improve the noninvasive diagnosis of active ABMR in kidney transplant patients. Patients with dd-cfDNA+/ DSA+ results have a high probability of active ABMR" (Jordan et al., 2018).

Gielis et al. (2020) obtained samples from 107 kidney transplant recipients to investigate the role of cell-free DNA in acute kidney rejection. Samples were collected between one day and three months after transplantation. The authors noted that increases in cell-free DNA "above a threshold value of 0.88% were significantly associated with the occurrence of episodes of acute rejection (P = 0.017), acute tubular necrosis (P = 0.011) and acute pyelonephritis (P = 0.032)" (Gielis et al., 2020). However, the authors also note that "Although increases in plasma ddcfDNA% are associated with graft injury, plasma ddcfDNA does not outperform the diagnostic capacity of the serum creatinine in the diagnosis of acute rejection" (Gielis et al., 2020).

Peabody et al. (2020) researched the clinical utility of the dd-cfDNA Prospera test by Natera to lower the rate of kidney graft loss. Simulated cases of 154 nephrologists were analyzed for this study; some physicians used dd-cfDNA testing and some did not. Results show that at baseline, there were no differences between primary diagnosis, biopsy decisions, or therapeutic management. However, after use of the cc-cfDNA test, "intervention nephrologists were more likely to arrive at the diagnosis of rejection (OR 4.00, 95% CI 1.93-8.30), make a correct decision on biopsy/transplant center referral (OR 11.07, 95% CI 4.87-25.16), and properly adjust therapeutic management (OR 2.37, 95% CI 1.07-5.24)" (Peabody et al., 2020).

Sigdel et al. (2018) evaluated an SNP-based assay's accuracy in identifying allograft rejection or injury. The assay is intended to identify rejection through measurement of donor-derived cell-free DNA (dd-cf DNA). A total of 193 unique renal transplant patients were included, with a total of 300 plasma samples provided. Of the 300

samples, 217 were biopsy-matched, 38 had active rejection (AR), 72 had borderline rejection (BL), 25 had other injury (Breda et al.), and 82 were stable (STA). The authors found that median dd-cfDNA was higher in biopsy-proven AR (2.3%) compared to BL (0.6%), OI (0.7%), and STA (0.4%). The assay was found to discriminate active rejection from non-rejection at an area under curve of 0.87, 88.7% sensitivity, and 72.6% specificity (at a cutoff of 1% dd-cfDNA). Of 13 patients with AR findings after six months, 12 tested positive by the assay. The authors concluded that their data supported the "feasibility of using this assay to detect disease prior to renal failure and optimize patient management in the case of allograft injury" (Sigdel et al., 2018).

Altug et al. (2019) performed an analytical validation of a "single-nucleotide polymorphism [SNP]-based donor-derived cell-free [cf] DNA assay for detecting rejection in kidney transplant patients". This test measured 13962 SNPs and was validated using 66 unique samples with 1064 replicates. The authors measured the cf-DNA fraction in related and unrelated (genetically related) donor-recipient pairs. The authors identified a "limit of blank" of 0.11% and a limit of detection and quantitation of 0.15% for unrelated donors. For related donors, a limit of blank of 0.23% and a limit of detection and quantitation of 0.29% was identified. Other metrics such as precision and linearity were found to be identical for both categories. The coefficient of variance was found to be 1.8%. The authors concluded that their findings were an adequate analytical validation of the assay (Altug et al., 2019).

In a retrospective, observational study, Rodgers et al. (2023) evaluated the correlation, classification, and prediction for AR across two donor-derived cell-free DNA tests and a blood gene-expression profile test in heart transplantation. Looking at patients who had an EMB and both standard and expanded SNP dd-cfDNA testing in the 14 days preceding the EMB, the authors found that "Both standard and expanded SNP dd-cfDNA tests showed no significant difference in testing accuracy for AR," as the sensitivities of standard SNP and expanded SNP were reported to be 39% and 39%, respectively ($p = 0.66$). The authors also reported that "Specificity with standard SNP was 82% and expanded SNP testing 84% ($p = .26$). PPV with standard SNP was 6.2% and expanded SNP testing 7.0% ($p = .70$). NPV with standard and expanded SNP testing were both 98% ($p = .76$)". Moreover, the researchers evaluated the screening performance of combining GEP with standard SNP testing (standard dd-cfDNA + GEP), commercially packaged as HeartCare® and determined that "specificity of standard dd-cfDNA + GEP was 47%, which was significantly lower when compared to either standard or expanded SNP dd-cfDNA tests individually ($pc < .001$)". Regarding the use of GEP in the detection of ACR, "we [the authors] showed GEP specificity was significantly lower than standard SNP dd-cfDNA ($pc < .001$) but otherwise did not show a significant difference for sensitivity, PPV, and NPV for detection of ACR" and, most notably, "Standard dd-cfDNA + GEP similarly showed significantly reduced

specificity versus standard SNP testing alone for detection of ACR (pc < .001)" (Rodgers et al., 2023). As such, the results suggest that additional coverage offered by expanded SNP assay as compared to the standard SNP assay does "not show a direct impact on discrimination of AR in HTx patients in this study" and "that dd-cfDNA testing, without GEP, is sufficient" for the detection of ACR (Rodgers et al., 2023).

Henricksen et al. (2023) conducted a single center, retrospective analysis of adult heart transplant recipients. There, they focused on survival and rejection-free survival one-year post-transplant as primary endpoints for "A GEP cohort, transplanted from January 1, 2015 through December 31, 2017 and eligible for rejection surveillance with GEP was compared to a paired testing cohort, transplanted July 1, 2018 through June 30, 2020, with surveillance from both dd-cfDNA and GEP". The authors found that "At 1-year, there were no differences between the paired testing and GEP groups in survival (98.4% vs. 94.7%, P = .23) or rejection-free survival (81.3% vs. 73.7% P = .28)," concluding that "Compared to post-transplant rejection surveillance with GEP alone, pairing dd-cfDNA and GEP testing was associated with similar survival and rejection-free survival at 1 year," with the benefit of requiring significantly fewer biopsies (Henricksen et al., 2023).

The use of RNA microarray analysis has also begun to garner attention. The INTERCOMEX (International Collaborative Microarray Study Extension) explored the feasibility of real-time MMDx kidney transplant biopsy assessment by comparing the central MMDx assessment with local stand-of-care in 10 experienced North American and European centers that followed Banff 2013 guidelines. 519 samples from 491 patients were deemed sufficient for RNA microarray analysis (3mm), which was used to determine agreement between the histologic diagnoses. The distribution of the principal diagnoses was as follows: antibody-mediated rejection (ABMR, n=88, 17%), ABMR suspected (n=10, 2%), T cell-mediated rejection (TCMR, n=29, 6%), acute kidney injury (AKI, n=43, 8%), "borderline" (n=31, 6%), unexplained atrophy/fibrosis (n=84, 16%), and "no major abnormalities" (n=141, 27%). According to the authors, the report sign-out diagnosis of rejection agreed with histology with a normalized accuracy of 76-77% overall. However, dissecting the accuracy assessment for MMDx yielded PPVs of 51%, 45%, and 90% for ABMR, TCMR, and rejection, respectively, and NPVs of 92%, 96%, and 54% for the same (Halloran et al., 2017). Nevertheless, the authors concluded that "Feedback received for 451 biopsy samples indicates that clinicians agreed with MMDx more often than with their local biopsy assessment and indicated that MMDx would give them more confidence for clinical management" (Halloran et al., 2017).

Alam et al. (2022) evaluated whether MMDx performs in agreement with other validated modalities. Two hundred and twenty-eight corresponding endomyocardial biopsies and MMDx specimens from 135 adult heart transplant patients were

retrospectively reviewed with correlating donor-derived cell-free DNA (dd-cfDNA). "Rejection was classified on endomyocardial biopsies in 29 specimens (TCMR ≥ 2R and/or AMR ≥ 1), on MMDx in 56 specimens, and in 74 values with dd-cfDNA ≥0.20%. Despite moderate agreement between endomyocardial biopsies and MMDx (84% agreement, Cohen's kappa, 0.48, p < .001), systematic differences were observed (McNemar's test, p < .001) where MMDx classified 32 of 37 discordant cases as rejection. MMDx and dd-cfDNA demonstrated slight agreement (72% agreement, Cohen's kappa, 0.39, p < .001); however, systematic differences were also apparent where MMDx classified 12 of 50 discordant specimens as rejection when dd-cfDNA was <0.20% (McNemar's test, p < .001)" (Alam et al., 2022). Alam et al. (2022) findings provide insight on the performance of MMDx relative to other modalities in a heart transplant cohort and have implications on the surveillance and workup of allograft rejection in heart transplantation.

More recently, the TruGraf blood test has emerged as another possible alternative to expensive and invasive biopsies. This proprietary microarray-based test relies on specific gene expression in the peripheral blood as a means of providing information on the adequacy of immunosuppression in transplant candidates with stable renal function, who may manifest subclinical acute rejection (subAR). A study conducted at the Northwestern University Comprehensive Transplant Center and five participating clinical centers for the Genomics for Kidney Transplantation Project reported that in the 125 candidates demonstrating stable renal function, the TruGraf classifier boasted a PPV of 86% (95% CI: 83-89%) and an NPV of 28% (95% CI 13-49%) (First et al., 2017). In another study, simultaneous blood tests and clinical assessments were conducted on 192 patients from seven transplant centers to evaluate the predictive power of TruGraf v1. Researchers found that the accuracy of TruGraf—defined here as the agreement between TruGraf result and clinical and/or histologic assessment—was 74% (142/192), with an NPV of 90% (Marsh et al., 2019). Given the data, the researchers assert that the use of the TruGraf classifier will eschew unnecessary surveillance biopsies with high confidence and lead to personalization of management in patient care. However, though these results seem promising, the predictive power of TruGraf in patients with graft dysfunction has yet to be evaluated.

Moayedi et al. (2019) published results from the Outcomes AlloMap Registry (OAR) study, a multi-center prospective observational study. The OAR study was comprised of the largest group of patients – 1,504

patients – who had undergone gene expression profiling (GEP) for surveillance purposes to-date. Patients were aged ≥ 15 years and ≥ 55 days post-cardiac transplant with a median age of 54 years old. Results indicated the prevalence of "moderate to severe acute cellular rejection (≥2R) was 2.0% from 2 to 6 months and 2.2% after 6

months." Post-transplant survival at one, two, and five years was 99%, 98%, and 94%, respectively. The authors concluded that patients selected for GEP surveillance had excellent survival outcomes as well as low rates of acute rejection, graft dysfunction, readmission, and death (Moayedi et al., 2019).

As part of the D-OAR (utility of donor-derived cell free DNA in association with gene expression profiling) trials, Khush et al. (2019) reported overall that "dd-cfDNA levels differed significantly between patients with and without AR," such that the "median level of dd-cfDNA in patients with AR was significantly higher (0.17%) than in the group of patient specimens without rejection (0.07%, P < .001)" but that "Median dd-cfDNA levels were 0.17% for both ACR and AMR" (Khush et al., 2019). When considering only the planned biopsy samples, the authors found that "Statistically different median dd-cfDNA levels were observed in patients with and without rejection" (p = 0.02, n = 254), while the surveillance biopsy analysis sample size (n = 587) was purportedly "too small to detect a statistically significant difference in median dd-cfDNA levels" (p =0.140) (Khush et al., 2019). Consequently, the researchers conclude that "We [the authors] demonstrated that dd-cfDNA levels are significantly higher in patients with AR compared with patients with no biopsy evidence of rejection" and that "Surveillance with dd-cfDNA may therefore detect early rejection and thereby trigger augmentation of immunosuppression to prevent a more severe rejection event that may result in irreversible graft damage." However, it should also be noted that many patients were enrolled later than two months posttransplant and that the technique used to evaluate dd-cfDNA test performance (i.e., endomyocardial biopsy) is not a true "gold standard" for the diagnosis of AR (Khush et al., 2019).

Knüttgen et al. (2022) analyzed dd-cfDNA percentage in 87 heart transplant recipients. The study goal was to determine the clinical validity of using graft-derived cell-free DNA as well as the association of dd-cfDNA measurements with cardiac allograft rejection. Per the results, sensitivity was 76% and specificity was 83% for cardiac rejection with approximately a 95% confidence interval. Overall, the authors concluded that dd-cfDNA plasma values were "significantly associated with cardiac rejection", but that pericardial effusions and/or improper sampling "should be considered" as having the ability to confound results in a clinical setting (Knüttgen et al., 2022).

Kamath et al. (2022) evaluated 72 adult heart transplant patients with at least three concurrent AlloMap/AlloSure results in a single-center retrospective study. The aim of the study was to evaluate the performance of AlloSure, AlloMap, and a combination approach in predicting mortality. During the 480-day follow-up period, five patients died. Higher AlloSure variability (HR 1.66, 95%CI 1.14 – 2.41) was associated with increased mortality risk. However, there was not a strong association (of mortality) with higher AlloMap variability, or of combination AlloSure/AlloMap variability. The authors

concluded that, "increased variability of dd-cfDNA in heart transplant patients is associated with both mortality risk and the presence of donor specific antibodies" (Kamath et al., 2022).

An observational study by Kim et al. (2022) aimed to capture the performance of the Prospera dd-cfDNA test in detecting AR in adult heart transplant recipients. The authors reported that "Median dd-cfDNA fraction was significantly higher in samples with a matched biopsy showing AR (median 0.58%, IQR, 0.13%–1.68%) compared to samples where matched biopsies did not show AR (median 0.04%, IQR, 0.01–0.11%, pc < 0.001". This was confirmed for this study population through ROC analysis, where "the AUC-ROC of the dd-cfDNA test to distinguish AR from non-AR was 0.86" and using a dd-cfDNA fraction threshold of 0.15%, "dd-cfDNA detected histologically-proven rejection with a sensitivity of 78.5% (95% CI, 60.7%–96.3%) and a specificity of 76.9% (95% CI, 71.1%–82.7%). A PPV of 25.1% (95% CI, 18.8%–31.5%) and NPV of 97.3% (95% CI, 95.1%–99.5%) were calculated for this cohort (Kim et al., 2022). However, the authors acknowledge that "there may have been a selection bias for patients with previous history or suspicion of AMR, who would be more frequently biopsied at our [the chosen] centers," the higher prevalence of AR in the cohort making it difficult to generalize to patient cohorts with a different risk of rejection, and the limited number of sequential samples (Kim et al., 2022).

Other techniques may be also be worth noting in the future. Montero et al. (2019) analyzed 12 studies and 1181 patients and found that pretransplant interferon-γ enzyme-linked immunospot (IFN-γ ELISPOT) was "significantly associated with increased AR risk (odds ratio: 3.29; 95% confidence interval (CI), 2.34-4.60); hierarchical summary receiver operating characteristic jointly estimated sensitivity and specificity values were 64.9% (95% CI, 53.7%-74.6%) and 65.8% (95% CI, 57.4%-73.5%), respectively, with moderate heterogeneity across studies". But though this is the case, "Prospective randomized clinical trials are warranted" given the "suboptimal predictive ability at an individual level" (Montero et al., 2019).

Holzhauser et al. (2023) reviewed the evidence supporting both GEP and dd-cfDNA testing in heart transplant rejection and provided practical guidance for clinicians to allow for a transition to less invasive options for monitoring rejection after heart transplantation, as well as answering some of the common questions about GEP and dd-cfDNA. One important question they answered was when GEP and dd-cfDNA monitoring should begin post-transplant. As mentioned by previous sources, 55 days post-heart transplant is the earliest timepoint at which GEP monitoring should begin, as the performance of the GEP can be affected by the administration of corticosteroid therapy. In contrast, the dd-cfDNA assay results stabilize approximately 14 days post-

heart transplantation and many centers are using dd-cfDNA surveillance as early as three to four weeks post-transplantation (Holzhauser et al., 2023).

VI. Guidelines and Recommendations

International Society of Heart and Lung Transplantation (ISHLT)

In 2023, the International Society of Heart and Lung Transplantation issued guidelines for the care of heart transplant recipients (Velleca et al., 2023). Below are the recommendations relevant to this policy.

- "It is reasonable to utilize EMB in a HT candidate suspected of having an infiltrative cardiomyopathy or an inflammatory process, such as giant cell myocarditis, amyloidosis, or sarcoidosis. Class IIa, Level of Evidence C
- It is reasonable to perform periodic EMB during the first three to 12 postoperative months for surveillance of rejection **Class IIa, Level of Evidence: C**
- The standard of care for adolescents should be similar to adults, including surveillance EMB for heart allograft rejection for 3 to 12 months after HT. In younger children, especially infants, the risks associated with EMB and required general anesthesia may outweigh the surveillance benefit for comparably rare acute rejection; therefore, it is reasonable to use a combination of noninvasive screening methods (echocardiography, ECG, biomarkers) instead. **Class IIa, Level of Evidence: C**
- After the first postoperative year, it is reasonable to continue EMB surveillance in patients who are at higher risk for late acute rejection. This group includes HT recipients with donor-specific antibodies (DSA), a history of recurrent acute rejection, calcineurin-inhibitor free immunosuppression, reduced immunosuppression due to post-transplant malignancy or chronic infection, African American descent. **Class IIa, Level of Evidence: C**
- Routine EMB later than 5 years after HT are not recommended. EMB should be performed only for cause in patients with signs or symptoms of cardiac allograft dysfunction.
  Class III, Level of Evidence: C
- Children receiving ABO incompatible cardiac allografts in the first 2 years of life with isohemagglutinin titers toward the donor blood group below 1:32 and without elevated titers post-transplant do not require more frequent EMB or non-invasive monitoring compared to recipients of ABO compatible organs. **Class IIa, Level of Evidence: B**

- Ventricular evoked responses (VER) monitoring for rejection surveillance is no longer recommended as the technology has become obsolete. **Class III, Level of Evidence: C**
- Gene Expression Profiling (GEP) (i.e., Allomap) of peripheral blood can be used in low-risk patients between 2 months and 5 years after HT to identify adult recipients who have low risk of current ACR to reduce the frequency of EMB. Data in children does not allow a general recommendation of GEP as a routine tool at present.
  Class IIa, Level of Evidence: B
- It is reasonable to integrate biomarkers such as BNP and high-sensitivity troponins into a rejection monitoring strategy to identify higher risk patients who may benefit from additional evaluation for ACR, AMR, or CAV.
- **Class IIb, Level of Evidence C**
- Post-transplant monitoring for DSA should be performed at 1, 3, 6, and 12 months postoperatively and annually thereafter. Sensitized patients should be monitored more frequently. **Class IIa, Level of Evidence C**
- The use of systemic inflammatory markers such as C-reactive protein (CRP) for acute heart allograft rejection monitoring is not recommended. **Class III, Level of Evidence: C**
- In younger children, especially infants, the risks associated with EMB and required general anesthesia may outweigh the surveillance benefit for comparably rare acute rejection; therefore, it is reasonable to use a combination of noninvasive screening methods (echocardiography, ECG, biomarkers) instead. **Class IIa, Level of Evidence: C**
- Use of the immune cell function assay (ImmuKnow) cannot be recommended in adult and pediatric heart transplant recipients for rejection monitoring. **Class III, Level of Evidence B**
- **Patient Management During a Pandemic**: Efforts should be made to reduce visits by clinically stable heart transplant patients to medical facilities by shifting blood testing to the patients' homes. Remote drawing of blood samples can include screening tests to determine if patients require endomyocardial biopsies using gene expression profiling and donor derived cell-free DNA assays. **Class I, Level of Evidence C"** (Velleca et al., 2023)

In a 2016 guideline discussing antibody-mediated rejection (AMR) of the lung, the ISHLT noted the lack of specific diagnostic criteria for AMR and listed allograft dysfunction, positive histology, positive C4d staining, and donor-specific anti–human

leukocyte antigen (HLA) antibodies (DSA) as potential diagnostic items for AMR (ISHLT, 2016).

Regarding chronic lung allograft dysfunction (CLAD), the society recommended the following:

- "In stable CLAD patients with stable FEV1 [forced expiratory volume in 1 second] or a very slow decline in FEV1 (so-called plateau phase), it is advised to have lung function measured at least every 3 or 4 months."
- "We recommend measuring TLC [total lung capacity] in LTx patients at 3 and 6 months after transplant and annually thereafter. TLC measurements should also be obtained if changes ≥10% from previous values. The "gold standard" technique to assess TLC is body plethysmography."
- "The initial CT scans (inspiratory views with a maximum width of 3-mm sections, and expiratory sections as well) without contrast media are recommended in all LTx patients at 6-month follow-up (when spirometry is usually optimal). Repeat CT studies should be obtained when CLAD is initially diagnosed to better visualize air trapping and various subtle opacities."
- "Transbronchial biopsy and bronchoalveolar lavage (BAL) have a major role in the detection of treatable causes prior to the diagnosis of definite CLAD and should be performed at the start of the diagnostic process to investigate the decline in lung function not explained by obvious, non-CLAD causes."
- "BAL should also be assessed for signs of aspiration, which is suggested by the presence of multinucleated giant cells or foreign organic material (such as meat and plant material), and/or of lipid, as demonstrated on a lipid stain such as oil red-O or Sudan black and/or bile acids (detected by enzymatic assay)" (Verleden et al., 2019).

American Society of Transplant Surgeons (ASTS)

A positional statement on the role of dd-cfDNA in transplant surveillance was approved by the ASTS Executive Committee in March of 2023. The statement aims to provide clarity for clinicians and to advocate for access to molecular diagnostic techniques to support improvement in long term allograft function. ASTS recommends the following, particularly for adult individuals:

- "We suggest that clinicians consider measuring serial dd-cfDNA levels in kidney transplant recipients with stable renal allograft function to exclude the presence of subclinical antibody mediated rejection.

- We recommend that clinicians measure dd-cfDNA levels in kidney transplant recipients with acute allograft dysfunction to exclude the presence of rejection, particularly antibody-mediated rejection (ABMR).
- We do not recommend the use of blood gene expression profiling (GEP) in kidney transplant recipients for the purpose of diagnosing or excluding sub-clinical rejection, as adequate evidence supporting such use is still lacking.
- We do not recommend the use of blood GEP to diagnose or exclude the presence of acute graft rejection in kidney transplant recipients with acute allograft dysfunction given the paucity of data to support this practice.
- We recommend that dd-cfDNA may be utilized to rule out subclinical rejection in heart transplant recipients.
- We recommend that clinicians utilize peripheral blood GEP as a non-invasive diagnostic tool to rule out acute cellular rejection in stable, low-risk, adult heart transplant recipients who are over 55 days status post heart transplantation.
- We recommend that there is still insufficient evidence to recommend dd-cfDNA or GEP testing in liver transplant recipients" (ASTS, 2023).

American Society of Transplantation (ASTS)

The AST's Thoracic and Critical Care Community published a review of currently available and emerging biomarkers to aid in the management of heart transplant recipients. The panel expressed a need for larger and more sophisticated studies to rigorously validate the growing number of potentially useful biomarkers in heart transplant medicine; this need is underscored by the growing awareness that the current gold-standard for detecting rejection (endomyocardial biopsy) has several limitations.

Based on the currently available data, over half the members of the expert panel agreed that Donor-specific HLA antibodies (DSA) and dd-cfDNA used in combination may be the optimal biomarkers to assess clinical outcomes for heart transplant recipients. Despite this potential, however, the committee cautions that current dd-cfDNA assays lack tissue specificity, cannot distinguish between ACR and AMR, and have unproven clinical utility in the context of heart transplant medicine (due to a lack of clinical utility trials) (Kobashigawa et al., 2023).

Heart Failure Association of the European Society of Cardiology (ESC)

The Heart Failure Association of the European Society of Cardiology published a position statement on Advanced Heart Failure (Crespo-Leiro et al., 2018) which states: "Post-transplant patients should undergo a pre-defined regimen of graft biopsies, titration of immunosuppressive and other therapies, rejection monitoring, assessment for infections, transplant coronary artery disease and/or cardiac allograft vasculopathy,

immunosuppression side effects, and other potential complications including neoplasia, and co-morbidities that require comprehensive treatment."

A joint position statement on endomyocardial biopsy (Moayedi et al.) by the Heart Failure Association, Heart Failure Society of America, and Japanese Heart Failure Society Position elaborates, stating that "EMB remains the gold standard for the detection of HTx rejection. EMB after HTx can be scheduled according to a protocol for routine surveillance EMB (rsEMB) in asymptomatic patients, and it is also performed in patients with worsening clinical status, as a symptom triggered EMB." However, it is admitted that though this is the case, "there is a lack of consensus on the optimal timing and frequency of rsEMB" (Seferović et al., 2021).

European Association of Urology (M. Crespo-Leiro et al.)

The European Association of Urology published guidelines on renal transplantation. In it, they state that "the ultimate standard for the diagnosis of rejection is transplant biopsy, because it is impossible to differentiate acute rejection solely on clinical indicators from other causes of renal dysfunction (e.g. acute tubular necrosis, infection, disease recurrence or CNI nephrotoxicity). Therefore, all rejections should be verified by renal biopsy" (Breda et al., 2022).

The EAU also asserted that when it comes to matching donors with recipients, the following recommendations apply:

- "Determine the ABO blood group and the human leukocyte antigen A, B, C and DR phenotypes for all candidates awaiting kidney transplantation."
- "Test both the donor and recipient for human leukocyte antigen DQ. Human leukocyte antigen DP testing may be performed for sensitised patients."
- "Perform thorough testing for HLA antibodies before transplantation."
- "Perform adequate cross-match tests to avoid hyper-acute rejection, before each kidney and combined kidney/pancreas transplantation" (Breda et al., 2022).

Renal Association (RA)

The Renal Association published guidelines regarding post-operative care for kidney transplant patients. These guidelines have been endorsed by the British Transplant Society (BTS). The assessment of rejection recommendations is provided below:

- "We recommend that a transplant renal biopsy should be carried out before treating an acute rejection episode unless this will substantially delay treatment or pose a significant risk to the patient."

- "We recommend that a protocol transplant renal biopsy, defined as a biopsy performed in a stable graft without clinical evidence of acute rejection, be considered in the setting of persisting delayed graft function."

Furthermore, in the rationale, the RA states that "Rejection episodes are characteristically associated with loss of graft function but diagnosis is best established by a percutaneous biopsy since it differentiates rejection clearly from other causes of graft dysfunction. Recognition of different forms of rejection may inform different treatment regimens (e.g. antibody mediated rejection)" (Baker et al., 2017).

American Association for the Study of Liver Diseases (AASLD) and the American Society of Transplantation (ASTS)

These joint guidelines provide guidance on the long-term management of liver transplants. Their recommendations concerning assessment of rejection are as follows:

- "Rejection can be reliably diagnosed only on the basis of liver histology; a biopsy sample should be taken before treatment initiation and classified according to the Banff criteria."
- The guidelines also note that "Both forms of rejection are, until the late stages, asymptomatic, and the diagnosis is made through the investigation of abnormal liver tests; the diagnosis can be confirmed only on the basis of histology" (Lucey et al., 2013).

Kidney Disease: Improving Global Outcomes (KDIGO)

The KDIGO does not list any gene expression or cell-free DNA techniques in their guideline for evaluating and managing transplant recipient patients (KDIGO, 2010, 2020).

European Society for Organ Transplantation (ESOT)

A working group from the European Society for Organ Transplantation submitted a Broad Scientific Advice request to the European Medicines Agency (EMA) outlining the alloimmune and non-alloimmune factors driving allograft rejection. ESOT proposes the following for alloimmune risk stratification in CDC-negative kidney transplantation:

- Transplantation performed with an HLA-identical donor carries a significantly lower immunological risk than transplantation from a donor of another HLA status.
- For the same allogeneic eplet load, grafts from living donors, which are better preserved and are therefore less immunogenic than grafts from deceased donors, are associated with a lower immunological risk.

- Patients with anti-donor serological memory at the time of or short time before transplantation (i.e., those with the presence of HLA-DSA) should be clearly differentiated from the others:
  - Patients with donor reactivity are likely to have immune reactions to the allograft, with a heightened risk of post-transplant AMR and poor allograft outcome.
  - SAB testing is the gold standard to establish the repertoire of serologic memory and define the presence of a recipient's circulating anti-HLA DSA.
    - Local transplant physicians and immunologists should determine the appropriate cut-off point, with a focus on plausibility of immunization history.
    - In absence of clinical validation, non–HLA-DSA routine screening assays should not be considered when evaluating immunological risk of a transplantation.
  - Using SAB testing, three risk groups can be identified (patients with non–donor-specific HLA antibodies and no previous exposure to donor antigen are considered as naïve patients):
    - Group 1: Patients with no signs of anti-HLA immune sensitization at any time point (very low risk).
    - Group 2: Patients with previous exposure to donor antigens or history of HLA-DSA positivity, but without HLA-DSA at time of transplantation (intermediate risk due to likely presence of memory T and/or B alloimmune response): T/B ELISpot assays could identify anti-donor memory cells, but without clinical validation these assays should not be considered when evaluating immunological risk of a transplantation.
    - Group 3: Patients with HLA-DSA at time of transplantation (high risk). There is ongoing discussion on the utility of MFI in SAB or FCXM as a potential surrogate to estimate the DSA titer and for individual risk stratification.
    - Molecular HLA mismatch analysis is likely to play a future role in better allocating more compatible allografts, as well as in stratifying the primary alloimmune risk. However, in the absence of consensus regarding what algorithm (and which cut-off) should be used to quantify the eplet load and whether the quality of eplet should also be considered, it is difficult to integrate such information immediately into clinical practice and clinical trial design. Further consensus building is necessary.
    - Although it is a fast-evolving field, no reliable test is currently available to measure innate immune alloreactivity in routine clinical practice. (Bestard et al., 2022)

In reviewing these conclusions, the Committee for Medicinal Products for Human Use (CHMP) of the European Medicines Agency (EMA) "agreed that several important issues need to be considered in assessing the alloimmune risk following kidney transplantation. These include general characteristics of the recipient and donor, as well as issues related to the transplanted organ and issues requiring further studies and/or consensus before adapting into general guidelines" (Bestard et al., 2022).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

AlloMap was approved by the FDA on August 26, 2008, as an In Vitro Diagnostic Multivariate Index assay (IVDMIA) test service, performed in a single laboratory, assessing the gene expression profile of RNA isolated from peripheral blood mononuclear cells (PBMC). AlloMap Testing is intended to aid in the identification of heart transplant recipients with stable allograft function who have a low probability of moderate/severe acute cellular rejection (ACR) at the time of testing in conjunction with standard clinical assessment (FDA, 2008).

The Heartsbreath test received approval on February 24, 2004, through the FDA's humanitarian device exemption. The order is described as being "indicated for use as an aid in the diagnosis of grade 3 heart transplant rejection in patients who have received heart transplants within the preceding year. The heartsbreath test is intended to be used as an adjunct to, and not as a substitute for, endomyocardial biopsy. The use of the device is limited to patients who have had endomyocardial biopsy within the previous month" (FDA, 2004b).

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by

the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81479	Unlisted molecular pathology procedure
81595	Cardiology (heart transplant), mRNA, gene expression profiling by real-time quantitative PCR of 20 genes (11 content and 9 housekeeping), utilizing subfraction of peripheral blood, algorithm reported as a rejection risk score Proprietary test: AlloMap® Lab/Manufacturer: CareDx, Inc.
81599	Unlisted multianalyte assay with algorithmic analysis
84999	Unlisted chemistry procedure
0055U	Cardiology (heart transplant), cell-free DNA, PCR assay of 96 DNA target sequences (94 single nucleotide polymorphism targets and two control targets), plasma Proprietary test: myTAIHEART Lab/Manufacturer: TAI Diagnostics, Inc.
0087U	Cardiology (heart transplant), mRNA gene expression profiling by microarray of 1283 genes, transplant biopsy tissue, allograft rejection and injury algorithm reported as a probability score Proprietary test: Molecular Microscope® MMDx-Heart Lab/Manufacturer: Kashi Clinical Laboratories
0088U	Transplantation medicine (kidney allograft rejection), microarray gene expression profiling of 1494 genes, utilizing transplant biopsy tissue, algorithm reported as a probability score for rejection Proprietary test: Molecular Microscope® MMDx-Kidney Lab/Manufacturer: Kashi Clinical Laboratories
0118U	Transplantation medicine, quantification of donor-derived cell-free DNA using whole genome next-generation sequencing, plasma, reported as percentage of donor-derived cell-free DNA in the total cell-free DNA Proprietary test: Viracor TRAC™ dd-cfDNA Lab/Manufacturer: Viracor Eurofins
0319U	Nephrology (renal transplant), RNA expression by select transcriptome sequencing, using pretransplant peripheral blood, algorithm reported as a risk score for early acute rejection Proprietary test: Clarava™ Lab/Manufacturer: Verici Dx/Verici Dx, Inc

CPT	Code Description
0320U	Nephrology (renal transplant), RNA expression by select transcriptome sequencing, using posttransplant peripheral blood, algorithm reported as a risk score for acute cellular rejection Proprietary test: Tuteva™ Lab/Manufacturer: Verici Dx/Verici Dx, Inc

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Agbor-Enoh, S., Wang, Y., Tunc, I., Jang, M. K., Davis, A., De Vlaminck, I., Luikart, H., Shah, P. D., Timofte, I., Brown, A. W., Marishta, A., Bhatti, K., Gorham, S., Fideli, U., Wylie, J., Grimm, D., Goodwin, N., Yang, Y., Patel, K., . . . Valantine, H. A. (2019). Donor-derived cell-free DNA predicts allograft failure and mortality after lung transplantation. *EBioMedicine*, *40*, 541-553. https://doi.org/10.1016/j.ebiom.2018.12.029

Alam, A., Van Zyl, J., Paul Milligan, G., Michelle McKean, S., Patel, R., & Anne Hall, S. (2022). Evolving the surveillance and workup of heart transplant rejection: A real-world analysis of the Molecular Microscope Diagnostic System. *Am J Transplant*, *22*(10), 2443-2450. https://doi.org/10.1111/ajt.17087

Altug, Y., Liang, N., Ram, R., Ravi, H., Ahmed, E., Brevnov, M., Swenerton, R. K., Zimmermann, B., Malhotra, M., Demko, Z. P., Billings, P. R., & Ryan, A. (2019). Analytical Validation of a Single-nucleotide Polymorphism-based Donor-derived Cell-free DNA Assay for Detecting Rejection in Kidney Transplant Patients. *Transplantation*, *103*(12). https://journals.lww.com/transplantjournal/Fulltext/2019/12000/Analytical_Validation_of_a_Single_nucleotide.31.aspx

ASTS. (2023). *ASTS Statement on donor-derived cell-free DNA (dd-cf-DNA)*. https://asts.org/docs/default-source/position-statements/dd-cfdna-position-statement.pdf

Baker, R. J., Mark, P. B., Patel, R. K., Stevens, K. K., & Palmer, N. (2017). Renal association clinical practice guideline in post-operative care in the kidney transplant recipient. *BMC Nephrol*, *18*(1), 174. https://doi.org/10.1186/s12882-017-0553-2

Bakir, M., Jackson, N. J., Han, S. X., Bui, A., Chang, E., Liem, D. A., Ardehali, A., Ardehali, R., Baas, A. S., Press, M. C., Cruz, D., Deng, M. C., DePasquale, E. C., Fonarow, G. C., Khuu, T., Kwon, M. H., Kubak, B. M., Nsair, A., Phung, J. L., . . . Cadeiras, M. (2018). Clinical phenomapping and outcomes after heart transplantation. *J Heart Lung Transplant*, *37*(8), 956-966. https://doi.org/10.1016/j.healun.2018.03.006

Bestard, O., Thaunat, O., Bellini, M. I., Böhmig, G. A., Budde, K., Claas, F., Couzi, L., Furian, L., Heemann, U., Mamode, N., Oberbauer, R., Pengel, L., Schneeberger, S., & Naesens, M. (2022). Alloimmune Risk Stratification for Kidney Transplant Rejection. *Transpl Int*, *35*, 10138. https://doi.org/10.3389/ti.2022.10138

Bloom, R. D., Bromberg, J. S., Poggio, E. D., Bunnapradist, S., Langone, A. J., Sood, P., Matas, A. J., Mehta, S., Mannon, R. B., Sharfuddin, A., Fischbach, B., Narayanan, M., Jordan, S. C., Cohen, D., Weir, M. R., Hiller, D., Prasad, P., Woodward, R. N., Grskovic, M., . . . Brennan, D. C. (2017). Cell-Free DNA and Active Rejection in Kidney Allografts. *J Am Soc Nephrol*, *28*(7), 2221-2232. https://doi.org/10.1681/asn.2016091034

Breda, A., Budde, K., Figueiredo, A., Lledó García, E., Olsburgh, J., Regele, H., Boissier, R., V. Hevia, V., Rodríguez Faba, O., Zakri, R. H., & Smith, E. J. (2022). European Association of Urology Guidelines on Renal Transplantation: Update 2021. *Eur Urol Focus*. https://d56bochluxqnz.cloudfront.net/documents/full-guideline/EAU-Guidelines-on-Renal-Transplantation-2022.pdf

Bromberg, J. S., Brennan, D. C., Poggio, E., Bunnapradist, S., Langone, A., Sood, P., Matas, A. J., Mannon, R. B., Mehta, S., Sharfuddin, A., Fischbach, B., Narayanan, M., Jordan, S. C., Cohen, D. J., Zaky, Z. S., Hiller, D., Woodward, R. N., Grskovic, M., Sninsky, J. J., . . . Bloom, R. D. (2017). Biological Variation of Donor-Derived Cell-Free DNA in Renal Transplant Recipients: Clinical Implications. *The Journal of Applied Laboratory Medicine: An AACC Publication*, *2*(3), 309-321. https://doi.org/10.1373/jalm.2016.022731

CareDX. (2024a). *AlloSure Kidney Care Test Info*. https://caredx.com/products-and-services/transplant-services/kidney/allosure/

CareDx. (2024b). *Allosure Lung*. https://caredx.com/products-and-services/transplant-services/lung/

CareDx. (2024c). *HeartCare* https://caredx.com/products-and-services/transplant-services/heart/heartcare/

CareDX. (2024d). *Identify Heart Transplant Injury With HeartCare*. https://caredx.com/products-and-services/transplant-services/heart/heartcare/

Carey, S. A., Tecson, K. M., Jamil, A. K., Felius, J., Wolf-Doty, T. K., & Hall, S. A. (2018). Gene expression profiling scores in dual organ transplant patients are similar to those in heart-only recipients. *Transpl Immunol*. https://doi.org/10.1016/j.trim.2018.03.003

CMS. (2009). *National Coverage Determination (NCD) for Heartsbreath Test for Heart Transplant Rejection (260.10)*. https://www.cms.gov/medicare-coverage-database/view/ncd.aspx?NCDId=325&ncdver=1&

Crespo-Leiro, M., Zuckermann, A., Stypmann, J., Mohacsi, P., Grskovic, M., Beausang, J., Hiller, D., Sit, R., Christie, B., Elechko, J., Woodward, R., Yee, J., & Vanhaecke, J. (2015). Increased Plasma Levels of Donor-Derived Cell-Free DNA Correlate With Rejection in

Heart Transplant Recipients: The CARGO II Multicenter Trial. *The Journal of Heart and Lung Transplantation*, *34*(4), S31-S32. https://doi.org/10.1016/j.healun.2015.01.075
Crespo-Leiro, M. G., Metra, M., Lund, L. H., Milicic, D., Costanzo, M. R., Filippatos, G., Gustafsson, F., Tsui, S., Barge-Caballero, E., De Jonge, N., Frigerio, M., Hamdan, R., Hasin, T., Hulsmann, M., Nalbantgil, S., Potena, L., Bauersachs, J., McDonagh, T., Seferovic, P., & Ruschitzka, F. (2018). Advanced heart failure: a position statement of the Heart Failure Association of the European Society of Cardiology. *Eur J Heart Fail*. https://doi.org/10.1002/ejhf.1236
Crespo-Leiro, M. G., Stypmann, J., Schulz, U., Zuckermann, A., Mohacsi, P., Bara, C., Ross, H., Parameshwar, J., Zakliczynski, M., Fiocchi, R., Hoefer, D., Colvin, M., Deng, M. C., Leprince, P., Elashoff, B., Yee, J. P., & Vanhaecke, J. (2016). Clinical usefulness of gene-expression profile to rule out acute rejection after heart transplantation: CARGO II. *Eur Heart J*, *37*(33), 2591-2601. https://doi.org/10.1093/eurheartj/ehv682
Crespo-Leiro, M. G., Stypmann, J., Schulz, U., Zuckermann, A., Mohacsi, P., Bara, C., Ross, H., Parameshwar, J., Zakliczynski, M., Fiocchi, R., Hoefer, D., Deng, M., Leprince, P., Hiller, D., Eubank, L., Deljkich, E., Yee, J. P., & Vanhaecke, J. (2015). Performance of gene-expression profiling test score variability to predict future clinical events in heart transplant recipients. *BMC Cardiovasc Disord*, *15*, 120. https://doi.org/10.1186/s12872-015-0106-1
Deng, M. C., Elashoff, B., Pham, M. X., Teuteberg, J. J., Kfoury, A. G., Starling, R. C., Cappola, T. P., Kao, A., Anderson, A. S., Cotts, W. G., Ewald, G. A., Baran, D. A., Bogaev, R. C., Shahzad, K., Hiller, D., Yee, J., & Valantine, H. A. (2014). Utility of gene expression profiling score variability to predict clinical events in heart transplant recipients. *Transplantation*, *97*(6), 708-714. https://doi.org/10.1097/01.TP.0000443897.29951.cf
Eurofins. (2023). How Do You Anticipate Allograft Rejection? https://transplantgenomics.com/biomarkers/
eurofins. (2024). *Viracor TRAC® Kidney dd-cfDNA*. https://www.eurofins-viracor.com/
Eurofins Viracor. (2024). *TruGraf® Blood Gene Expression Test Test Code: 30929*. https://www.eurofins-viracor.com/
FDA. (2004a). The Heartsbreath Test: Product Labeling for Physicians. https://www.accessdata.fda.gov/cdrh_docs/pdf3/H030004c.pdf
FDA. (2004b). *SUMMARY OF SAFETY AND PROBABLE BENEFIT. Menssana Research, Inc. Hearts breath Test for Grade 3 Heart Transplant Rejection* U.S. Food and Drug Administration Retrieved from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfHDE/hde.cfm?id=375517
FDA. (2008). 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION https://www.accessdata.fda.gov/cdrh_docs/reviews/k073482.pdf
First, M. R., Whisenant, T., Friedewald, J. J., Lewis, P., Rose, S., Lee, D., Pierry, D., Kurian, S. M., Gelbart, T., & Abecassis, M. M. (2017). Clinical Utility of Peripheral Blood Gene

Expression Profiling of Kidney Transplant Recipients to Assess the Need for Surveillance Biopsies in Subjects with Stable Renal Function. *Journal of Transplantation Technologies & Research*, *7*(3). https://doi.org/10.4172/2161-0991.1000177

Fujita, B., Prashovikj, E., Schulz, U., Borgermann, J., Sunavsky, J., Fuchs, U., Gummert, J., & Ensminger, S. (2017). Predictive value of gene expression profiling for long-term survival after heart transplantation. *Transpl Immunol*, *41*, 27-31. https://doi.org/10.1016/j.trim.2017.02.001

Gielis, E. M., Ledeganck, K. J., De Winter, B. Y., Del Favero, J., Bosmans, J. L., Claas, F. H., Abramowicz, D., & Eikmans, M. (2015). Cell-Free DNA: An Upcoming Biomarker in Transplantation. *Am J Transplant*, *15*(10), 2541-2551. https://doi.org/10.1111/ajt.13387

Gielis, E. M., Ledeganck, K. J., Dendooven, A., Meysman, P., Beirnaert, C., Laukens, K., De Schrijver, J., Van Laecke, S., Van Biesen, W., Emonds, M. P., De Winter, B. Y., Bosmans, J. L., Del Favero, J., & Abramowicz, D. (2020). The use of plasma donor-derived, cell-free DNA to monitor acute rejection after kidney transplantation. *Nephrol Dial Transplant*, *35*(4), 714-721. https://doi.org/10.1093/ndt/gfz091

Grskovic, M., Hiller, D. J., Eubank, L. A., Sninsky, J. J., Christopherson, C., Collins, J. P., Thompson, K., Song, M., Wang, Y. S., Ross, D., Nelles, M. J., Yee, J. P., Wilber, J. C., Crespo-Leiro, M. G., Scott, S. L., & Woodward, R. N. (2016). Validation of a Clinical-Grade Assay to Measure Donor-Derived Cell-Free DNA in Solid Organ Transplant Recipients. *J Mol Diagn*, *18*(6), 890-902. https://doi.org/10.1016/j.jmoldx.2016.07.003

Halloran, P. F., Reeve, J., Akalin, E., Aubert, O., Bohmig, G. A., Brennan, D., Bromberg, J., Einecke, G., Eskandary, F., Gosset, C., Duong Van Huyen, J. P., Gupta, G., Lefaucheur, C., Malone, A., Mannon, R. B., Seron, D., Sellares, J., Weir, M., & Loupy, A. (2017). Real Time Central Assessment of Kidney Transplant Indication Biopsies by Microarrays: The INTERCOMEX Study. *Am J Transplant*, *17*(11), 2851-2862. https://doi.org/10.1111/ajt.14329

Halloran, P. F., Reeve, J., Madill-Thomsen, K. S., Demko, Z., Prewett, A., & Billings, P. (2022). The Trifecta Study: Comparing Plasma Levels of Donor-derived Cell-Free DNA with the Molecular Phenotype of Kidney Transplant Biopsies. *J Am Soc Nephrol*, *33*(2), 387-400. https://doi.org/10.1681/asn.2021091191

Henricksen, E. J., Moayedi, Y., Purewal, S., Twiggs, J. V., Waddell, K., Luikart, H., Han, J., Feng, K., Wayda, B., Lee, R., Shudo, Y., Jimenez, S., Khush, K. K., & Teuteberg, J. J. (2023). Combining donor derived cell free DNA and gene expression profiling for non-invasive surveillance after heart transplantation. *Clin Transplant*, *37*(3), e14699. https://doi.org/10.1111/ctr.14699

Holzhauser, L., DeFilippis, E. M., Nikolova, A., Byku, M., Contreras, J. P., De Marco, T., Hall, S., Khush, K. K., & Vest, A. R. (2023). The End of Endomyocardial Biopsy?: A Practical

Guide for Noninvasive Heart Transplant Rejection Surveillance. *JACC Heart Fail*, *11*(3), 263-276. https://doi.org/10.1016/j.jchf.2022.11.002

Huang, E., Sethi, S., Peng, A., Najjar, R., Mirocha, J., Haas, M., Vo, A., & Jordan, S. C. (2019). Early clinical experience using donor-derived cell-free DNA to detect rejection in kidney transplant recipients. *Am J Transplant*, *19*(6), 1663-1670. https://doi.org/10.1111/ajt.15289

ISHLT. (2016). Antibody-mediated rejection of the lung: A consensus report of the International Society for Heart and Lung Transplantation. https://www.jhltonline.org/article/S1053-2498(16)01277-8/pdf

Jordan, S. C., Bunnapradist, S., Bromberg, J. S., Langone, A. J., Hiller, D., Yee, J. P., Sninsky, J. J., Woodward, R. N., & Matas, A. J. (2018). Donor-derived Cell-free DNA Identifies Antibody-mediated Rejection in Donor Specific Antibody Positive Kidney Transplant Recipients. *Transplant Direct*, *4*(9), e379. https://doi.org/10.1097/txd.0000000000000821

Kamath, M., Shekhtman, G., Grogan, T., Hickey, M. J., Silacheva, I., Shah, K. S., Shah, K. S., Hairapetian, A., Gonzalez, D., Godoy, G., Reed, E. F., Elashoff, D., Bondar, G., & Deng, M. C. (2022). Variability in Donor-Derived Cell-Free DNA Scores to Predict Mortality in Heart Transplant Recipients - A Proof-of-Concept Study. *Front Immunol*, *13*, 825108. https://doi.org/10.3389/fimmu.2022.825108

KDIGO. (2010). Managing KIDNEY TRANSPLANT RECIPIENTS. https://kdigo.org/wp-content/uploads/2017/02/KDIGO_TX_NephsTool-Managing-Kidney-Transplant-Recipients.pdf

KDIGO. (2020). KDIGO Clinical Practice Guideline on the Evaluation and Management of Candidates for Kidney Transplantation. *Official Journal of the Tranplantation Society and International Liver Transplantation Society*, *104*(4S). https://kdigo.org/wp-content/uploads/2018/08/KDIGO-Txp-Candidate-GL-FINAL.pdf

Keller, M., Sun, J., Mutebi, C., Shah, P., Levine, D., Aryal, S., Iacono, A., Timofte, I., Mathew, J., Varghese, A., Giner, C., & Agbor-Enoh, S. (2022). Donor-derived cell-free DNA as a composite marker of acute lung allograft dysfunction in clinical care. *J Heart Lung Transplant*, *41*(4), 458-466. https://doi.org/10.1016/j.healun.2021.12.009

Khush, K. K., Patel, J., Pinney, S., Kao, A., Alharethi, R., DePasquale, E., Ewald, G., Berman, P., Kanwar, M., Hiller, D., Yee, J. P., Woodward, R. N., Hall, S., & Kobashigawa, J. (2019). Noninvasive detection of graft injury after heart transplant using donor-derived cell-free DNA: A prospective multicenter study. *Am J Transplant*, *19*(10), 2889-2899. https://doi.org/10.1111/ajt.15339

Kim, P. J., Olymbios, M., Siu, A., Wever Pinzon, O., Adler, E., Liang, N., Swenerton, R., Sternberg, J., Kaur, N., Ahmed, E., Chen, Y. A., Fehringer, G., Demko, Z. P., Billings, P. R., & Stehlik, J. (2022). A novel donor-derived cell-free DNA assay for the detection of acute rejection in heart transplantation. *J Heart Lung Transplant*, *41*(7), 919-927. https://doi.org/10.1016/j.healun.2022.04.002

Knight, S. R., Thorne, A., & Lo Faro, M. L. (2019). Donor-specific Cell-free DNA as a Biomarker in Solid Organ Transplantation. A Systematic Review. *Transplantation*, *103*(2), 273-283. https://doi.org/10.1097/tp.0000000000002482

Knüttgen, F., Beck, J., Dittrich, M., Oellerich, M., Zittermann, A., Schulz, U., Fuchs, U., Knabbe, C., Schütz, E., Gummert, J., & Birschmann, I. (2022). Graft-derived Cell-free DNA as a Noninvasive Biomarker of Cardiac Allograft Rejection: A Cohort Study on Clinical Validity and Confounding Factors. *Transplantation*, *106*(3), 615-622. https://doi.org/10.1097/tp.0000000000003725

Kobashigawa, J., Hall, S., Shah, P., Fine, B., Halloran, P., Jackson, A. M., Khush, K. K., Margulies, K. B., Sani, M. M., Patel, J. K., Patel, N., Peyster, E., & conference, p. (2023). The evolving use of biomarkers in heart transplantation: Consensus of an expert panel. *Am J Transplant*, *23*(6), 727-735. https://doi.org/10.1016/j.ajt.2023.02.025

Kobashigawa, J., Patel, J., Azarbal, B., Kittleson, M., Chang, D., Czer, L., Daun, T., Luu, M., Trento, A., Cheng, R., & Esmailian, F. (2015). Randomized pilot trial of gene expression profiling versus heart biopsy in the first year after heart transplant: early invasive monitoring attenuation through gene expression trial. *Circ Heart Fail*, *8*(3), 557-564. https://doi.org/10.1161/circheartfailure.114.001658

Lucey, M. R., Terrault, N., Ojo, L., Hay, J. E., Neuberger, J., Blumberg, E., & Teperman, L. W. (2013). Long-Term Management of the Successful Adult Liver Transplant: 2012 Practice Guideline by AASLD and the AST. https://www.aasld.org/sites/default/files/2019-06/141022_Guideline_Adult-LT_Management_4UFb.pdf

Marsh, C. L., Kurian, S. M., Rice, J. C., Whisenant, T. C., David, J., Rose, S., Schieve, C., Lee, D., Case, J., Barrick, B., Peddi, V. R., Mannon, R. B., Knight, R., Maluf, D., Mandelbrot, D., Patel, A., Friedewald, J. J., Abecassis, M. M., & First, M. R. (2019). Application of TruGraf v1: A Novel Molecular Biomarker for Managing Kidney Transplant Recipients With Stable Renal Function. *Transplantation Proceedings*, *51*(3), 722-728. https://doi.org/https://doi.org/10.1016/j.transproceed.2019.01.054

Moayedi, Y., Foroutan, F., Miller, R. J. H., Fan, C. S., Posada, J. G. D., Alhussein, M., Tremblay-Gravel, M., Oro, G., Luikart, H. I., Yee, J., Shullo, M. A., Khush, K. K., Ross, H. J., & Teuteberg, J. J. (2019). Risk evaluation using gene expression screening to monitor for acute cellular rejection in heart transplant recipients. *J Heart Lung Transplant*, *38*(1), 51-58. https://doi.org/10.1016/j.healun.2018.09.004

Montero, N., Farouk, S., Gandolfini, I., Crespo, E., Jarque, M., Meneghini, M., Torija, A., Maggiore, U., Cravedi, P., & Bestard, O. (2019). Pretransplant Donor-specific IFNγ ELISPOT as a Predictor of Graft Rejection: A Diagnostic Test Accuracy Meta-analysis. *Transplant Direct*, *5*(5), e451. https://doi.org/10.1097/txd.0000000000000886

Natera. (2024). *Prospera*. https://www.natera.com/organ-health/prospera-organ-transplantation-assessment

Oellerich, M., Budde, K., Osmanodja, B., Bornemann-Kolatzki, K., Beck, J., Schütz, E., & Walson, P. D. (2022). Donor-derived cell-free DNA as a diagnostic tool in transplantation. *Front Genet*, *13*, 1031894. https://doi.org/10.3389/fgene.2022.1031894

Oellerich, M., Sherwood, K., Keown, P., Schütz, E., Beck, J., Stegbauer, J., Rump, L. C., & Walson, P. D. (2021). Liquid biopsies: donor-derived cell-free DNA for the detection of kidney allograft injury. *Nat Rev Nephrol*, *17*(9), 591-603. https://doi.org/10.1038/s41581-021-00428-0

One Lambda. (2024). *MMDx® for Molecular Biopsy Assessment*. https://www.thermofisher.com/onelambda/us/en/post-transplant/molecular-biopsy-assessment.html

Pai, A., Swan, J. T., Wojciechowski, D., Qazi, Y., Dholakia, S., Shekhtman, G., Abou-Ismail, A., & Kumar, D. (2021). Clinical Rationale for a Routine Testing Schedule Using Donor-Derived Cell-Free DNA After Kidney Transplantation. *Ann Transplant*, *26*, e932249. https://doi.org/10.12659/AOT.932249

Pattar, S., & Greenway, S. (2020). Monitoring the Health of Solid Organs After Transplantation Using Cell-Free DNA. *AACC*. https://www.aacc.org/publications/cln/articles/2020/june/monitoring-the-health-of-solid-organs-after-transplantation-using-cell-free-dna

Peabody, J., Billings, P., Valdenor, C., Demko, Z., Moshkevich, S., Tran, M., & Paculdo, D. (2020). Randomized clinical trial of a novel donor-derived cfDNA test to detect rejection in CPV-simulated renal transplant patients. *Int Urol Nephrol*, *52*(8), 1593-1601. https://doi.org/10.1007/s11255-020-02491-1

Peddi, V. R., Patel, P. S., Schieve, C., Rose, S., & First, M. R. (2020). Serial Peripheral Blood Gene Expression Profiling to Assess Immune Quiescence in Kidney Transplant Recipients with Stable Renal Function. *Ann Transplant*, *25*, e920839. https://doi.org/10.12659/aot.920839

Pham, M. X., Teuteberg, J. J., Kfoury, A. G., Starling, R. C., Deng, M. C., Cappola, T. P., Kao, A., Anderson, A. S., Cotts, W. G., Ewald, G. A., Baran, D. A., Bogaev, R. C., Elashoff, B., Baron, H., Yee, J., & Valantine, H. A. (2010). Gene-expression profiling for rejection surveillance after cardiac transplantation. *N Engl J Med*, *362*(20), 1890-1900. https://doi.org/10.1056/NEJMoa0912965

Phillips, M., Boehmer, J. P., Cataneo, R. N., Cheema, T., Eisen, H. J., Fallon, J. T., Fisher, P. E., Gass, A., Greenberg, J., Kobashigawa, J., Mancini, D., Rayburn, B., & Zucker, M. J. (2004). Heart allograft rejection: detection with breath alkanes in low levels (the HARDBALL study). *J Heart Lung Transplant*, *23*(6), 701-708.

Rodgers, N., Gerding, B., Cusi, V., Vaida, F., Tada, Y., Morris, G. P., Adler, E. D., Stehlik, J., & Kim, P. J. (2023). Comparison of two donor-derived cell-free DNA tests and a blood gene-expression profile test in heart transplantation. *Clin Transplant*, *37*(6), e14984. https://doi.org/10.1111/ctr.14984

Schutz, E., Fischer, A., Beck, J., Harden, M., Koch, M., Wuensch, T., Stockmann, M., Nashan, B., Kollmar, O., Matthaei, J., Kanzow, P., Walson, P. D., Brockmoller, J., & Oellerich, M. (2017). Graft-derived cell-free DNA, a noninvasive early rejection and graft damage marker in liver transplantation: A prospective, observational, multicenter cohort study. *PLoS Med*, *14*(4), e1002286. https://doi.org/10.1371/journal.pmed.1002286

Seferović, P. M., Tsutsui, H., McNamara, D. M., Ristić, A. D., Basso, C., Bozkurt, B., Cooper, L. T., Filippatos, G., Ide, T., Inomata, T., Klingel, K., Linhart, A., lyon, A. R., Mehra, M. R., Polovina, M., Milinković, I., Nakamura, K., Anker, S. D., Veljić, I., . . . Starling, R. C. (2021). Heart Failure Association, Heart Failure Society of America, and Japanese Heart Failure Society Position Statement on Endomyocardial Biopsy. *Journal of Cardiac Failure*, *27*(7), 727-743. https://doi.org/https://doi.org/10.1016/j.cardfail.2021.04.010

Sigdel, T. K., Archila, F. A., Constantin, T., Prins, S. A., Liberto, J., Damm, I., Towfighi, P., Navarro, S., Kirkizlar, E., Demko, Z. P., Ryan, A., Sigurjonsson, S., Sarwal, R. D., Hseish, S. C., Chan-On, C., Zimmermann, B., Billings, P. R., Moshkevich, S., & Sarwal, M. M. (2018). Optimizing Detection of Kidney Transplant Injury by Assessment of Donor-Derived Cell-Free DNA via Massively Multiplex PCR. *J Clin Med*, *8*(1). https://doi.org/10.3390/jcm8010019

Vella, J. (2024, August 12, 2022). *Transplantation immunobiology*. https://www.uptodate.com/contents/transplantation-immunobiology

Velleca, A., Shullo, M. A., Dhital, K., Azeka, E., Colvin, M., DePasquale, E., Farrero, M., García-Guereta, L., Jamero, G., Khush, K., Lavee, J., Pouch, S., Patel, J., Michaud, C. J., Shullo, M. A., Schubert, S., Angelini, A., Carlos, L., Mirabet, S., . . . Reinhardt, Z. (2023). The International Society for Heart and Lung Transplantation (ISHLT) guidelines for the care of heart transplant recipients. *J Heart Lung Transplant*, *42*(5), e1-e141. https://doi.org/10.1016/j.healun.2022.10.015

Verleden, G. M., Glanville, A. R., Lease, E. D., Fisher, A. J., Calabrese, F., Corris, P. A., Ensor, C. R., Gottlieb, J., Hachem, R. R., Lama, V., Martinu, T., Neil, D. A. H., Singer, L. G., Snell, G., & Vos, R. (2019). Chronic lung allograft dysfunction: Definition, diagnostic criteria, and approaches to treatment-A consensus report from the Pulmonary Council of the ISHLT. *J Heart Lung Transplant*, *38*(5), 493-503. https://doi.org/10.1016/j.healun.2019.03.009

Use of Common Genetic Variants (single nucleotide polymorphisms) to Predict Risk of Non-Familial Breast Cancer

Policy Number: AHS – M2126 – Use of Common Genetic Variants (single nucleotide polymorphisms) to Predict Risk of Non-Familial Breast Cancer	Prior Policy Name and Number, as applicable:
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY |SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Single nucleotide polymorphisms (SNPs) are single-base pair changes that achieve a population frequency of at least one percent. They represent the most common form of genetic variation and are responsible for much of the heritable phenotypic variation observed in human populations, but are not clearly deleterious (Attia, 2024; MedlinePlus, 2024).

II. Related Policies

Policy Number	Policy Title
AHS-M2020	Molecular Diagnostics for Breast Cancer Prognosis

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

1) For all situations, testing for one or more single nucleotide polymorphisms (SNPs) to estimate an individual's risk for developing breast cancer (e.g., OncoArray,

TruSight®, and BREVAGenplus™ breast cancer tests; tests offered directly to consumers) **DOES NOT MEET COVERAGE CRITERIA**.

IV. Table of Terminology

Term	Definition
ASCO	American Society of Clinical Oncology
ATM	*ATM serine/threonine kinase*
AUC	Area under the curve
BCRAT	Breast cancer risk assessment tool
BCSC	Breast cancer surveillance consortium
BMI	Body mass index
BRCA1	*BRCA1 DNA repair associated*
BRCA2	*BRCA2 DNA repair associated*
CHEK2	*Checkpoint kinase 2*
CLIA '88	Clinical Laboratory Improvement Amendments Of 1988
CMS	Centers For Medicare and Medicaid Services
CRISPLD2	*Cysteine rich secretory protein LCCL domain containing 2*
DA	Dense tissue
ER	Estrogen receptor
FDA	Food and Drug Administration
GWAS	Genome-wide association studies
HER2	Human epidermal growth factor 2
IL-13	*Interleukin 13*
IQ-QR	Interquartile range odds ratio
LDT	Laboratory developed test
MD	Mammographic density
NCCN	National Comprehensive Cancer Network
NDA	Non-dense tissue
PALB2	*Partner and localizer of BRCA2*
PMD	Percent density
PR	Progesterone receptor
PRS	Polygenic risk score
SNP88	Single nucleotide polymorphism risk score
SNP	Single nucleotide polymorphism
SHOX2	*Short stature homeobox 2*
TWAS	Transcriptome-wide association study
USPSTF	United States Preventive Services Task Force
VUS	Variant of uncertain significance

V. Scientific Background

In the United States, breast cancer is the second most diagnosed cancer, following skin cancer, and, the second most common cause of cancer death in women, following lung cancer. Approximately one in eight women will develop breast cancer in their lifetime (ACS, 2024).

Breast cancer risk is strongly associated with both genetic and environmental factors. Familial aggregation and twin studies have shown that inherited susceptibility plays a substantial role in risk of developing breast cancer (Lichtenstein et al., 2000; Peto & Mack, 2000). Many genetic loci are known to contribute to this risk. These loci fall into three categories: genes with high-penetrance mutations (notably *BRCA1* and *BRCA2*), moderate-risk alleles in genes such as *ATM*, *CHEK2* and *PALB2*, and common lower penetrance alleles (Michailidou et al., 2013), of which almost 80 have been identified, principally through genome-wide association studies (GWAS) (Ahmed et al., 2009; Antoniou et al., 2010; Bojesen et al., 2013; Cox et al., 2007; Easton et al., 2007; Fletcher et al., 2011; French et al., 2013; Garcia-Closas et al., 2013; Ghoussaini et al., 2012; Haiman et al., 2011; Michailidou et al., 2013; Stacey et al., 2007; Stacey et al., 2008; Thomas et al., 2009; Turnbull et al., 2010; Vachon et al., 2015; Zheng et al., 2009). GWAS continues to identify additional risk loci, with 65 loci identified by Michailidou et al. (2017). Coupled with established risk factors, these loci are likely to increase the utility and accuracy of clinical risk prediction.

For sporadic (nonfamilial) breast cancer, the Breast Cancer Risk Assessment Tool (BCRAT), most often referred to as the Gail model (Gail et al., 1989) is commonly used to produce individual risk estimates in women. The model incorporates individual risk factors including age, family history (breast cancer among first-degree relatives), personal reproductive history (age at menarche and at first live birth), and personal medical history (number of previous breast biopsies and presence of biopsy-confirmed atypical hyperplasia) to identify women who have an increased 5-year risk and lifetime risk of invasive breast cancer and who may benefit from risk reduction with selective estrogen receptor (ER) modulators (Kinsinger et al., 2002; Visvanathan et al., 2009). While this model has implications for primary prevention of invasive breast cancer, both the discriminatory accuracy of the Gail model and its calibration in certain populations have been challenged (Mealiffe et al., 2010). In 2018, Wang et al. (2018) systematically reviewed and analyzed the performance of different versions of the Gail model. They did find that the original Gail model 1 and the Caucasian-American Gail model was well calibrated in American and European women. However, in contrast, the Caucasian-American and Asian-American Gail models likely overestimate the risk in Asian females, providing a risk roughly double that of their actual risk (Wang et al., 2018).

It has been noted that the "effect of single SNPs in complex disease to date has been small (i.e., odds ratios in the 1.1-1.6 range)" (Attia, 2024). However, previous studies have analyzed the potential impact of adding genetic information from a panel of single nucleotide polymorphisms (SNPs) associated with breast cancer risk to the Gail model (Gail, 2008, 2009). SNPs are specific locations in the genome where a nucleotide differs between individuals. A study that compared classification of risk using the Gail model or the Gail model plus 10 common genetic susceptibility variants, excluding those associated with *BRCA1* or *BRCA2*, found that inclusion of these genetic factors only modestly improved performance of the BCRAT (Wacholder et al., 2010). Another study evaluated the inclusion of a SNP risk score combined with the Gail model, basing the SNP risk score on seven SNPs associated with risk for breast cancer (Mealiffe et al., 2010). These showed that real gains, albeit modest, could be achieved in reclassification of risk. Other studies have found modest potential clinical gains from combining SNP information with clinical risk factors (Gail, 2008, 2009; Pharoah et al., 2008; Wacholder et al., 2010). However, these studies have either been theoretical in nature (Gail, 2008, 2009; Pharoah et al., 2008) or they combined model building with evaluation (Wacholder et al., 2010), which may complicate evaluating the results in a clinical context. Incorporating genetic information has the greatest improvement in risk assessment in subsets of women that are at an intermediate risk based on their clinical risk factors (Mealiffe et al., 2010). Moreover, it should be cautioned that though "Success in creating risk scores with a handful of SNPs has led some to try creating risk scores with tens or hundreds of thousands of SNPs, hoping to increase predictive power", the predictive power of these attempts tend to level off very rapidly. In one instance, increasing the number of SNPs examined from tens to millions only explained two to four percent of the variance in disease risk (Attia, 2024; Khera et al., 2018).

Proprietary Testing

Proprietary tests exist for the assessment of SNPs in breast cancer risk. TruSight evaluates 94 genes and 284 SNPs related to common and rare cancers, including breast cancer (Illumina, 2024b); BREVAGen*plus*, now GeneType for Breast Cancer, measures 66 genes and 77 loci for Caucasian women, 74 for African American women, and 71 for Hispanic women (GeneType, 2019, 2024); Infinium OncoArray-500k covers over 500,000 SNPs associated with many types of cancer, as well as other features such as ancestry and pharmacogenetics (Illumina, 2024a). Additionally, companies, such as 23andMe, can offer direct-to-consumer SNP testing for risk of breast cancer (23andme, 2024; Begley, 2018; FDA, 2024). The number of possible assessments and combinations of SNPs are virtually infinite.

Clinical Utility and Validity

A 76-locus polygenic risk score (PRS) was incorporated into the Breast Cancer Surveillance Consortium (BCSC) risk-prediction model to assess its attributable risk, comparing five-year absolute risk predictions between models within three studies (1643 case patients, 2397 control patients). The PRS was found to be an independent risk factor across all three studies and improved discriminatory accuracy for area under the curve (AUC) from AUC = 0.66 to AUC = 0.69. The study concluded that the set of 76 SNPs improves the identification of women at the highest risk. Along with the increase seen in AUC, there was a net-reclassification of 11% of case patients (95% CI = 7% to 15%) to a risk level where women are more likely to benefit from chemoprevention. This suggests that SNPs could be clinically useful. However, independent cohort data are needed to test calibration in the general population (Vachon et al., 2017; Vachon et al., 2015).

Michailidou et al. (2017) performed a GWAS on breast cancer, encompassing "122,977 cases and 105,974 controls of European ancestry and 14,068 cases and 13,104 controls of East Asian ancestry." Overall, they identified 65 new loci associated at a genome-wide level with overall breast cancer risk (defined as $P < 5 \times 10^{-8}$). The authors concluded that "these results provide further insight into genetic susceptibility to breast cancer and will improve the use of genetic risk scores for individualized screening and prevention" (Michailidou et al., 2017).

Cuzick et al. (2017) developed a SNP risk score (SNP88) using the Illumina OncoArray, which includes most known breast-cancer risk SNPs (previously validated and directly available or with close surrogates on the OncoArray) in women receiving preventative treatment. They found that "SNP88 was predictive of breast cancer risk overall (interquartile range odds ratio [IQ-OR], 1.37), but mainly for estrogen receptor-positive disease (IQ-OR, 1.44) versus estrogen receptor-negative disease. However, the observed risk of SNP88 was only 46% of expected. No significant interaction was observed with treatment arm. SNP88 was independent of TC (Spearman rank-order correlation, 0.012) and when combined multiplicatively, a "substantial" improvement was seen (IQ-OR, 1.64)" (Cuzick et al., 2017).

Mavaddat et al. (2015) evaluated the value of using 77 breast cancer related SNPs for risk stratification. A total of 33,673 breast cancer cases and 33,381 controls were analyzed. All possible pair-wise multiplicative interactions were examined, and a 77-SNP polygenic risk score (PRS) was created for estrogen receptor (ER) status, as well as breast cancer overall. The authors found that women in the highest 1% of the PRS had a "three-fold increased risk" compared to women in the middle quintile (odds ratio = 3.36). Lifetime risk of breast cancer for women without a family history that had a PRS in the lowest and highest quintiles were 5.2% and 16.6%, respectively (Mavaddat et al., 2015).

Rudolph et al. (2018) investigated the integration of PRS into risk prediction models, combining PRS and environmental risk factors. The authors performed a retrospective review of 20 studies and evaluated joint associations of the 77-SNP PRS with several environmental factors such as body mass index (BMI) and alcohol use. They found that "the strongest evidence for a non-multiplicative joint association with the 77-SNP PRS was for alcohol consumption, adult height, and current use of combined menopausal hormone therapy in ER-positive disease. Risk associations for these factors by percentiles of PRS did not follow a clear dose-response. In addition, global and tail-based goodness of fit tests showed little evidence for departures from a multiplicative risk model, with alcohol consumption showing the strongest evidence for ER-positive disease (P = 0.013 for global and 0.18 for tail-based tests)" (Rudolph et al., 2018). They concluded that "the combined effects of the 77-SNP PRS and environmental risk factors for breast cancer are generally well described by a multiplicative model" (Rudolph et al., 2018).

Schuetz et al. (2019) researched genetic variants and the relationship between inflammation, apoptosis, and autophagy in breast cancer risk. In total, 206 SNPs were tested in 54 genes related to inflammation, apoptosis, and autophagy in a population-based breast cancer study; this study included women of both European descent (658 with breast cancer and 795 controls) and East Asian descent (262 with breast cancer and 127 controls). The researchers report that "although no SNP was associated with breast cancer risk among women of European descent, we found evidence for an association among East Asians for rs1800925 (IL-13) and breast cancer risk (OR = 2.08; 95% CI: 1.32-3.28; p = 0.000779), which remained statistically significant after multiple testing correction" (Schuetz et al., 2019). The researchers also report that "This association was replicated in a meta-analysis of 4305 cases and 4194 controls in the Shanghai Breast Cancer Genetics Study" (Schuetz et al., 2019).

Kapoor et al. (2020) assessed potential interactions between 205 breast cancer susceptibility loci and 13 established breast cancer risk factors. A total of 28,176 cases and 32,209 controls were analyzed with the iCOGS array (a custom SNP genotyping array), and 44,109 cases and 48,145 controls were genotyped using the OncoArray. An interaction with less than or equal to 1% prior probability was found with three different SNP risk factor pairs. "SNP rs4442975 was associated with a greater reduction of risk of ER-positive breast cancer… in current users of estrogen-progesterone therapy compared with non-users. This finding was supported by replication using OncoArray data of the previously reported interaction between rs13387042 (r2 = 0.93 with rs4442975) and current estrogen-progesterone therapy for overall disease (Pint = 0.004). The two other interactions suggested stronger associations between SNP rs6596100 and ER-negative breast cancer with increasing parity and younger age at first birth" (Kapoor et al., 2020).

Shu et al. (2020) performed a meta- analysis of data from GWAS conducted in Asians (24,206 cases, 24,775 controls) and European descendants (122,977 cases, 105,974). The focus of their study was identifying additional genetic susceptibility loci for breast cancer, as currently known risk variants only explain a small portion of breast cancer heritability, particularly in Asian women. In this study, they identified 31 potential novel risk loci, with the lead variant showing an associate with breast cancer risk at $p<5x10^{-8}$. Of note, "the associations for 10 of these loci were replicated in an independent sample of 16,787 cases and 16,680 controls of Asian women ($P < 0.05$). In addition, we replicated the associations for 78 of the 166 known risk variants at $P < 0.05$ in Asians. These findings improve our understanding of breast cancer genetics and etiology and extend previous findings from studies of European descendants to Asian women"(Shu et al., 2020).

Zhang et al. (2020) note that "breast cancer susceptibility variants frequently show heterogeneity in associations by tumor subtype... defined by combinations of ER, [progesterone receptor] PR, [human epidermal growth factor 2] HER2 and grade: (1) luminal A-like, (2) luminal B/HER2-negative-like, (3) luminal B-like, (4) HER2-enriched-like and (5) triple-negative or basal-like." To identify novel breast cancer loci, they performed a GWAS (133,384 breast cancer cases, 113,789 controls, plus 18,908 *BRCA1* mutation carriers, 9,414 of them with breast cancer) on patients with European ancestry. They identified 32 novel susceptibility loci ($p<5x10^{-8}$), 15 of which showed associations with at least one tumor feature. Five loci showed opposite associations ($p<0.05$) between luminal- and non-luminal subtypes. They also found that "the genetic correlations between five intrinsic-like subtypes ranged from 0.35 to 0.80. The proportion of genome-wide chip heritability explained by all known susceptibility loci was 37.6% for triple-negative and 54.2% for luminal A-like disease. The odds ratios of polygenic risk scores (PRSs), which included 330 variants, for the highest 1% quantiles compared to middle quantiles were 5.63 and 3.02 for luminal A-like and triple-negative disease, respectively. These findings provide an improved understanding of genetic predisposition to breast cancer subtypes and will inform the development of subtype-specific polygenic risk scores"(Zhang et al., 2020).

Adedokun et al. (2021) used a cross-ancestry GWAS approach to describe breast cancer risk loci. They identified breast cancer variants in individuals from African ancestry GWAS (9,421 cases, 10,193 controls) and meta-analyzed them with European ancestry GWAS data (122,977 cases, 105,974 controls). The identified "four loci for overall breast cancer risk [1p13.3, 5q31.1, 15q24 (two independent signals), and 15q26.3] and two loci for estrogen receptor-negative disease (1q41 and 7q11.23) at genome-wide significance." This study suggests that replication across multiple ancestry populations will "help improve the understanding of breast cancer genetics and identify causal variants" (Adedokun et al., 2021).

Chen et al. (2022) conducted a "genome-wide association study, as well as a transcriptome-wide association study (TWAS), of age- and BMI- adjusted DA, NDA, and PMD in up to 27,900 European-ancestry women from the MODE/BCAC consortia." In their results they identified 28 genome-wide significant loci for MD phenotypes and found that 45% of all known breast cancer SNPs were associated with at least one MD phenotype. Also, "TWAS identified two novel genes (*SHOX2* and *CRISPLD2*) whose genetically predicted expression was significantly associated with MD phenotypes." In conclusion, their findings provided insight into the genetic background of MD phenotypes, and further demonstrated their shared genetic basis with breast cancer (Chen et al., 2022).

Jia et al. (2023) studied the association between SNPs and overall breast cancer risk in the Chinese female population, stating that "frequencies vary across ethnic group." Thirty-four SNPs were included in the study, all of which had been previously identified by GWAS. The authors conducted an association analysis, including 1848 patients with breast cancer and 709 healthy controls. The authors identified a a significant association between SNP rs12493607 and breast cancer risk, as well as invasive carcinoma, estrogen receptor (ER)-positive, progesterone receptor (PR)-positive, HER2-negative, and young (aged younger than 45) breast cancer. The authors also identified "less conservatively" significant association with rs4784227 and rs2046210 and breast cancer risk. Overall, the authors concluded that the results "shed light on the relationship between SNPs and breast cancer susceptibility within a vast Chinese cohort, supporting the development of polygenetic risk scores for the Chinese population" (Jia et al., 2023).

VI. Guidelines and Recommendations

American Society of Clinical Oncology (ASCO)

An update from the ASCO included recommendations for genetic and genomic testing for cancer susceptibility. These guidelines state, "ASCO recognizes that concurrent multigene testing (i.e., panel testing) may be efficient in circumstances that require evaluation of multiple high-penetrance genes of established clinical utility as possible explanations for a patient's personal or family history of cancer. Depending on the specific genes included on the panel employed, panel testing may also identify mutations in genes associated with moderate or low cancer risks and mutations in high-penetrance genes that would not have been evaluated based on the presented personal or family history. Multigene panel testing will also identify variants of uncertain significance (VUSs) in a substantial proportion of patient cases, simply as a result of the multiplicity of genes tested. ASCO affirms that it is sufficient for cancer risk

assessment to evaluate genes of established clinical utility that are suggested by the patient's personal and/or family history" (Robson et al., 2015).

National Comprehensive Cancer Network (NCCN)

Prior to 2020, the NCCN guidelines focused largely on testing *BRCA1/2*. However, the NCCN has since updated their guidelines based on "strong evidence that genes beyond *BRCA1/2*, *TP53*, and *PTEN* confer markedly increased risk of breast and/or ovarian cancers" (NCCN, 2024). These NCCN guidelines acknowledge that "Multi-gene testing can detect P/LP variants not found in single-gene testing", but "Since more than one gene can explain an inherited cancer syndrome, phenotype-directed testing based on personal and family history through a multi-gene panel test is often more efficient and/or cost-effective" (NCCN, 2024). Furthermore, "Multi-gene testing may also be considered for those who tested negative for one particular syndrome, but whose personal and family history is suggestive of an inherited susceptibility." The NCCN also stated that "Multi-gene tests also increase the likelihood of detecting a VUS." The NCCN recommends that "for individuals potentially meeting established criteria for one or more of the hereditary cancer syndromes, genetic testing should be considered along with appropriate pre- and post-test counseling" (NCCN, 2024).

The NCCN Panel recommends that "Multi-gene testing may be considered for individuals who meet testing criteria and who previously underwent single-gene and/or absent deletion duplication analysis but tested negative. Both first- and second-degree relatives of individuals who meet these testing criteria are also eligible for testing, except for second-degree relatives of individuals with pancreatic cancer or prostate cancer, for whom prior probability of a high-penetrance cancer susceptibility gene is low in the absence of additional family history of cancer; only first-degree relatives of these affected individuals should be offered testing, unless indicated based on additional family history" (NCCN, 2024). It should be noted that "Carriers of a P/LP variant should be encouraged to participate in clinical trials or genetic registries. Carriers should be encouraged to recontact their genetics providers every few years for updates, as laboratories may issue amended reports as the knowledge base surrounding hereditary cancer risk expands" (NCCN, 2024).

However, "A major dilemma regarding multi-gene testing is that there are limited data and a lack of clear guidelines regarding degree of cancer risk associated with some of the genes assessed, and how to communicate and manage risk for carriers of these genes." This issue is exacerbated by the "low incidence rates of hereditary disease, leading to a difficulty in conducting adequately powered studies," and the fact that "Multi-gene tests include moderate-penetrance genes, and they often also include

low-penetrance genes for which there are little available data regarding degree of cancer risk and guidelines for risk management"(NCCN, 2024).

The NCCN states, "Reports regarding germline findings that may impact medical management should come from laboratories that are certified by the College of American Pathologists (CAP) and Clinical Laboratory Improvement Amendments (CLIA), with some U.S. states (eg, New York) having additional reporting requirements.. The testing typically used by companies providing ancestry information directly to consumers is microarray-based single nucleotide polymorphism (SNP) testing that has not been validated for clinical use. These companies do not provide comprehensive genetic analysis that includes gross deletion or duplication analysis. Third-party services are available to assist patients with interpreting their raw data, but these services are not government-regulated. In addition to the errors inherent in working with raw data from DTC labs, other limitations of these services include inadequate informed consent process, uncertain clinical validity and utility, and lack of medical oversight" (NCCN, 2024). As such, "Given the limitations of the information obtained from DTC services, confirmatory germline testing by a certified laboratory is clinically indicated, and changes to medical management based solely on DTC testing results are not recommended" (NCCN, 2024).

Finally, "Confirmatory germline testing through an appropriately certified laboratory is clinically indicated when a potential P/LP variant is identified through various data sources" as listed below:

- "Commercial entities providing ancestry (and sometimes health) information typically do so through microarray-based single nucleotide polymorphism (SNP) testing that has not been validated for clinical use. Third-party software applications can be used by consumers to obtain an interpretation of the raw data provided by these companies. Raw data and third-party software are not able to provide information that is appropriate for medical management, as these services are not subject to quality-control processes and recent research suggests that the error rate (40%) is substantial. In addition, the current tests only provide limited founder pathogenic variants results without the benefit of family history. More comprehensive genetic counseling and testing for pathogenic variants in other inherited cancer risk genes may be appropriate at the time of confirmation testing."
- "Commercial laboratories utilizing consumer-initiated or direct-to-consumer (DTC) marketing of DNA sequence-based cancer predisposition tests vary substantially in providing information necessary to make informed decisions regarding results and may vary in accuracy in their variant interpretation"

- "Research: Patients may have participated in research studies that included germline genomic analysis. In such cases, it is clinically indicated to review the patient's findings with a genetics professional and/or the reporting laboratory to establish whether the original report was generated by an appropriately certified laboratory, or whether confirmatory testing is clinically indicated" (NCCN, 2024).

The United States Preventive Services Task Force (USPSTF)

The USPSTF published recommendations related to genetic testing for breast cancer. In particular, "The USPSTF found adequate evidence that the benefits of risk assessment, genetic counseling, and genetic testing are moderate in women whose family history is associated with an increased risk for harmful mutations in the BRCA1/2 genes," whereas for women without such family history, it stated that the benefits are small to none (USPSTF, 2019). They concluded with moderate certainty that the net benefit of these procedures outweighs the harms in women both with and without a familial risk of potentially harmful mutations. The USPSTF does not address the use of SNPs as a screening method for cancer (USPSTF, 2019).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81599	Unlisted multianalyte assay with algorithmic analysis

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

23andme. (2024). *DO YOU SPEAK BRCA?* 23andMe, Inc. https://www.23andme.com/brca/

ACS. (2024). Key Statistics for Breast Cancer. https://www.cancer.org/cancer/breast-cancer/about/how-common-is-breast-cancer.html

Adedokun, B., Du, Z., Gao, G., Ahearn, T. U., Lunetta, K. L., Zirpoli, G., Figueroa, J., John, E. M., Bernstein, L., Zheng, W., Hu, J. J., Ziegler, R. G., Nyante, S., Bandera, E. V., Ingles, S. A., Press, M. F., Deming-Halverson, S. L., Rodriguez-Gil, J. L., Yao, S., . . . Huo, D. (2021). Cross-ancestry GWAS meta-analysis identifies six breast cancer loci in African and European ancestry women. *Nat Commun*, *12*(1), 4198. https://doi.org/10.1038/s41467-021-24327-x

Ahmed, S., Thomas, G., Ghoussaini, M., Healey, C. S., Humphreys, M. K., Platte, R., Morrison, J., Maranian, M., Pooley, K. A., Luben, R., Eccles, D., Evans, D. G., Fletcher, O., Johnson, N., dos Santos Silva, I., Peto, J., Stratton, M. R., Rahman, N., Jacobs, K., . . . Easton, D. F. (2009). Newly discovered breast cancer susceptibility loci on 3p24 and 17q23.2. *Nat Genet*, *41*(5), 585-590. https://doi.org/10.1038/ng.354

Antoniou, A. C., Wang, X., Fredericksen, Z. S., McGuffog, L., Tarrell, R., Sinilnikova, O. M., Healey, S., Morrison, J., Kartsonaki, C., Lesnick, T., Ghoussaini, M., Barrowdale, D., Peock, S., Cook, M., Oliver, C., Frost, D., Eccles, D., Evans, D. G., Eeles, R., . . . Couch, F. J. (2010). A locus on 19p13 modifies risk of breast cancer in BRCA1 mutation carriers and is associated with hormone receptor-negative breast cancer in the general population. *Nat Genet*, *42*(10), 885-892. https://doi.org/10.1038/ng.669

Attia, J. (2024, May 20). *Genetic association and GWAS studies: Principles and applications*. https://www.uptodate.com/contents/genetic-association-and-gwas-studies-principles-and-applications

Begley, S. (2018). FDA approves first direct-to-consumer test for breast cancer risk. *STAT*. https://www.statnews.com/2018/03/06/fda-approves-test-breast-cancer/

Bojesen, S. E., Pooley, K. A., Johnatty, S. E., Beesley, J., Michailidou, K., Tyrer, J. P., Edwards, S. L., Pickett, H. A., Shen, H. C., Smart, C. E., Hillman, K. M., Mai, P. L., Lawrenson, K., Stutz, M. D., Lu, Y., Karevan, R., Woods, N., Johnston, R. L., French, J. D., . . . Dunning, A. M. (2013). Multiple independent variants at the TERT locus are associated with telomere length and risks of breast and ovarian cancer. *Nat Genet*, *45*(4), 371-384, 384e371-372. https://doi.org/10.1038/ng.2566

Cox, A., Dunning, A. M., Garcia-Closas, M., Balasubramanian, S., Reed, M. W., Pooley, K. A., Scollen, S., Baynes, C., Ponder, B. A., Chanock, S., Lissowska, J., Brinton, L.,

Peplonska, B., Southey, M. C., Hopper, J. L., McCredie, M. R., Giles, G. G., Fletcher, O., Johnson, N., . . . Easton, D. F. (2007). A common coding variant in CASP8 is associated with breast cancer risk. *Nat Genet*, *39*(3), 352-358. https://doi.org/10.1038/ng1981

Cuzick, J., Brentnall, A. R., Segal, C., Byers, H., Reuter, C., Detre, S., Lopez-Knowles, E., Sestak, I., Howell, A., Powles, T. J., Newman, W. G., & Dowsett, M. (2017). Impact of a Panel of 88 Single Nucleotide Polymorphisms on the Risk of Breast Cancer in High-Risk Women: Results From Two Randomized Tamoxifen Prevention Trials. *J Clin Oncol*, *35*(7), 743-750. https://doi.org/10.1200/jco.2016.69.8944

Easton, D. F., Pooley, K. A., Dunning, A. M., Pharoah, P. D. P., Thompson, D., Ballinger, D. G., Struewing, J. P., Morrison, J., Field, H., Luben, R., Wareham, N., Ahmed, S., Healey, C. S., Bowman, R., Luccarini, C., Conroy, D., Shah, M., Munday, H., Jordan, C., . . . Ponder, B. A. J. (2007). Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature*, *447*(7148), 1087-1093. https://doi.org/10.1038/nature05887

FDA. (2024, 07/29/2019). *DEN170046*. U.S. Food & Drug Administration. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/denovo.cfm?ID=DEN170046

Fletcher, O., Johnson, N., Orr, N., Hosking, F. J., Gibson, L. J., Walker, K., Zelenika, D., Gut, I., Heath, S., Palles, C., Coupland, B., Broderick, P., Schoemaker, M., Jones, M., Williamson, J., Chilcott-Burns, S., Tomczyk, K., Simpson, G., Jacobs, K. B., . . . Peto, J. (2011). Novel breast cancer susceptibility locus at 9q31.2: results of a genome-wide association study. *J Natl Cancer Inst*, *103*(5), 425-435. https://doi.org/10.1093/jnci/djq563

French, J. D., Ghoussaini, M., Edwards, S. L., Meyer, K. B., Michailidou, K., Ahmed, S., Khan, S., Maranian, M. J., O'Reilly, M., Hillman, K. M., Betts, J. A., Carroll, T., Bailey, P. J., Dicks, E., Beesley, J., Tyrer, J., Maia, A. T., Beck, A., Knoblauch, N. W., . . . Dunning, A. M. (2013). Functional variants at the 11q13 risk locus for breast cancer regulate cyclin D1 expression through long-range enhancers. *Am J Hum Genet*, *92*(4), 489-503. https://doi.org/10.1016/j.ajhg.2013.01.002

Gail, M. H. (2008). Discriminatory accuracy from single-nucleotide polymorphisms in models to predict breast cancer risk. *J Natl Cancer Inst*, *100*(14), 1037-1041. https://doi.org/10.1093/jnci/djn180

Gail, M. H. (2009). Value of adding single-nucleotide polymorphism genotypes to a breast cancer risk model. *J Natl Cancer Inst*, *101*(13), 959-963. https://doi.org/10.1093/jnci/djp130

Gail, M. H., Brinton, L. A., Byar, D. P., Corle, D. K., Green, S. B., Schairer, C., & Mulvihill, J. J. (1989). Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. *J Natl Cancer Inst*, *81*(24), 1879-1886.

Garcia-Closas, M., Couch, F. J., Lindstrom, S., Michailidou, K., Schmidt, M. K., Brook, M. N., Orr, N., Rhie, S. K., Riboli, E., Feigelson, H. S., Le Marchand, L., Buring, J. E., Eccles, D.,

Miron, P., Fasching, P. A., Brauch, H., Chang-Claude, J., Carpenter, J., Godwin, A. K., . . . Kraft, P. (2013). Genome-wide association studies identify four ER negative-specific breast cancer risk loci. *Nat Genet*, *45*(4), 392-398, 398e391-392. https://doi.org/10.1038/ng.2561

GeneType. (2019, May 2019). *Product Specification: GeneType for Breast Cancer*. Retrieved Jul 28, 2021 from https://e13609b8-ed51-46c1-8570-6819a0110aba.filesusr.com/ugd/70aa06_079e41335b874beebc288a6fe4582276.pdf

GeneType. (2024). Brest Cancer Test https://doi.org/https://genetype.com/for-medical-practitioners/breast-cancer-test-practitioners-au/

Ghoussaini, M., Fletcher, O., Michailidou, K., Turnbull, C., Schmidt, M. K., Dicks, E., Dennis, J., Wang, Q., Humphreys, M. K., Luccarini, C., Baynes, C., Conroy, D., Maranian, M., Ahmed, S., Driver, K., Johnson, N., Orr, N., dos Santos Silva, I., Waisfisz, Q., . . . Easton, D. F. (2012). Genome-wide association analysis identifies three new breast cancer susceptibility loci. *Nat Genet*, *44*(3), 312-318. https://doi.org/10.1038/ng.1049

Haiman, C. A., Chen, G. K., Vachon, C. M., Canzian, F., Dunning, A., Millikan, R. C., Wang, X., Ademuyiwa, F., Ahmed, S., Ambrosone, C. B., Baglietto, L., Balleine, R., Bandera, E. V., Beckmann, M. W., Berg, C. D., Bernstein, L., Blomqvist, C., Blot, W. J., Brauch, H., . . . Couch, F. J. (2011). A common variant at the TERT-CLPTM1L locus is associated with estrogen receptor-negative breast cancer. *Nat Genet*, *43*(12), 1210-1214. https://doi.org/10.1038/ng.985

Illumina. (2024a). Infinium OncoArray-500K BeadChip. https://www.illumina.com/products/by-type/microarray-kits/infinium-oncoarray-500k.html

Illumina. (2024b). TruSight Cancer. https://www.illumina.com/products/by-type/clinical-research-products/trusight-cancer.html

Jia, Z., Huang, Y., Liu, J., Liu, G., Li, J., Xu, H., Jiang, Y., Zhang, S., Wang, Y., Chen, G., Qiao, G., & Li, Y. (2023). Single nucleotide polymorphisms associated with female breast cancer susceptibility in Chinese population. *Gene*, *884*, 147676. https://doi.org/10.1016/j.gene.2023.147676

Kapoor, P. M., Lindström, S., Behrens, S., Wang, X., Michailidou, K., Bolla, M. K., Wang, Q., Dennis, J., Dunning, A. M., Pharoah, P. D. P., Schmidt, M. K., Kraft, P., García-Closas, M., Easton, D. F., Milne, R. L., & Chang-Claude, J. (2020). Assessment of interactions between 205 breast cancer susceptibility loci and 13 established risk factors in relation to breast cancer risk, in the Breast Cancer Association Consortium. *Int J Epidemiol*, *49*(1), 216-232. https://doi.org/10.1093/ije/dyz193

Khera, A. V., Chaffin, M., Aragam, K. G., Haas, M. E., Roselli, C., Choi, S. H., Natarajan, P., Lander, E. S., Lubitz, S. A., Ellinor, P. T., & Kathiresan, S. (2018). Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. *Nat Genet*, *50*(9), 1219-1224. https://doi.org/10.1038/s41588-018-0183-z

Kinsinger, L. S., Harris, R., Woolf, S. H., Sox, H. C., & Lohr, K. N. (2002). Chemoprevention of breast cancer: a summary of the evidence for the U.S. Preventive Services Task Force. *Ann Intern Med*, *137*(1), 59-69.

Lichtenstein, P., Holm, N. V., Verkasalo, P. K., Iliadou, A., Kaprio, J., Koskenvuo, M., Pukkala, E., Skytthe, A., & Hemminki, K. (2000). Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. *N Engl J Med*, *343*(2), 78-85. https://doi.org/10.1056/nejm200007133430201

Mavaddat, N., Pharoah, P. D. P., Michailidou, K., Tyrer, J., Brook, M. N., Bolla, M. K., Wang, Q., Dennis, J., Dunning, A. M., Shah, M., Luben, R., Brown, J., Bojesen, S. E., Nordestgaard, B. G., Nielsen, S. F., Flyger, H., Czene, K., Darabi, H., Eriksson, M., . . . Garcia-Closas, M. (2015). Prediction of Breast Cancer Risk Based on Profiling With Common Genetic Variants. *J Natl Cancer Inst*, *107*(5). https://doi.org/10.1093/jnci/djv036

Mealiffe, M. E., Stokowski, R. P., Rhees, B. K., Prentice, R. L., Pettinger, M., & Hinds, D. A. (2010). Assessment of Clinical Validity of a Breast Cancer Risk Model Combining Genetic and Clinical Information. *J Natl Cancer Inst*, *102*(21), 1618-1627. https://doi.org/10.1093/jnci/djq388

MedlinePlus. (2024, March 22). *What are single nucleotide polymorphisms (SNPs)?* National Library of Medicine. https://medlineplus.gov/genetics/understanding/genomicresearch/snp/

Michailidou, K., Hall, P., Gonzalez-Neira, A., Ghoussaini, M., Dennis, J., Milne, R. L., Schmidt, M. K., Chang-Claude, J., Bojesen, S. E., Bolla, M. K., Wang, Q., Dicks, E., Lee, A., Turnbull, C., Rahman, N., Fletcher, O., Peto, J., Gibson, L., Silva Idos, S., . . . Easton, D. F. (2013). Large-scale genotyping identifies 41 new loci associated with breast cancer risk. *Nat Genet*, *45*(4), 353-361e352. https://doi.org/10.1038/ng.2563

Michailidou, K., Lindstrom, S., Dennis, J., Beesley, J., Hui, S., Kar, S., Lemacon, A., Soucy, P., Glubb, D., Rostamianfar, A., Bolla, M. K., Wang, Q., Tyrer, J., Dicks, E., Lee, A., Wang, Z., Allen, J., Keeman, R., Eilber, U., . . . Easton, D. F. (2017). Association analysis identifies 65 new breast cancer risk loci. *Nature*, *551*(7678), 92-94. https://doi.org/10.1038/nature24284

NCCN. (2024). *Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic Version 3.2024 — February 12, 2024* https://www.nccn.org/professionals/physician_gls/pdf/genetics_bop.pdf

Peto, J., & Mack, T. M. (2000). High constant incidence in twins and other relatives of women with breast cancer. *Nat Genet*, *26*(4), 411-414. https://doi.org/10.1038/82533

Pharoah, P. D., Antoniou, A. C., Easton, D. F., & Ponder, B. A. (2008). Polygenes, risk prediction, and targeted prevention of breast cancer. *N Engl J Med*, *358*(26), 2796-2803. https://doi.org/10.1056/NEJMsa0708739

Robson, M. E., Bradbury, A. R., Arun, B., Domchek, S. M., Ford, J. M., Hampel, H. L., Lipkin, S. M., Syngal, S., Wollins, D. S., & Lindor, N. M. (2015). American Society of Clinical

Oncology Policy Statement Update: Genetic and Genomic Testing for Cancer Susceptibility. *J Clin Oncol*, *33*(31), 3660-3667. https://doi.org/10.1200/jco.2015.63.0996

Rudolph, A., Song, M., Brook, M. N., Milne, R. L., Mavaddat, N., Michailidou, K., Bolla, M. K., Wang, Q., Dennis, J., Wilcox, A. N., Hopper, J. L., Southey, M. C., Keeman, R., Fasching, P. A., Beckmann, M. W., Gago-Dominguez, M., Castelao, J. E., Guenel, P., Truong, T., . . . Garcia-Closas, M. (2018). Joint associations of a polygenic risk score and environmental risk factors for breast cancer in the Breast Cancer Association Consortium. *Int J Epidemiol*, *47*(2), 526-536. https://doi.org/10.1093/ije/dyx242

Schuetz, J. M., Grundy, A., Lee, D. G., Lai, A. S., Kobayashi, L. C., Richardson, H., Long, J., Zheng, W., Aronson, K. J., Spinelli, J. J., & Brooks-Wilson, A. R. (2019). Genetic variants in genes related to inflammation, apoptosis and autophagy in breast cancer risk. *PLoS One*, *14*(1), e0209010. https://doi.org/10.1371/journal.pone.0209010

Shu, X., Long, J., Cai, Q., Kweon, S. S., Choi, J. Y., Kubo, M., Park, S. K., Bolla, M. K., Dennis, J., Wang, Q., Yang, Y., Shi, J., Guo, X., Li, B., Tao, R., Aronson, K. J., Chan, K. Y. K., Chan, T. L., Gao, Y. T., . . . Zheng, W. (2020). Identification of novel breast cancer susceptibility loci in meta-analyses conducted among Asian and European descendants. *Nat Commun*, *11*(1), 1217. https://doi.org/10.1038/s41467-020-15046-w

Stacey, S. N., Manolescu, A., Sulem, P., Rafnar, T., Gudmundsson, J., Gudjonsson, S. A., Masson, G., Jakobsdottir, M., Thorlacius, S., Helgason, A., Aben, K. K., Strobbe, L. J., Albers-Akkers, M. T., Swinkels, D. W., Henderson, B. E., Kolonel, L. N., Le Marchand, L., Millastre, E., Andres, R., . . . Stefansson, K. (2007). Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer. *Nat Genet*, *39*(7), 865-869. https://doi.org/10.1038/ng2064

Stacey, S. N., Manolescu, A., Sulem, P., Thorlacius, S., Gudjonsson, S. A., Jonsson, G. F., Jakobsdottir, M., Bergthorsson, J. T., Gudmundsson, J., Aben, K. K., Strobbe, L. J., Swinkels, D. W., van Engelenburg, K. C., Henderson, B. E., Kolonel, L. N., Le Marchand, L., Millastre, E., Andres, R., Saez, B., . . . Stefansson, K. (2008). Common variants on chromosome 5p12 confer susceptibility to estrogen receptor-positive breast cancer. *Nat Genet*, *40*(6), 703-706. https://doi.org/10.1038/ng.131

Thomas, G., Jacobs, K. B., Kraft, P., Yeager, M., Wacholder, S., Cox, D. G., Hankinson, S. E., Hutchinson, A., Wang, Z., Yu, K., Chatterjee, N., Garcia-Closas, M., Gonzalez-Bosquet, J., Prokunina-Olsson, L., Orr, N., Willett, W. C., Colditz, G. A., Ziegler, R. G., Berg, C. D., . . . Hunter, D. J. (2009). A multistage genome-wide association study in breast cancer identifies two new risk alleles at 1p11.2 and 14q24.1 (RAD51L1). *Nat Genet*, *41*(5), 579-584. https://doi.org/10.1038/ng.353

Turnbull, C., Ahmed, S., Morrison, J., Pernet, D., Renwick, A., Maranian, M., Seal, S., Ghoussaini, M., Hines, S., Healey, C. S., Hughes, D., Warren-Perry, M., Tapper, W., Eccles, D., Evans, D. G., Hooning, M., Schutte, M., van den Ouweland, A., Houlston, R.,

. . . Easton, D. F. (2010). Genome-wide association study identifies five new breast cancer susceptibility loci. *Nat Genet*, *42*(6), 504-507. https://doi.org/10.1038/ng.586

USPSTF. (2019, August 20). *BRCA-Related Cancer: Risk Assessment, Genetic Counseling, and Genetic Testing*. https://www.uspreventiveservicestaskforce.org/uspstf/recommendation/brca-related-cancer-risk-assessment-genetic-counseling-and-genetic-testing

Vachon, C. M., Affiliations of authors: Department of Health Sciences Research, D. o. E., Mayo Clinic (CMV, VSP, CGS, MRJ, JEO, ADN, FJC), Department of Gynecology and Obstetrics, U. H. E. F.-A.-U. E.-N., Comprehensive Cancer Center Erlangen-EMN , Erlangen , Germany (LH, KH, CCH, SMJ, MWB, PAF), Department of Medicine, I. f. H. G., Helen Diller Family Comprehensive Cancer Center, University of California , San Francisco, CA (EZ), Departments of Medicine and Epidemiology and Biostatistics and General Internal Medicine Section, D. o. V. A. a. D. o. G. I. M. E., JAT, KK), Division of Breast Imaging, D. o. R., Mayo Clinic College of Medicine , Rochester, MN (KRB, DHW), Institute of Diagnostic Radiology, U. H. E., Friedrich-Alexander University Erlangen-Nuremberg , Erlangen , Germany (RS-W), Division of Experimental Pathology, D. o. L. M. a. P., Mayo Clinic College of Medicine , Rochester, MN (JMC, FJC), Wayne State University School of Medicine and Karmanos Cancer Institute , D., MI (KSP), University of Cambridge, C. f. C. G. E., Cambridge, UK (DFE), Moffitt Cancer Center, T., Florida (TAS), University of California at Los Angeles, D. o. M., Division Hematology/Oncology, David Geffen School of Medicine , Los Angeles, CA (PAF)., Pankratz, V. S., Affiliations of authors: Department of Health Sciences Research, D. o. E., Mayo Clinic (CMV, VSP, CGS, MRJ, JEO, ADN, FJC), Department of Gynecology and Obstetrics, U. H. E. F.-A.-U. E.-N., Comprehensive Cancer Center Erlangen-EMN , Erlangen , Germany (LH, KH, CCH, SMJ, MWB, PAF), Department of Medicine, I. f. H. G., Helen Diller Family Comprehensive Cancer Center, University of California , San Francisco, CA (EZ), Departments of Medicine and Epidemiology and Biostatistics and General Internal Medicine Section, D. o. V. A. a. D. o. G. I. M. E., JAT, KK), Division of Breast Imaging, D. o. R., Mayo Clinic College of Medicine , Rochester, MN (KRB, DHW), Institute of Diagnostic Radiology, U. H. E., Friedrich-Alexander University Erlangen-Nuremberg , Erlangen , Germany (RS-W), . . . University of California at Los Angeles, D. o. M., Division Hematology/Oncology, David Geffen School of Medicine , Los Angeles, CA (PAF). (2017). The Contributions of Breast Density and Common Genetic Variation to Breast Cancer Risk. *JNCI: Journal of the National Cancer Institute*, *107*(5). https://doi.org/10.1093/jnci/dju397

Vachon, C. M., Pankratz, V. S., Scott, C. G., Haeberle, L., Ziv, E., Jensen, M. R., Brandt, K. R., Whaley, D. H., Olson, J. E., Heusinger, K., Hack, C. C., Jud, S. M., Beckmann, M. W., Schulz-Wendtland, R., Tice, J. A., Norman, A. D., Cunningham, J. M., Purrington, K. S., Easton, D. F., . . . Couch, F. J. (2015). The contributions of breast density and common

genetic variation to breast cancer risk. *J Natl Cancer Inst*, *107*(5). https://doi.org/10.1093/jnci/dju397

Visvanathan, K., Chlebowski, R. T., Hurley, P., Col, N. F., Ropka, M., Collyar, D., Morrow, M., Runowicz, C., Pritchard, K. I., Hagerty, K., Arun, B., Garber, J., Vogel, V. G., Wade, J. L., Brown, P., Cuzick, J., Kramer, B. S., & Lippman, S. M. (2009). American society of clinical oncology clinical practice guideline update on the use of pharmacologic interventions including tamoxifen, raloxifene, and aromatase inhibition for breast cancer risk reduction. *J Clin Oncol*, *27*(19), 3235-3258. https://doi.org/10.1200/jco.2008.20.5179

Wacholder, S., Hartge, P., Prentice, R., Garcia-Closas, M., Feigelson, H. S., Diver, W. R., Thun, M. J., Cox, D. G., Hankinson, S. E., Kraft, P., Rosner, B., Berg, C. D., Brinton, L. A., Lissowska, J., Sherman, M. E., Chlebowski, R., Kooperberg, C., Jackson, R. D., Buckman, D. W., . . . Hunter, D. J. (2010). Performance of common genetic variants in breast-cancer risk models. *N Engl J Med*, *362*(11), 986-993. https://doi.org/10.1056/NEJMoa0907727

Wang, X., Huang, Y., Li, L., Dai, H., Song, F., & Chen, K. (2018). Assessment of performance of the Gail model for predicting breast cancer risk: a systematic review and meta-analysis with trial sequential analysis. *Breast Cancer Res*, *20*(1), 18. https://doi.org/10.1186/s13058-018-0947-5

Zhang, H., Ahearn, T. U., Lecarpentier, J., Barnes, D., Beesley, J., Qi, G., Jiang, X., O'Mara, T. A., Zhao, N., Bolla, M. K., Dunning, A. M., Dennis, J., Wang, Q., Ful, Z. A., Aittomaki, K., Andrulis, I. L., Anton-Culver, H., Arndt, V., Aronson, K. J., . . . Garcia-Closas, M. (2020). Genome-wide association study identifies 32 novel breast cancer susceptibility loci from overall and subtype-specific analyses. *Nat Genet*, *52*(6), 572-581. https://doi.org/10.1038/s41588-020-0609-2

Zheng, W., Long, J., Gao, Y. T., Li, C., Zheng, Y., Xiang, Y. B., Wen, W., Levy, S., Deming, S. L., Haines, J. L., Gu, K., Fair, A. M., Cai, Q., Lu, W., & Shu, X. O. (2009). Genome-wide association study identifies a new breast cancer susceptibility locus at 6q25.1. *Nat Genet*, *41*(3), 324-328. https://doi.org/10.1038/ng.318

Venous and Arterial Thrombosis Risk Testing

Policy Number: AHS – M2041 – Venous and Arterial Thrombosis Risk Testing	Prior Policy Name and Number, as applicable: • AHS – M2041 – Venous Thrombosis Risk Testing
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

A thrombosis, also known as a blood clot, occurs within blood vessels in the body. The two main types of thrombosis include venous thrombosis, which is when a vein is blocked due to a blood clot, and arterial thrombosis, which is when an artery is blocked due to a blood clot. Thrombophilias refer to hereditary and/or acquired abnormalities of hemostasis that predispose patients to thrombosis (Stevens et al., 2016). The most common presentations of venous thromboembolism (VTE) are deep vein thrombosis (DVT) and pulmonary embolism (PE) (Bartholomew, 2017).

Terms such as male and female are used when necessary to refer to sex assigned at birth.

II. Related Policies

Policy Number	Policy Title
AHS-M2141	Testing of Homocysteine Metabolism-Related Conditions
AHS-M2180	Genetic Markers for Assessing Risk of Cardiovascular Disease

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For individuals without recurrent venous thromboembolism (VTE) risk factors (e.g., surgery, prolonged immobilization, collagen vascular disease, malignancy, certain hematologic disorders), genetic testing for Factor V Leiden and prothrombin gene G20210A mutations and plasma testing for protein C deficiency, protein S deficiency, and antithrombin III deficiency (see Note 1) **MEETS COVERAGE CRITERIA** in **any** of the following situations:
    a) For individuals less than 50 years of age who have experienced any deep venous thrombosis (DVT) or pulmonary embolism (PE).
    b) For individuals who have experienced a DVT in unusual sites (e.g., hepatic, mesenteric, or cerebral veins).
    c) For individuals who have experienced a DVT and who have a strong family history of thrombotic disease.
    d) For individuals who are pregnant or taking oral contraceptives and who have experienced a DVT.
    e) For first- and second-degree relatives (see Note 2) of individuals who experienced a DVT before 50 years of age.
    f) For women under the age of 50 who smoke and who have suffered a myocardial infarction.
    g) Before the administration of oral contraceptives, targeted testing of individuals with a personal or family history of DVT.
    h) For pediatric individuals who have suffered from a pediatric arterial ischemic stroke.
2) For individuals with warfarin-induced skin necrosis or for infants who develop neonatal purpura fulminans, plasma testing for protein C deficiency and protein S deficiency (see Note 1) **MEETS COVERAGE CRITERIA**.
3) For individuals with a first-degree relative (see Note 2) with a known Factor V Leiden or prothrombin gene G20210A mutation, genetic testing for the known familial mutation **MEETS COVERAGE CRITERIA** in any of the following situations:
    a) For individuals who are considering the use of oral contraceptives.
    b) For individuals who were assigned female at birth and who are considering hormone replacement therapy.
4) For hypercoagulable evaluation or for "at risk" family members, *MTHFR* genetic testing **DOES NOT MEET COVERAGE CRITERIA**.

5) For individuals with recurrent thrombotic events who are receiving a lifelong anticoagulation regimen, genetic testing for Factor V Leiden and prothrombin gene G20210A mutations **DOES NOT MEET COVERAGE CRITERIA**.

6) Venous thrombosis risk testing for superficial venous thrombosis (including superficial thrombophlebitis and varicosities) **DOES NOT MEET COVERAGE CRITERIA**.

7) For all situations, activated protein C (aPC) resistance assay **DOES NOT MEET COVERAGE CRITERIA**.

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

8) Genetic testing for inherited thrombophilia **DOES NOT MEET COVERAGE CRITERIA** for any of the following situations:
   a) For the evaluation of recurrent fetal loss, placental abruption, preeclampsia, or fetal growth restriction.
   b) For the evaluation of arterial thrombosis not attributable to paradoxical emboli.
   c) As a routine screen for the general population.
   d) As a routine screen for asymptomatic individuals considering oral contraceptive use or hormone replacement therapy.
   e) As a routine screen for asymptomatic pregnant individuals.
   f) For prenatal or preimplantation testing.
   g) As a routine newborn screen.

9) Genetic testing for inherited thrombophilia more than once per lifetime **DOES NOT MEET COVERAGE CRITERIA**.

10) Testing for other genetic thrombophilia risk factors (e.g., factor V HR2 variant, prothrombin G1199A variant, factor VII R353Q variant, factor 13B V34L variant, PAI-1), as well as multi-gene panel testing **DOES NOT MEET COVERAGE CRITERIA**.

11) DVT risk testing as part of a pre-transplant evaluation test DOES NOT MEET COVERAG**E CRITERIA**.

12) To determine arterial thrombosis risk, genetic testing for Factor V Leiden mutation and prothrombin gene G20210A mutation **DOES NOT MEET COVERAGE CRITERIA**.

NOTES:

Note 1: Plasma testing for protein C deficiency, protein S deficiency, and antithrombin III deficiency should be performed at least six weeks after the acute thrombotic event and while the patient is not taking anticoagulants. Assays for clotting inhibitors amount and function should be performed prior to any molecular testing.

Note 2: First-degree relatives include parents, full siblings, and children of the individual. Second-degree relatives include grandparents, aunts, uncles, nieces, nephews, grandchildren, and half-siblings of the individual.

IV. Table of Terminology

Term	Definition
ACC	American College of Cardiology
ACMG	American College of Medical Genetics and Genomics
ACOG	American College of Obstetricians and Gynecologists
ACR	American College of Radiology
AHA/ASA	American Heart Association/American Stroke Association
aPC	Activated protein C
APS	Antiphospholipid syndrome
ASCP	American Society for Clinical Pathology
ASH	American Society of Hematology
COC	Combined oral contraceptives
CTPA	Computed tomography pulmonary angiography
DVT	Deep vein thrombosis
EGAPP	Evaluation of genomic applications in practice and prevention
ESC	European Society of Cardiology
FVL	Factor V Leiden
HRT	Hormone replacement therapy
NAFT	The North American Thrombosis Forum
OC	Oral contraceptives
PE	Pulmonary embolism
PGM	Prothrombin 20210A gene mutation
SIGN	Scottish Intercollegiate Guidelines Network

SPESI	Simplified pulmonary embolism severity index
SVM	Society for Vascular Medicine
VQ	Ventilation perfusion
VTE	Venous thromboembolism
VUS	Variant of unknown significance

V. Scientific Background

A thrombus is "an aggregate of coagulated blood within the vascular system or heart which contains platelets, fibrin, leukocytes, and red blood cells in varying amounts" (Herrmann, 2018). This aggregate of blood can be problematic as it may obstruct normal blood circulation throughout the body and even travel to peripheral areas. The primary manifestations of venous thromboembolisms (VTE) are deep vein thrombosis and pulmonary embolism. These conditions affect an estimated one million individuals in the United States annually (Bartholomew, 2017).

Thrombosis is widely theorized to develop due to Virchow's Triad, which consists of abnormalities in blood flow, a vascular endothelial injury, and alterations in the blood constituents. Changes in any of these characteristics may cause the clot to form (Bauer & Lip, 2024). For example, sickle red blood cells may cause increased clumping or decreased adhesion to the vessel walls (Byrnes & Wolberg, 2017). There are two main types of thrombosis: venous thrombosis (when a vein is blocked due to a blood clot) and arterial thrombosis (when an artery is blocked due to a blood clot).

A deep vein thrombosis (DVT) refers to a thrombus in a "deep" vein whereas a pulmonary embolism (PE) refers to an obstruction of the pulmonary artery (or one of its branches) by foreign material (Bauer, 2024a; Thompson, 2023). DVT of the lower extremities may cause symptoms, such as swelling or edema in the lower extremities, pain, and warmth in the affected area (Bauer, 2024a). This thrombus may travel to the lungs (becoming an embolus) and cause a PE. A PE has similar symptoms to DVT but may include pulmonary issues, such as shortness of breath. The risk factors for VTE, PE, and DVT are similar (Thompson, 2023). The two primary categories of risk factors for VTE are hereditary and acquired, and the genetic tendency toward VTE is referred to as inherited thrombophilia. Hereditary risk factors include genetic mutations such as Factor V Leiden (FVL) mutations. The five most common genetic risk factors for VTE are FVL mutations, prothrombin mutations, protein S defect, protein C defect, and antithrombin defect (Bauer & Lip, 2024). Approximately 50–60% of the variance in VTE incidence are attributed to genetic effects (Crous-Bou et al., 2016).

A modified activated partial thromboplastin time (aPTT) assay detects the anticoagulant activity of activated protein C (aPC). FVL mutations cause coagulation

factor V to be unresponsive to aPC and initially, these changes were termed "aPC resistance" due to the reduced activity of aPC on a modified aPTT assay. A single nucleotide change (G1691A) results in a point mutation of glutamine to arginine at position 506. Approximately 99% of carriers of this mutation are heterozygous, and only 5% of these heterozygotes will experience a VTE in their lifetime. These mutations are often suspected in patients experiencing a VTE at a young age (under 50), a VTE in unusual areas such as a portal vein, or recurrent VTEs (Bauer, 2023a). Protein C may also be genetically deficient, but this mutation is only seen in 2-5% of individuals with a VTE (Bauer, 2024a). Protein S, a cofactor for the aPC control mechanism, and deficiencies in this protein may also confer additional risk for VTE (Bauer, 2024b).

The second most common inherited thrombophilia is the G20210A mutation of prothrombin. This mutation is a gain of function mutation where clotting activity is increased by creating more thrombin and fibrin. The overall prevalence of this mutation is about 2% (Bauer, 2023c). Genetic defects of antithrombin (an inhibitor of thrombin) may also occur, but the estimated prevalence of antithrombin defects is only a maximum of 0.2% (Bauer, 2023b).

Acquired risk factors or predisposing conditions for thrombosis include a prior thrombotic event, recent major surgery, presence of a central venous catheter, trauma, immobilization, malignancy, pregnancy, the use of oral contraceptives or heparin, myeloproliferative disorders, antiphospholipid syndrome (APS), and a number of other major medical illnesses (Bauer & Lip, 2024). Patients with acquired hypercoagulability have an increased risk of venous thrombosis, arterial thrombosis, or both; however, there is a low risk of recurrence, regardless of thrombophilia status (Connors, 2017). A rare complication of warfarin treatment, warfarin-induced skin necrosis is commonly due to protein C deficiency, with rare cases of protein S deficiency or PVL having been reported (Bauer & Lip, 2023).

Risk factors for arterial thrombosis are lesser known. The relationship between FVL and arterial thrombosis is controversial with studies reporting varying results; overall, FVL is not currently considered a major risk factor for arterial thrombosis (Carroll & Piazza, 2018; Kujovich, 2011). Kujovich (2018) states that FVL testing should not be performed on persons with any type of arterial thrombosis including myocardial infarction and stroke in children or adults. It has also been reported that while inherited antithrombin, protein S and protein C deficiencies are important risk factors for venous thrombosis, "they have little or no effect on arterial thrombosis" (Previtali et al., 2011). Further, prothrombin gene mutation is not consistently shown to increase the risk of an arterial thromboembolism, and "There is no association of antithrombin deficiency with arterial thrombosis" (Carroll & Piazza, 2018).

It has been proposed that venous thrombosis risk testing may be beneficial as a pre-transplant evaluation test. However, no studies have been identified suggesting this. The North American Thrombosis Forum (NAFT) states that even though certain genetic conditions predispose a small proportion of the population to the development of blood clots, "few people with thrombophilias develop symptoms"; further, there is no cost-effective, safe or long-term method to prevent a blood clot from forming even if a genetic predisposition is identified (NATF, 2019).

Thrombotic events such as thrombophilia and stroke have become increasingly documented in hospitalized pediatric patients with underlying medical conditions such as prematurity, cancer, and congenital heart disease, but they are rarely identified in healthy children. Furthermore, in most cases of pediatric venous thromboembolism, there exist other underlying risk factors such as indwelling central venous catheter and inherited thrombophilia that are worthy of further investigation. The incidence of venous thromboembolisms is highest in neonates and infants, but there is a second peak recorded in adolescence, coinciding with the use of oral contraceptives. However, as in the case with adults, little to no evidence suggests that the use of venous thrombosis risk testing in children will affect the acute management of venous thromboembolisms. In a study including a total of 271 children with VTE, it was found that the relative frequencies of individually inherited thrombophilias were low—for example, the highest recorded frequency of IT disorders was of Factor V Leiden, occurring in only five to 10 percent of the samples. Moreover, a study of 52 children with thromboembolic events during the acute phase did not urge any changes to acute management, regardless of the result of the test (Raffini et al., 2023).

Venous thrombosis risk testing has also been entertained as a manner of combatting pediatric stroke, which can be characterized in a variety of ways, such as by age and by presentation. Arterial ischemic infarctions are the most common, comprising approximately 80% of all perinatal strokes, and this form of stroke can occur in up to one in 3500 of newborns. However, though it would seem reasonable for venous thrombosis risk testing to be employed here, recent prospective case-control studies suggest that routine thrombophilia testing is not warranted. The study showed that conditions associated with thrombophilia rarely coincided with arterial ischemic strokes, and these conditions included, but were not limited to, decreased levels of protein C, protein S, or prothrombin, and genotyping of factor V Leiden (FVL) and factor II (FII, prothrombin) G20210A. Of the 14 parameters examined, 12 showed no difference, including all common thrombophilias examined, with specific mention that FVL and FII were comparable to population norms (Curtis et al., 2017; Ferriero et al., 2019). Subsequent evaluation deemed thrombophilia evaluation in neonates as having limited clinical utility because "levels of protein C, protein S, antithrombin, and factor XI are normally decreased to 30% of adults levels, and these levels only approach adult

levels at various time points during childhood". Therefore, the use of thrombophilia testing for these proteins may be misleading in the neonatal period, and MRIs instead should be used to diagnose the thrombosis (Ferriero et al., 2019). Moreover, studies focusing on the roles of thrombophilia, arteriopathy, and cardiac abnormalities in perinatal ischemic stroke find that these risk factors were at best unclear, weakening what predictive power they were believed to contain for even recurrent events after perinatal stroke and leading researchers to conclude that thrombophilia evaluation should rarely be considered in cases of perinatal stroke (Lehman et al., 2017).

While the initial aPTT assays used unaltered plasma (first-generation assays), some versions were neither sensitive nor specific for FVL. Modifications to this test resulted in second generation functional aPC resistance assays that correlate well with the presence of FVL. However, in rare cases, functional assays for aPC resistance can give misleading results (e.g., the presence of a lupis anticoagulant can cause falsely abnormal results in some assays; therapy with a direct thrombin inhibitor or oral factor Za inhibitor can cause falsely normal results). In addition, while FVL can be detected by genetic testing or a second-generation functional coagulation test for aPC resistance, individuals with a positive aPC resistance assay would still need to receive genetic testing to confirm a diagnosis (Bauer, 2023a). Due to difficulty with interpretation, a need for confirmatory genetic testing, and the overall declining cost of genetic testing, aPC resistance assays are performed infrequently. When performed, they are simply reported as positive, borderline, or negative (Middeldrop, 2023).

The term FVL paradox describes the different risk of DVT and PE in FVL carriers; there is data to suggest that FLV carriers are less likely to experience isolated PE (without DVT) than the general population (Bauer, 2023a). de Moerloose et al. (2000) studied the prevalence of FVL in people suspected of DVT and/or PE. The 99 participants with PE were categorized based on "those with PE but without DVT (n = 57) and those with PE and DVT (n = 42)." The odds ratio for the prevalence of FVL was higher (19.1%) in patients with DVT and PE than the odds ratio for the prevalence of FVL in patients with only PE (10.5%), suggesting that "patients with primary PE are less often affected by the factor V Leiden mutation." In another study by (Mäkelburg et al., 2010), the relative risk of DVT in FVL carriers compared to non-carriers was 7.0, while the relative risk of PE in FVL carriers compared to non-carriers was 2.8.

Analytical Validity

Murphy and Sabath (2019) have compared the accuracy and reliability of two tests: a genotypic assay which identifies FVL mutations, and a phenotypic aPC resistance assay. Data from 1596 patients was analyzed; each patient had received both types of testing. The authors state that the phenotypic testing exhibited both high sensitivity and

specificity compared to genotypic testing. “Phenotypic assays had close to total concordance with genotypic assays over 16 years of testing. Changing ordering practices could result in up to an 80% reduction in testing costs” (Murphy & Sabath, 2019).

A systematic review and meta-analysis by Chiasakul et al. (2019) researched the relationship between inherited thrombophilia and the risk of arterial ischemic stroke in adults. Inherited thrombophilias included FVL, protein C and S deficiency, antithrombin deficiency and prothrombin G20210A mutation. For this study, 11,916 stroke patients and 96,057 controls were identified. The authors concluded that “Compared with controls, patients with arterial ischemic stroke were significantly more likely to have the following inherited thrombophilias: factor V Leiden (OR, 1.25; 95% CI, 1.08-1.44; I2=0%), prothrombin G20210A mutation (OR, 1.48; 95% CI, 1.22-1.80; I2=0%), protein C deficiency (OR, 2.13; 95% CI, 1.16-3.90; I2=0%), and protein S deficiency (OR, 2.26; 95% CI, 1.34-3.80; I2=8.8%)” (Chiasakul et al., 2019). Antithrombin deficiency did not reach statistical significance in this study. Hence, in this review, inherited thrombophilias were found to be associated with an increased risk of arterial ischemic stroke in adults.

In a systematic review, Ortega studied the predictive value of D-dimer testing for venous thrombosis diagnosis in unusual locations. 3378 patients from 23 articles with thrombosis in unusual sites, such as upper extremity deep vein thrombosis (DVT), cerebral vein thrombosis (CVT) and splanchnic vein thrombosis (SVT), were studied. 12 articles on CVT concluded that timing of D-dimer testing is important and patients with short duration of symptoms displayed higher D-dimer levels. Sensitivity and specificity in these patients ranged from 58% to 97% and from 77% to 97.5%, respectively. The authors conclude that "D-dimer testing should not be currently recommended for the diagnosis of thrombosis in unusual sites as a first line diagnostic tool. The development of algorithms combining biomarkers such as D-dimer and clinical decision tools could improve the diagnosis" (Ordieres-Ortega et al., 2020).

Clinical Utility and Validity

A D-dimer assay is a blood test that is used in clinical practice to assist in identifying if a patient has a DVT or PE; this test may also help patients experiencing unprovoked VTE to determine if anticoagulation treatment should continue or halt after initial treatment is complete (Linkins & Takach Lapner, 2017). A D-dimer assay may vary greatly based on the type of antibody used, the method of capture, calibration, and instrumentation. Currently, 30 different assays as available which use 20 different monoclonal antibodies; various studies have reported a broad sensitivity and specificity range for D-dimer assays from 69-97% and 43-99% respectively (Linkins & Takach

Lapner, 2017). Hence, all D-dimer assays differ and need to be validated within the population of interest. Because of this, comparing study results is challenging.

Factor VIII is a blood clotting protein encoded by the F8 gene. A case report by Algahtani and Stuckey (2019) suggests that high factor VIII levels may also assist in risk factor determination for thrombosis or ischemic heart disease. "We conclude that high factor VIII levels are a risk factor for thrombosis, with a greater impact on venous than on arterial thrombosis. However, due to a lack of international consensus on methods for the laboratory testing of factor VIII levels in plasma, we would not currently recommend the measurement of factor VIII levels as part of routine thrombophilia screening" (Algahtani & Stuckey, 2019). This relationship has been shown previously as elevated levels of coagulation factor VIII:C were identified in a retrospective study of 584 first-degree relatives of 177 patients with high coagulation factor VIII:C levels; the researchers found that 40% of first degree relatives also had high VIII:C levels and were at an increased risk for VTE and arterial thrombosis when compared to other first-degree relatives with normal VIII:C levels (Bank et al., 2005).

Lee et al. (2017) performed whole exome sequencing on 64 patients with VTE to assess the types of mutations of inherited thrombophilias. Of these 64 patients, 39 of them were found to have a pathogenic variant or variant of unknown significance (VUS). Further, eight were found to have a Factor V mutation (six with FVL and two with less common mutations), two were found to have a prothrombin G20210A mutation, six were found to have a protein S mutation, two were found to have a protein C mutation, and three were found to have an antithrombin mutation (Lee et al., 2017).

Segal et al. (2009) reviewed the utility of FVL and prothrombin G20210A testing. The authors reviewed 124 articles and concluded that although genetic testing for these two risk factors is very accurate (valid), the clinical utility is lacking due to lack of evidence demonstrating improvement in clinical outcomes (Segal et al., 2009).

Onda studied the clinical utility of a new diagnostic algorithm based on serum D-dimer levels for VTE after hepatectomy. A total of 742 patients who underwent hepatectomy were enrolled in the study and measured for serum D-dimer levels post-op. CT scan was performed for patients who had a D-dimer level of greater than 20 µg/mL. Based on D-dimer and CT scan, VTE was diagnosed in 26 patients and pulmonary embolism (PE) was diagnosed in 18 patients. Multivariate analysis also showed that a resected liver weight of more than 120 grams is a significant predictor of VTE. Overall, "patients who undergo hepatectomy are at high risk for VTE, especially when the resected liver weight is high. The proposed diagnostic algorithm based on serum D-dimer levels for VTE after hepatectomy can be useful for early diagnosis" (Onda et al., 2021).

VI. Guidelines and Recommendations

American Heart Association/American Stroke Association (AHA/ASA)

The AHA/ASA has issued a scientific statement for the management of stroke in neonates and children, wherein testing for thrombophilic abnormalities are discussed. The AHA/ASA admits that due to the lack of "an adequately powered study to detect the impact of genetic thrombophilia on recurrence risk in pediatric AIS [arterial ischemic stroke], definite recommendations about evaluation remain challenging", but acknowledges that "laboratory testing outside of clinical studies may provide guidance for long-term management of the patient". For cases of thrombophilia the AHA/ASA provides an algorithm for the "Targeted Evaluation of a Child With AIS for Rare Causes or Causes Requiring Additional Evaluation" that includes the examination of Factor VIII level, Lipoprotein(a),
MTHFR mutation, and homocysteine levels, and it is suggested that "non-DNA testing may need to be repeated when the child is older to ensure that adult levels of proteins have been attained" and "measurement of proteins or homocysteine levels in the acute phase of stroke may not be accurate and should be repeated after the acute event". Finally, for the evaluation of a child with AIS, it is believed that "A thrombophilia evaluation is helpful in every case of childhood stroke, especially if there is no identifiable cause, medical history of thrombosis, or a first-degree relative with thrombosis history" (Ferriero et al., 2019).

In 2021, the AHA released guidelines on stroke prevention. The AHA brushes on testing for hematologic traits in the context of secondary stroke prevention. "If in certain clinical scenarios (eg, paradoxical emboli caused by venous thrombosis or recurrent venous thrombosis) testing for thrombophilic states is considered, testing for protein C, protein S, or antithrombin levels should be deferred or repeated at least 4 to 6 weeks (or up to 6 months for factor VIII609) after the acute stroke given that these protein levels may be altered during the acute stroke phase" (Kleindorfer et al., 2021).

American College of Medical Genetics and Genomics (ACMG)

ACMG has released guidelines for laboratory testing of venous thromboembolism (VTE). This 2018 edition superseded the 2005 edition. The guidelines are as follows:

Testing for factor V Leiden and factor II c.*97G>A (this mutation is also known as G20210A) is recommended in the following circumstances:

- A first unprovoked VTE, especially <50 years old
- VTE at unusual sites (such as hepatic portal, mesenteric, and cerebral veins)
- Recurrent VTE
- Personal history of VTE with (a) two or more family members with a history of VTE or (b) one first-degree relative with VTE at a young age

- Patients with low aPC resistance activity

Testing may be considered in the following circumstances:

- Females under the age of 50 who smoke tobacco and have a history of acute myocardial infarction
- Siblings of individuals known to be homozygous for factor V Leiden or factor II c.*97G>A, because they have a one in four chance of being a homozygote
- Asymptomatic pregnant individual or individual contemplating pregnancy, with a first-degree relative with unprovoked VTE or VTE provoked by pregnancy or contraceptive use
- Pregnant individual or individual contemplating pregnancy or estrogen use who has a first-degree relative with a history of VTE and is a known carrier for factor V Leiden and/or factor II c.97*G>A variant
- Pregnant individual or individual contemplating pregnancy with a previous non-estrogen-related VTE or VTE provoked by a minor risk factor, because knowledge of the factor V Leiden or factor II c.*97G>A status may alter pregnancy related thrombophylaxis (Zhang et al., 2018).

The ACMG found several clinical scenarios requiring special considerations worth mentioning, involving different populations. One involved the testing of asymptomatic versus symptomatic individuals, in which they assert that "It is generally not recommended to test asymptomatic minors as VTE rarely occurs before young adulthood even in the homozygous state." For prenatal testing and population screening, the ACMG suggests that "prenatal testing and population screening are not indicated due to the low penetrance of these variants, later age of onset, and lack of genotype-directed prophylaxis". Lastly, in individuals considering taking estrogen-containing oral contraceptives (OC) or hormone replacement therapy (HRT), the ACMG indicates that "A family and personal history of thrombosis should be carefully evaluated for all women before initiating HRT and a positive history may warrant thrombophilia screening" (Zhang et al., 2018).

ACMG does not support testing for *MTHFR* variants in thrombophilia assessment due to the lack of correlation with negative pregnancy outcomes (Hickey et al., 2013). This statement was reaffirmed in 2020 (Bashford et al., 2020).

American Society of Hematology (ASH)

The 2013 ASH recommends against testing "for thrombophilia in adult patients with venous thromboembolism (VTE) occurring in the setting of major transient risk factors (surgery, trauma or prolonged immobility)" (ASH, 2013).

In 2018, ASH released their guidelines for management of venous thromboembolism, which included the following recommendations (Lim et al., 2018). These guidelines were reviewed in 2022 by an ASH expert working group and agreed to "continue monitoring the supporting evidence rather than revise or retire these guidelines at this time."

- "*Recommends* using a strategy starting with D-dimer for excluding PE in a population with low prevalence/PTP (≤5%), followed by ventilation-perfusion (VQ) scan or computed tomography pulmonary angiography (CTPA) for patients requiring additional testing.
- *Recommends against* using a positive D-dimer alone to diagnose PE, and against additional testing following negative CTPA or normal VQ scan in a population with low prevalence/PTP (≤5%).
- S*uggests* using a strategy starting with D-dimer for excluding PE in a population with intermediate prevalence/PTP (~20%), followed by VQ scan or CTPA for patients requiring additional testing.
- *Recommends against* using a positive D-dimer alone to diagnose PE, and against additional testing following negative CTPA or normal VQ scan in a population with intermediate prevalence/PTP (~20%).
- *Recommends against* using a positive D-dimer alone to diagnose PE, and against using D-dimer as a subsequent test following a negative CT scan in a population with high prevalence/PTP (≥50%).
- *Suggests* using a strategy starting with D-dimer for excluding recurrent PE in a population with unlikely PTP.
- *Recommends* using a strategy starting with D-dimer for excluding DVT in a population with low prevalence/PTP (≤10%), followed by proximal lower extremity ultrasound or whole-leg ultrasound for patients requiring additional testing.
- *Recommends against* using a positive D-dimer alone to diagnose DVT, and against additional testing following negative proximal or whole-leg ultrasound in a population with low prevalence/PTP (≤10%).
- *Recommends against* using a positive D-dimer alone to diagnose DVT in a population with intermediate prevalence/PTP (~25%).
- *Recommends against* using a positive D-dimer alone to diagnose DVT in a population with high prevalence/PTP (≥50%).
- *Suggests* using a strategy starting with D-dimer for excluding recurrent DVT in a population with unlikely PTP.
- *Suggests* a strategy starting with D-dimer for excluding upper extremity DVT in a population with low prevalence/unlikely PTP (10%), followed by duplex ultrasound if D-dimer is positive.

- *Recommends against* using a positive D-dimer alone to diagnose upper extremity DVT in a population with low prevalence/unlikely PTP (10%).
- *Suggests* a strategy of either D-dimer followed by duplex ultrasound/serial duplex ultrasound, or duplex ultrasound/serial duplex ultrasound alone for assessing patients suspected of having upper extremity DVT in a population with high prevalence/likely PTP (40%).
- *Recommends against* using a positive D-dimer alone to diagnose upper extremity DVT in a population with high prevalence/likely PTP (40%)" (Lim et al., 2018).

In 2020, the ASH released guidelines on management of venous thromboembolism. ASH suggest "against the routine use of prognostic scores, D-dimer testing, or venous ultrasound to guide the duration of anticoagulation" (Ortel et al., 2020).

In 2023, the ASH released guidelines thrombophilia testing for the management of venous thromboembolism. "The panel issued a strong recommendation against testing the general population before starting combined oral contraceptives (COCs) and conditional recommendations for thrombophilia testing in the following scenarios: (a) patients with VTE associated with nonsurgical major transient or hormonal risk factors; (b) patients with cerebral or splanchnic venous thrombosis, in settings where anticoagulation would otherwise be discontinued; (c) individuals with a family history of antithrombin, protein C, or protein S deficiency when considering thromboprophylaxis for minor provoking risk factors and for guidance to avoid COCs/hormone replacement therapy; (d) pregnant women with a family history of high-risk thrombophilia types; and (e) patients with cancer at low or intermediate risk of thrombosis and with a family history of VTE. For all other questions, the panel provided conditional recommendations against testing for thrombophilia." The panel also listed the following 23 recommendations (Middeldorp et al., 2023):

- "For patients with unprovoked VTE who have completed primary short-term treatment, the ASH guideline panel suggests not to perform thrombophilia testing to guide the duration of anticoagulant treatment (conditional recommendation based on very low certainty in the evidence about effects)."
- "For patients with VTE provoked by surgery who have completed primary short-term treatment, the ASH guideline panel suggests not to perform thrombophilia testing to determine the duration of anticoagulant treatment (conditional recommendation based on very low certainty in the evidence about effects)."
- "For patients with VTE provoked by a nonsurgical major transient risk factor who have completed primary short-term treatment, the ASH guideline panel suggests testing for thrombophilia to guide anticoagulant treatment duration. The panel suggests indefinite anticoagulant treatment for patients with thrombophilia and

stopping anticoagulant treatment for patients without thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."

- "For women with VTE provoked by pregnancy or postpartum who have completed primary treatment, the ASH guideline panel suggests thrombophilia testing to guide anticoagulant treatment duration. The panel suggests indefinite anticoagulant treatment for women with thrombophilia and stopping anticoagulant treatment for women without thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."
- "For women with VTE associated with COCs who have completed primary short-term treatment, the ASH guideline panel suggests testing for thrombophilia to guide anticoagulant treatment duration. The panel suggests indefinite anticoagulant treatment for women with thrombophilia and stopping anticoagulant treatment for women without thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."
- "For patients with an unspecified type of VTE who have completed primary short-term treatment, the ASH guideline panel suggests not performing thrombophilia testing to guide anticoagulant treatment duration (conditional recommendation based on very low certainty in the evidence about effects)."
- "For patients with CVT who have completed primary treatment in a setting where anticoagulation would be discontinued, the ASH guideline panel suggests thrombophilia testing to guide anticoagulant treatment duration. The panel suggests indefinite anticoagulation for patients with thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."
- "For patients with CVT who have completed primary treatment in a setting where anticoagulation would be continued indefinitely, the ASH guideline panel suggests not to perform thrombophilia testing to guide anticoagulant treatment duration (conditional recommendation based on very low certainty in the evidence about effects)."
- "For patients with splanchnic venous thrombosis who have completed primary treatment in a setting where anticoagulation would be discontinued, the ASH guideline panel suggests thrombophilia testing to guide anticoagulant treatment duration. The panel suggests indefinite anticoagulation for patients with thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."
- "For patients with splanchnic venous thrombosis who have completed primary treatment in a setting where anticoagulation would be continued indefinitely, the ASH guideline panel suggests not performing thrombophilia testing to guide anticoagulant treatment duration (conditional recommendation based on very low certainty in the evidence about effects)."

- "For individuals with a family history of VTE and known FVL or PGM [prothrombin 20210A gene mutation] (low-risk thrombophilia) who have a minor provoking risk factor for VTE (eg, immobility or minor injury, illness, or infection), the ASH guideline panel suggests not testing for the known familial thrombophilia to guide thromboprophylaxis (conditional recommendation based on very low certainty in the evidence about effects). For individuals with a family history of VTE and known antithrombin, protein C, or protein S deficiency (high-risk thrombophilia) who have a minor provoking risk factor for VTE, the ASH guideline panel suggests testing for the known familial thrombophilia. The panel suggests thromboprophylaxis in individuals with thrombophilia and no thromboprophylaxis in individuals without thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."
- "For individuals with a family history of VTE and known FVL or PGM (low-risk thrombophilia) who have a minor provoking risk factor for VTE (eg, immobility or minor injury, illness, or infection), the ASH guideline panel suggests not testing for all hereditary thrombophilias to guide thromboprophylaxis (conditional recommendation based on very low certainty in the evidence about effects). For individuals with a family history of VTE and known antithrombin, protein C, or protein S deficiency (high-risk thrombophilia) who have a minor provoking risk factor for VTE, the ASH guideline panel suggests testing for all hereditary thrombophilias (using a panel of tests). The panel suggests thromboprophylaxis in individuals with thrombophilia and no thromboprophylaxis for a minor provoking risk factor in individuals without thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."
- "For individuals with a family history of VTE and unknown thrombophilia status in the family who have a minor provoking risk factor for VTE (eg, immobility or minor injury, illness, or infection), the ASH guideline panel suggests not testing for all hereditary thrombophilias (using a panel of tests) to guide thromboprophylaxis (conditional recommendation based on very low certainty in the evidence about effects)."
- "For individuals with a family history of FVL or PGM (low-risk thrombophilia) but no family history of VTE who have a minor provoking risk factor for VTE (eg, immobility or minor injury, illness, or infection), the ASH guideline panel suggests not testing for the known thrombophilia to guide thromboprophylaxis (conditional recommendation based on very low certainty in the evidence about effects). For individuals with a first-degree family history of antithrombin, protein C, or protein S deficiency (high-risk thrombophilia) but no family history of VTE who have a minor provoking risk factor for VTE, the ASH guideline panel suggests testing for the known thrombophilia. The panel suggests thromboprophylaxis in individuals with thrombophilia and no thromboprophylaxis in individuals without

thrombophilia (conditional recommendation based on very low certainty in the evidence about effects). For individuals with a second-degree family history of antithrombin, protein C, or protein S deficiency (high-risk thrombophilia) but no family history of VTE who have a minor provoking risk factor for VTE, the ASH guideline panel suggests either testing for the known thrombophilia or not testing for thrombophilia to guide the use of thromboprophylaxis (conditional recommendation based on very low certainty in the evidence about effects)."

- "For women from the general population who are considering using COCs, the ASH guideline panel recommends not performing thrombophilia testing to guide the use of COC (strong recommendation based on low certainty in the evidence about effects)."
- "For women from the general population who are considering using HRT, the ASH guideline panel suggests not performing thrombophilia testing to guide the use of HRT (conditional recommendation based on low certainty in the evidence about effects)."
- "For women with a family history of VTE and unknown thrombophilia status in the family who are considering using COCs, the ASH guideline panel suggests not testing for hereditary thrombophilia (using a panel of tests) to guide the use of COC (conditional recommendation based on very low certainty in the evidence about effects)."
- "For women with a family history of VTE and unknown thrombophilia in the family who are considering using HRT, the ASH guideline panel suggests not performing thrombophilia testing for any hereditary thrombophilia to guide the use of HRT (conditional recommendation based on very low certainty in the evidence about effects)."
- "For women with a family history of VTE and known FVL or PGM in the family (low-risk thrombophilia), the ASH guideline panel suggests not testing for the known familial thrombophilia to guide the use of COC (conditional recommendation based on very low certainty in the evidence about effects). For women with a family history of VTE and known antithrombin, protein C, or protein S deficiency in the family (high-risk thrombophilia), the ASH guideline panel suggests testing for the known familial thrombophilia. The panel suggests avoidance of COCs for women with high-risk thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."
- "For women with a family history of VTE and known FVL or PGM in the family (low-risk thrombophilia), the ASH guideline panel suggests not testing for the known familial thrombophilia to guide the use of HRT (conditional recommendation based on very low certainty in the evidence about effects). For women with a family history of VTE and known antithrombin, protein C, or protein S deficiency in the family (high-risk thrombophilia), the ASH guideline

panel suggests testing for the known familial thrombophilia. The panel suggests avoidance of HRT for women with high-risk thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."

- "For women with a family history of VTE and known homozygous FVL, a combination of FVL and PGM, or an antithrombin deficiency in the family, the ASH guideline panel suggests testing for the known familial thrombophilia. The panel suggests antepartum thromboprophylaxis for women with the same familial thrombophilia (ie, homozygous FVL, combination of FVL and PGM, or antithrombin deficiency) and no antepartum prophylaxis for women without the same familial thrombophilia (conditional recommendation based on very low certainty in the evidence about effects). For women with a family history of VTE and a known protein C or protein S deficiency in the family, the ASH guideline panel suggests either testing for the known familial thrombophilia or not testing for thrombophilia to guide antepartum prophylaxis (conditional recommendation based on very low certainty in the evidence about effects)."
- "For women with a first-degree family history of VTE and known homozygous FVL, a combination of FVL and PGM, antithrombin deficiency, protein C deficiency, or protein S deficiency in the family, the ASH guideline panel suggests testing for the known familial thrombophilia. The panel suggests postpartum thromboprophylaxis for women with the same familial thrombophilia (ie, homozygous FVL, combination of FVL and PGM, or antithrombin deficiency) and no postpartum prophylaxis for women without the same familial thrombophilia (conditional recommendation based on very low certainty in the evidence about effects). For women with a second-degree family history of VTE and a known combination of FVL and PGM, or antithrombin deficiency in the family, the ASH guideline panel suggests testing for the known familial thrombophilia. The panel suggests postpartum thromboprophylaxis for women with thrombophilia and no postpartum prophylaxis for women without thrombophilia (conditional recommendation based on very low certainty in the evidence about effects). For women with a second-degree family history of VTE and a known protein C or protein S deficiency in the family, the ASH guideline panel suggests either testing for the known familial thrombophilia or not testing for thrombophilia to guide postpartum thromboprophylaxis (conditional recommendation based on very low certainty in the evidence about effects)."
- "For ambulatory patients with cancer receiving systemic therapy who have a family history of VTE and are otherwise determined to be at low or intermediate risk for VTE, the ASH guideline panel suggests testing for hereditary thrombophilia. The panel suggests ambulatory thromboprophylaxis for patients with thrombophilia and no thromboprophylaxis for patients without

thrombophilia (conditional recommendation based on very low certainty in the evidence about effects)."

American College of Obstetricians and Gynecologists

The 2013 ACOG clinical management guidelines recommend that screening for inherited thrombophilia "may be considered in the following clinical settings:

1. "A personal history of venous thromboembolism that was associated with a non-recurrent risk factor
2. A first degree relative (parent or sibling) with a history of high-risk thrombophilia" (ACOG, 2013).

The 2018 ACOG Practice Bulletin Summary Number 197 supersedes the above 2013 guidelines (Practice Bulletin Number 138). In this update, the ACOG makes the following recommendations regarding screening based on "limited or inconsistent scientific evidence":

"Screening for inherited thrombophilias is not recommended for women with a history of fetal loss or adverse pregnancy outcomes including abruption, preeclampsia, or fetal growth restriction because there is insufficient clinical evidence that antepartum prophylaxis with unfractionated heparin or low molecular-weight heparin prevents recurrence in these patients."

"Because of the lack of association between either heterozygosity or homozygosity for the *MTHFR* C677T polymorphism and any negative pregnancy outcomes, including any increased risk of VTE, screening with either *MTHFR* mutation analyses or fasting homocysteine levels is not recommended."

The 2018 ACOG recommends the following screening guideline based on "consensus and expert opinion":

"Among women with personal histories of VTE, recommended screening tests for inherited thrombophilias should include factor V Leiden mutation; prothrombin G20210A mutation; and antithrombin, protein S, and protein C deficiencies" (ACOG, 2018).

Evaluation of Genomic Applications in Practice and Prevention

"The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group found adequate evidence to recommend against *routine* testing for Factor V Leiden (FVL) and/or prothrombin 20210G>A (Gupta et al.) in the following circumstances: (1) adults with idiopathic venous thromboembolism (VTE). In such

cases, longer term secondary prophylaxis to avoid recurrence offers similar benefits to patients with and without one or more of these mutations. (2) Asymptomatic adult family members of patients with VTE and an FVL or PT mutation, for the purpose of considering primary prophylactic anticoagulation. Potential benefits are unlikely to exceed potential harms. The evidence was insufficient to determine whether FVL/PT testing might have clinical utility in some circumstances, such as for identifying FVL homozygosity among asymptomatic family members of adults with idiopathic VTE or counseling patients about the risks and benefits of antithrombotic therapy. The recommendations do not extend to patients with other risk factors for thrombosis, such as contraceptive use, as the evidence review that serves as the basis for the recommendations focused primarily on idiopathic VTE" (EGAPP, 2011).

The Anticoagulation Forum

The Anticoagulation Forum published guidance in the Journal of Thrombosis and Thrombolysis on (Stevens et al., 2016):

- "Do not perform thrombophilia testing following an episode of provoked VTE. A positive thrombophilia evaluation is not a sufficient basis to offer extended anticoagulation following an episode of provoked VTE.
- Do not perform thrombophilia testing in patients following an episode of unprovoked VTE. If a patient with unprovoked VTE and low bleeding risk is planning to stop anticoagulation, test for thrombophilia if test results would change this decision. A negative thrombophilia evaluation is not a sufficient basis to stop anticoagulants following an episode of unprovoked VTE in a patient with low bleeding risk and willingness to continue therapy.
  Heterozygosity for FVL or PGM does not increase the predicted risk of recurrence after unprovoked VTE to a clinically significant degree.
- Do not test for thrombophilia in asymptomatic family members of patients with VTE or hereditary thrombophilia. As a family history of VTE confers an excess risk of thrombosis, relatives should be counseled regarding use of prophylaxis in high-risk situations.
- Do not test for thrombophilia in asymptomatic family members of patients with VTE or hereditary thrombophilia who are contemplating use of estrogen. If an [individual] is contemplating estrogen use has a first-degree relative with VTE and a known hereditary thrombophilia, test for that thrombophilia if the result would change the decision to use estrogen.
- Do not perform thrombophilia testing at the time of VTE diagnosis or during the initial 3-month course of anticoagulant therapy. When testing for thrombophilias following VTE, use either a 2-stage testing approach or perform testing after a minimum of 3 months of anticoagulant therapy has been completed, and

anticoagulants have been held.

- Do not test for thrombophilia in asymptomatic family members of patients with VTE or hereditary thrombophilia who are contemplating pregnancy. If [an individual] contemplating pregnancy has a first-degree relative with VTE and a known hereditary thrombophilia… test for that thrombophilia if the result would change VTE prophylaxis decisions" (Stevens et al., 2016).

American College of Cardiology (ACC)

In 2017, guidance published in the New England Journal of Medicine by Gupta was summarized by Barnes for the American College of Cardiology:

1. "Venous thromboembolism (VTE) affects an estimated 300,000-600,000 patients annually in the United States.
2. The risk of VTE recurrence is best predicted by whether the initial VTE episode was provoked or unprovoked, not the results of inherited thrombophilia testing.
3. Most patients with a provoked VTE have recently undergone surgery, immobility, trauma, or have a concurrent cancer diagnosis. Concurrent use of hormones (e.g., estrogen-containing contraceptive pills) is also frequently considered a provoking factor for VTE development.
4. For patients with a first provoked VTE event, guidelines recommend anticoagulation for only 3 months (not longer). Prolonged anticoagulation is associated with an increased risk of bleeding that outweighs the risk of VTE recurrence for these patients.
5. Patients with an unprovoked VTE (none of the provoking risk factors listed above) require longer anticoagulation due to a higher risk of recurrence that outweighs the risk of bleeding associated with long-term anticoagulation therapy.
6. Thrombophilia testing performed in the setting of an acute clot or ongoing anticoagulation therapy will often result in spurious results (usually false positive). For example, natural anticoagulants (e.g., protein C and S, antithrombin) are consumed during an acute thrombotic event and the levels can be reduced by ongoing anticoagulant therapy.
7. A recent study identified that up to 55% of Medicare patients with provoked VTE had undergone inappropriate thrombophilia testing, associated with significant cost to the healthcare system.
8. While thrombophilia testing rarely impacts management decisions about anticoagulation therapy, it may be beneficial for genetic testing purposes in patients presenting with a first unprovoked VTE at a young age (e.g., <45 years) or at an unusual site.
9. For patients with unprovoked VTE at a young age, VTE at an unusual site, arterial thrombosis, or pregnancy morbidity, testing for antiphospholipid antibodies,

JAK2 mutation, and paroxysmal nocturnal hemoglobinuria may be beneficial.
10. There is no role for extensive cancer screening (e.g., computed tomography scanning) in patients with VTE. Only routine, age-appropriate cancer screening is recommended" (G. Barnes, 2017; Gupta et al., 2017).

Again in 2017, key points—inclusive of guiding points—published in the New England Journal of Medicine by Connors were captured by Barnes for the American College of Cardiology:

1. "The majority of patients with venous thromboembolism (VTE) should not be tested for thrombophilia. Data supporting clinical usefulness and benefits are limited or nonexistent.
2. Most patients with inherited thrombophilia can be identified by coagulation experts based on the patient's personal and family history of VTE. Thrombophilia testing is usually not required.
3. Factors associated with an inherited thrombophilia include VTE at a young age (<40-50 years), a strong family history of VTE, VTE in conjunction with weak provoking factors at a young age, recurrent VTE, and VTE in an unusual site (e.g., cerebral or splanchnic veins).
4. Do not perform thrombophilia testing at the time of a VTE event, as it can be inaccurate (often false positive). Perform testing (when indicated) after completion of initial therapy and if it might change management strategies.
5. Do not perform thrombophilia testing while a patient is receiving anticoagulation. Instead, wait until 2 weeks after discontinuing warfarin, or 2 days for direct oral anticoagulants and heparin.
6. The goal of thrombophilia testing should be to aid decision making regarding future VTE prophylaxis, to guide testing of family members, and to determine the cause in severe or fatal VTE. Test results alone should not be used to decide on the duration of anticoagulation therapy.
7. Most VTE recurrence risk tools do not incorporate thrombophilia test results into their risk stratification schemes.
8. For patients with provoked VTE, even if they have homozygous factor V Leiden, prothrombin gene mutations, or deficiencies of protein S, C, or antithrombin, they do not require lifelong anticoagulation.
9. Currently available thrombophilia tests are insufficient to identify inherited risks of VTE. Therefore, a negative test should not be interpreted as a patient being free of thrombophilia.
10. Testing for the antiphospholipid antibody syndrome may be useful in patients with unprovoked VTE if there is clinical equipoise about extended anticoagulation courses. It can also be useful to determine warfarin versus direct oral anticoagulant therapy" (G. D. Barnes, 2017; Connors, 2017).

European Society of Cardiology

The ESC has published guidelines for the diagnosis and management of acute PE. These guidelines state:

- "D-dimer measurement and clinical prediction rules should be considered to rule out PE during pregnancy or the post-partum period
- Plasma D-dimer measurement, preferably using a highly sensitive assay, is recommended in outpatients/emergency department patients with low or intermediate clinical probability, or those that are PE-unlikely, to reduce the need for unnecessary imaging and irradiation
- A D-dimer test, using an age-adjusted cut-off or adapted to clinical probability, should be considered as an alternative to the fixed cut-off level
- D-dimer measurement is not recommended in patients with high clinical probability, as a normal result does not safely exclude PE, even when using a highly sensitive assay
- Assessment of the RV [right ventricle] by imaging methods or laboratory biomarkers should be considered, even in the presence of a low PESI [Pulmonary Embolism Severity Index] or a negative sPESI [simplified Pulmonary Embolism Severity Index]" (Konstantinides et al., 2019).

In 2021, the ESC Working Group released guidelines on diagnosis and management of acute deep vein thrombosis. These guidelines suggest that "ELISA D-dimer or highly sensitive immunoturbidimetric tests should be measured in 'unlikely' clinical probability patients to exclude DVT diagnosis" (Mazzolai et al., 2022).

World Health Organization (WHO)

In the WHO medical eligibility criteria for contraceptive use, known thrombogenic mutations (e.g. factor V Leiden; prothrombin mutation; protein S, protein C, and antithrombin deficiencies) are "a condition which represents an unacceptable health risk if the contraceptive method is used" when considering combined hormonal contraceptives, and the method is "not to be used." The WHO notes that "among women with thrombogenic mutations, COC users had a 2- to 20-fold higher risk of thrombosis than non-users." When considering progestogen-only contraceptives, intrauterine devices, barrier methods, or female surgical sterilization, known thrombogenic mutations are "a condition for which there is no restriction for the use of the contraceptive method" or "the advantages of using the method generally outweigh the theoretical or proven risks." In all cases, the WHO clarifies that "routine screening is not appropriate because of the rarity of the conditions and the high cost of screening" (WHO, 2015).

National Institute for Health and Care Excellence (NICE)

In the NICE venous thromboembolic disease guidelines, for thrombophilia testing, it is recommended:

- "Do not offer testing for hereditary thrombophilia to people who are continuing anticoagulation treatment.
- "Do not offer thrombophilia testing to people who have had provoked DVT or PE."
- "Consider testing for antiphospholipid antibodies in people who have had unprovoked DVT or PE if it is planned to stop anticoagulation treatment, but be aware that these tests can be affected by anticoagulants and specialist advice may be needed."
- "Consider testing for hereditary thrombophilia in people who have had unprovoked DVT or PE and who have a first-degree relative who has had DVT or PE if it is planned to stop anticoagulation treatment, but be aware that these tests can be affected by anticoagulants and specialist advice may be needed."

"Do not routinely offer thrombophilia testing to first-degree relatives of people with a history of DVT or PE and thrombophilia" (NICE, 2023).

American College of Radiology (ACR)

The ACR has guidelines for suspected pulmonary embolisms (PE). The ARC prefaced that their publication "focuses on the initial evaluation for clinically suspected PE, recognizing that as many as 80% of PE cases are associated with DVT" and that "PE also may occur without detectable DVT." The guidelines do not mention any laboratory or genetic testing, although some of the imaging recommendations are based on positive or negative D-dimer results (Kirsch et al., 2022).

Thrombosis Canada

Thrombosis Canada released guidelines on the diagnosis of PE (Thrombosis Canada, 2023). They state: "The constellation of symptoms and signs may be suggestive of PE but do not alone have the sensitivity or specificity to rule in or rule out the diagnosis. When the diagnosis of PE is considered, the clinical stability of the patient and associated pre-test probability will dictate the diagnostic approach." The guidelines further recommend:

- "In patients without hypotension (SBP >90 mmHg), pre-test probability can be assessed by a validated clinical prediction rule such as the Well's score. In patients who are <50 years of age, with a low pretest probability of PE, further testing (such as d-dimer measurement or diagnostic imaging) is not necessary

provided all clinical features/criteria in the Pulmonary Embolism Rule-out Criteria (PERC) are present."

- "In cases with PE unlikely pre-test probability, a negative high sensitivity D-dimer result rules out the diagnosis of PE. However, a positive D-dimer test MUST be followed up with a definitive test to confirm/refute the diagnosis of PE.
- When a high-sensitive d-dimer assay is used, age-adjusted D-dimer levels can increase the specificity of D-dimer testing without sacrificing sensitivity. In patients over the age of 50, a D-dimer result is considered negative if it is less than the patient age multiplied by 10 (for example, in a 76-year-old, a negative result is less than 760 µg/L). For patients under the age of 50, a D-dimer value less than 500 µg/L remains the cutoff for a negative result.
- With a high PE pre-test probability (Well's score > 4.5), there is no role for ordering a D-dimer, as the clinical likelihood of PE remains unacceptably high among those with a negative D-dimer result. Therefore, when the Wells score is 4.5 or greater, one should go directly to imaging to establish the diagnosis" (Thrombosis Canada, 2023).

The Wells Score is a total score of: clinical symptoms and signs of DVT (3 points), previous DVT or PE (1.5 points), immobilization of over three days or surgery within four weeks (1.5 points), heart rate over 100 beats per minute (1.5 points), hemoptysis (1 point), malignancy (1 point), and no alternative diagnosis more likely than PE (3 points). A total score under 4.5 equates to PE unlikely, and a toal score of 4.5 or over equates to PE likely (Thrombosis Canada, 2023).

The PE Rule-out Criteria (PERC) for patients with low pretest probability for PE are: age less than 50, initial heart rate of less than 100 beats per minute, initial SaO2 of over 94% on room air, no unilateral leg swelling, no hemoptysis, no surgery or trauma in the past four weeks, no history of VTE and no estrogen use (Thrombosis Canada, 2023).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, please visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81240	F2 (prothrombin, coagulation factor III) (eg, hereditary hypercoagulability) gene analysis, 20210G>A variant
81241	F5 (coagulation factor V) (eg, hereditary hypercoagulability) gene analysis, Leiden variant
81291	MTHFR (5,10-methylenetetrahydrofolate reductase) (eg, hereditary hypercoagulability) gene analysis, common variants (eg, 677T, 1298C)
81400	Molecular pathology procedure, Level 1 (eg, identification of single germline variant [eg, SNP]) by techniques such as restriction enzyme digestion or melt curve analysis.
81479	Unlisted molecular pathology procedure
85300	Clotting inhibitors or anticoagulants; antithrombin III, activity
85301	Clotting inhibitors or anticoagulants; antithrombin III, antigen assay
85302	Clotting inhibitors or anticoagulants; protein C, antigen
85303	Clotting inhibitors or anticoagulants; protein C, activity
85305	Clotting inhibitors or anticoagulants; protein S, total
85306	Clotting inhibitors or anticoagulants; protein S, free
85307	Activated Protein C (APC) resistance assay
0278U	Hematology (genetic thrombosis), genomic sequence analysis of 14 genes, blood, buccal swab, or amniotic fluid Proprietary test: Versiti™ Thrombosis Panel Lab/Manufacturer: Versiti™

Current Procedural Terminology© American Medical Association. All Rights reserved.
Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

ACOG. (2013). ACOG Practice Bulletin No. 138: Inherited thrombophilias in pregnancy. *Obstet Gynecol*, *122*(3), 706-717. https://doi.org/10.1097/01.AOG.0000433981.36184.4e

ACOG. (2018). ACOG Practice Bulletin No. 197 Summary: Inherited Thrombophilias in Pregnancy. *Obstet Gynecol*, *132*(1), 249-251. https://doi.org/10.1097/aog.0000000000002705

Algahtani, F. H., & Stuckey, R. (2019). High factor VIII levels and arterial thrombosis: illustrative case and literature review. *Ther Adv Hematol*, *10*, 2040620719886685. https://doi.org/10.1177/2040620719886685

ASCLS. (2021). American Society for Clinical Laboratory Science. https://www.choosingwisely.org/clinician-lists/ascls7-do-not-order-a-homocysteine-assay-as-part-of-the-thrombophilia-work-up/

ASCP. (2017). *American Society for Clinical Pathology*. http://www.choosingwisely.org/clinician-lists/ascp-testing-for-protein-c-protein-s-or-antithrombin-during-active-clotting-event/

ASCP. (2019). *American Society of Clinical Pathology*. American Society of Clinical Pathology. https://www.choosingwisely.org/clinician-lists/ascp-hypercoagulable-workup/

ASH. (2013). *ASH - Testing for thromboembolism | Choosing Wisely* http://www.choosingwisely.org/clinician-lists/american-society-hematology-testing-for-thrombophilia-in-adults/

Bank, I., Libourel, E. J., Middeldorp, S., Hamulyak, K., van Pampus, E. C., Koopman, M. M., Prins, M. H., van der Meer, J., & Buller, H. R. (2005). Elevated levels of FVIII:C within families are associated with an increased risk for venous and arterial thrombosis. *J Thromb Haemost*, *3*(1), 79-84. https://doi.org/10.1111/j.1538-7836.2004.01033.x

Barnes, G. (2017, 06/05/2017). *Thrombophilia Testing for Provoked VTE*. American College of Cardiology. Retrieved 02/13/2019 from https://www.acc.org/latest-in-cardiology/ten-points-to-remember/2017/06/05/12/46/thrombophilia-testing-in-provoked-venous-thromboembolism

Barnes, G. D. (2017). *Thrombophilia Testing and Venous Thrombosis*. American College of Cardiology. https://www.acc.org/latest-in-cardiology/ten-points-to-remember/2017/10/20/11/18/thrombophilia-testing-and-venous-thrombosis.

Bartholomew, J. R. (2017). Update on the management of venous thromboembolism. *Cleve Clin J Med*, *84*(12 Suppl 3), 39-46. https://doi.org/10.3949/ccjm.84.s3.04

Bashford, M. T., Hickey, S. E., Curry, C. J., Toriello, H. V., American College of Medical, G., Genomics Professional, P., & Guidelines, C. (2020). Addendum: ACMG Practice Guideline: lack of evidence for MTHFR polymorphism testing. *Genetics in Medicine*, *22*(12), 2125-2125. https://doi.org/10.1038/s41436-020-0843-0

Bauer, K. (2023a, 03/30/2023). *Factor V Leiden and activated protein C resistance*. https://www.uptodate.com/contents/factor-v-leiden-and-activated-protein-c-resistance

Bauer, K. (2023b, 10/18/2023). *Protein C deficiency*. https://www.uptodate.com/contents/protein-c-deficiency

Bauer, K. (2023c, 02/18/2023). *Prothrombin G20210A mutation*. https://www.uptodate.com/contents/prothrombin-g20210a-mutation

Bauer, K. (2024a, 03/8/2024). *Clinical presentation and diagnosis of the nonpregnant adult with suspected deep vein thrombosis of the lower extremity*. https://www.uptodate.com/contents/clinical-presentation-and-diagnosis-of-the-nonpregnant-adult-with-suspected-deep-vein-thrombosis-of-the-lower-extremity

Bauer, K. (2024b, 03/05/2024). *Protein S deficiency*. https://www.uptodate.com/contents/protein-s-deficiency

Bauer, K., & Lip, G. (2023, 05/18/2023). *Evaluating adult patients with established venous thromboembolism for acquired and inherited risk factors*. https://www.uptodate.com/contents/evaluating-adult-patients-with-established-venous-thromboembolism-for-acquired-and-inherited-risk-factors

Bauer, K., & Lip, G. (2024, 06/18/2024). *Overview of the causes of venous thrombosis*. https://www.uptodate.com/contents/overview-of-the-causes-of-venous-thrombosis

Byrnes, J. R., & Wolberg, A. S. (2017). Red blood cells in thrombosis. *Blood*, *130*(16), 1795-1799. https://doi.org/10.1182/blood-2017-03-745349

Carroll, B. J., & Piazza, G. (2018). Hypercoagulable states in arterial and venous thrombosis: When, how, and who to test? *Vasc Med*, *23*(4), 388-399. https://doi.org/10.1177/1358863x18755927

Chiasakul, T., De Jesus, E., Tong, J., Chen, Y., Crowther, M., Garcia, D., Chai-Adisaksopha, C., Messe, S. R., & Cuker, A. (2019). Inherited Thrombophilia and the Risk of Arterial Ischemic Stroke: A Systematic Review and Meta-Analysis. *J Am Heart Assoc*, *8*(19), e012877. https://doi.org/10.1161/jaha.119.012877

Connors, J. M. (2017). Thrombophilia Testing and Venous Thrombosis. In *N Engl J Med* (Vol. 377, pp. 2298). https://doi.org/10.1056/NEJMc1713797

Crous-Bou, M., Harrington, L. B., & Kabrhel, C. (2016). Environmental and genetic risk factors associated with venous thromboembolism. *Semin Thromb Hemost*, *42*(8), 808-820. https://doi.org/10.1055/s-0036-1592333

Curtis, C., Mineyko, A., Massicotte, P., Leaker, M., Jiang, X. Y., Floer, A., & Kirton, A. (2017). Thrombophilia risk is not increased in children after perinatal stroke. *Blood*, *129*(20), 2793-2800. https://doi.org/10.1182/blood-2016-11-750893

de Moerloose, P., Reber, G., Perrier, A., Perneger, T., & Bounameaux, H. (2000). Prevalence of factor V Leiden and prothrombin G20210A mutations in unselected patients with venous thromboembolism. *Br J Haematol*, *110*(1), 125-129. https://doi.org/10.1046/j.1365-2141.2000.02039.x

EGAPP. (2011). Recommendations from the EGAPP Working Group: routine testing for Factor V Leiden (R506Q) and prothrombin (20210G>A) mutations in adults with a history of idiopathic venous thromboembolism and their adult family members. *Genet Med*, *13*(1), 67-76. https://doi.org/10.1097/GIM.0b013e3181fbe46f

Ferriero, D. M., Fullerton, H. J., Bernard, T. J., Billinghurst, L., Daniels, S. R., DeBaun, M. R., deVeber, G., Ichord, R. N., Jordan, L. C., Massicotte, P., Meldau, J., Roach, E. S., Smith, E. R., & Nursing, A. H. A. S. C. a. C. o. C. a. S. (2019). Management of Stroke in Neonates and Children: A Scientific Statement From the American Heart Association/American Stroke Association. *Stroke*, *50*(3). https://doi.org/10.1161/str.0000000000000183

Gupta, A., Sarode, R., & Nagalla, S. (2017). Thrombophilia Testing in Provoked Venous Thromboembolism: A Teachable Moment. *JAMA Internal Medicine*, *177*(8), 1195-1196. https://doi.org/10.1001/jamainternmed.2017.1815

Herrmann, J. (2018). *Clinical Cardio-Oncology*. Elsevier. https://doi.org/10.1016/C2015-0-01414-9

Hickey, S. E., Curry, C. J., & Toriello, H. V. (2013). ACMG Practice Guideline: lack of evidence for MTHFR polymorphism testing. *Genet Med*, *15*(2), 153-156. https://doi.org/10.1038/gim.2012.165

Kirsch, J., Wu, C. C., Bolen, M. A., Henry, T. S., Rajiah, P. S., Brown, R. K. J., Galizia, M. S., Lee, E., Rajesh, F., Raptis, C. A., Rybicki, F. J., Sams, C. M., Verde, F., Villines, T. C., Wolf, S. J., Yu, J., Donnelly, E. F., & Abbara, S. (2022). ACR Appropriateness Criteria® Suspected Pulmonary Embolism: 2022 Update. *J Am Coll Radiol*, *19*(11s), S488-s501. https://doi.org/10.1016/j.jacr.2022.09.014

Kleindorfer, D. O., Towfighi, A., Chaturvedi, S., Cockroft, K. M., Gutierrez, J., Lombardi-Hill, D., Kamel, H., Kernan, W. N., Kittner, S. J., Leira, E. C., Lennon, O., Meschia, J. F., Nguyen, T. N., Pollak, P. M., Santangeli, P., Sharrief, A. Z., Smith, S. C., Jr., Turan, T. N., & Williams, L. S. (2021). 2021 Guideline for the Prevention of Stroke in Patients With Stroke and Transient Ischemic Attack: A Guideline From the American Heart Association/American Stroke Association. *Stroke*, *52*(7), e364-e467. https://doi.org/10.1161/str.0000000000000375

Konstantinides, S. V., Meyer, G., Becattini, C., Bueno, H., Geersing, G. J., Harjola, V. P., Huisman, M. V., Humbert, M., Jennings, C. S., Jimenez, D., Kucher, N., Lang, I. M., Lankeit, M., Lorusso, R., Mazzolai, L., Meneveau, N., Ainle, F. N., Prandoni, P., Pruszczyk, P., . . . Zamorano, J. L. (2019). 2019 ESC Guidelines for the diagnosis and management of acute pulmonary embolism developed in collaboration with the European Respiratory Society (ERS): The Task Force for the diagnosis and management of acute pulmonary embolism of the European Society of Cardiology (ESC). *Eur Respir J*, *54*(3). https://doi.org/10.1183/13993003.01647-2019

Kujovich, J. L. (2011). Factor V Leiden thrombophilia. *Genet Med*, *13*(1), 1-16. https://doi.org/10.1097/GIM.0b013e3181faa0f2

Kujovich, J. L. (2018). Factor V Leiden Thrombophilia. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. H. Bean, K. Stephens, & A. Amemiya (Eds.), *GeneReviews((R))*. University of Washington, Seattle. https://www.ncbi.nlm.nih.gov/books/NBK1368/

Lee, E. J., Dykas, D. J., Leavitt, A. D., Camire, R. M., Ebberink, E., García de Frutos, P., Gnanasambandan, K., Gu, S. X., Huntington, J. A., Lentz, S. R., Mertens, K., Parish, C. R., Rezaie, A. R., Sayeski, P. P., Cromwell, C., Bar, N., Halene, S., Neparidze, N., Parker, T. L., . . . Lee, A. I. (2017). Whole-exome sequencing in evaluation of patients with venous thromboembolism. *Blood Adv*, *1*(16), 1224-1237. https://doi.org/10.1182/bloodadvances.2017005249

Lehman, L. L., Beaute, J., Kapur, K., Danehy, A. R., Bernson-Leung, M. E., Malkin, H., Rivkin, M. J., & Trenor, C. C. (2017). Workup for Perinatal Stroke Does Not Predict Recurrence. *Stroke*, *48*(8), 2078-2083. https://doi.org/10.1161/STROKEAHA.117.017356

Leung, A. N., Bull, T. M., Jaeschke, R., Lockwood, C. J., Boiselle, P. M., Hurwitz, L. M., James, A. H., McCullough, L. B., Menda, Y., Paidas, M. J., Royal, H. D., Tapson, V. F., Winer-Muram, H. T., Chervenak, F. A., Cody, D. D., McNitt-Gray, M. F., Stave, C. D., & Tuttle, B. D. (2011). An official American Thoracic Society/Society of Thoracic Radiology clinical practice guideline: evaluation of suspected pulmonary embolism in pregnancy. *Am J Respir Crit Care Med*, *184*(10), 1200-1208. https://doi.org/10.1164/rccm.201108-1575ST

Lim, W., Le Gal, G., Bates, S. M., Righini, M., Haramati, L. B., Lang, E., Kline, J. A., Chasteen, S., Snyder, M., Patel, P., Bhatt, M., Patel, P., Braun, C., Begum, H., Wiercioch, W., Schünemann, H. J., & Mustafa, R. A. (2018). American Society of Hematology 2018 guidelines for management of venous thromboembolism: diagnosis of venous thromboembolism. *Blood Adv*, *2*(22), 3226. https://doi.org/10.1182/bloodadvances.2018024828

Linkins, L. A., & Takach Lapner, S. (2017). Review of D-dimer testing: Good, Bad, and Ugly. *Int J Lab Hematol*, *39 Suppl 1*, 98-103. https://doi.org/10.1111/ijlh.12665

Mäkelburg, A. B., Veeger, N. J., Middeldorp, S., Hamulyák, K., Prins, M. H., Büller, H. R., & Lijfering, W. M. (2010). Different risk of deep vein thrombosis and pulmonary embolism in carriers with factor V Leiden compared with non-carriers, but not in other thrombophilic defects. Results from a large retrospective family cohort study. *Haematologica*, *95*(6), 1030-1033. https://doi.org/10.3324/haematol.2009.017061

Mazzolai, L., Ageno, W., Alatri, A., Bauersachs, R., Becattini, C., Brodmann, M., Emmerich, J., Konstantinides, S., Meyer, G., Middeldorp, S., Monreal, M., Righini, M., & Aboyans, V. (2022). Second consensus document on diagnosis and management of acute deep vein thrombosis: updated document elaborated by the ESC Working Group on aorta and peripheral vascular diseases and the ESC Working Group on pulmonary circulation and right ventricular function. *European Journal of Preventive Cardiology*. https://doi.org/10.1093/eurjpc/zwab088

Middeldorp, S., Nieuwlaat, R., Baumann Kreuziger, L., Coppens, M., Houghton, D., James, A. H., Lang, E., Moll, S., Myers, T., Bhatt, M., Chai-Adisaksopha, C., Colunga-Lozano, L. E., Karam, S. G., Zhang, Y., Wiercioch, W., Schünemann, H. J., & Iorio, A. (2023).

American Society of Hematology 2023 guidelines for management of venous thromboembolism: thrombophilia testing. *Blood Adv*, *7*(22), 7101-7138. https://doi.org/10.1182/bloodadvances.2023010177

Middeldrop, S. (2023, May 30 2023). *Lab Interpretation: Positive factor V Leiden or abnormal activated protein C resistance in adults*. https://www.uptodate.com/contents/factor-v-leiden-and-activated-protein-c-resistance

Murphy, C. H., & Sabath, D. E. (2019). Comparison of Phenotypic Activated Protein C Resistance Testing With a Genetic Assay for Factor V Leiden. *Am J Clin Pathol*, *151*(3), 302-305. https://doi.org/10.1093/ajcp/aqy142

NATF. (2019). *Genetic Risk Factors for Blood Clots and the Role of Genetic Testing*. https://natfonline.org/2019/01/genetic-risk-factors-blood-clots-role-genetic-testing/

NICE. (2023). Venous thromboembolic diseases: diagnosis, management and thrombophilia testing. https://www.nice.org.uk/guidance/ng158/chapter/Recommendations#thrombophilia-testing

Onda, S., Furukawa, K., Haruki, K., Hamura, R., Shirai, Y., Yasuda, J., Shiozaki, H., Gocho, T., Shiba, H., & Ikegami, T. (2021). d-dimer-based screening for early diagnosis of venous thromboembolism after hepatectomy. *Langenbeck's Archives of Surgery*, *406*(3), 883-892. https://doi.org/10.1007/s00423-020-02058-9

Ordieres-Ortega, L., Demelo-Rodríguez, P., Galeano-Valle, F., Kremers, B. M. M., ten Cate-Hoek, A. J., & ten Cate, H. (2020). Predictive value of D-dimer testing for the diagnosis of venous thrombosis in unusual locations: A systematic review. *Thrombosis Research*, *189*, 5-12. https://doi.org/10.1016/j.thromres.2020.02.009

Ortel, T. L., Neumann, I., Ageno, W., Beyth, R., Clark, N. P., Cuker, A., Hutten, B. A., Jaff, M. R., Manja, V., Schulman, S., Thurston, C., Vedantham, S., Verhamme, P., Witt, D. M., D. Florez, I., Izcovich, A., Nieuwlaat, R., Ross, S., J. Schünemann, H., . . . Zhang, Y. (2020). American Society of Hematology 2020 guidelines for management of venous thromboembolism: treatment of deep vein thrombosis and pulmonary embolism. *Blood Adv*, *4*(19), 4693-4738. https://doi.org/10.1182/bloodadvances.2020001830

Previtali, E., Bucciarelli, P., Passamonti, S. M., & Martinelli, I. (2011). Risk factors for venous and arterial thrombosis. *Blood Transfus*, *9*(2), 120-138. https://doi.org/10.2450/2010.0066-10

Raffini, L., Mahoney, D. H., & Armsby, C. (2023). Thrombophilia testing in children and adolescents. *UpToDate*. https://www.uptodate.com/contents/thrombophilia-testing-in-children-and-adolescents

Segal, J. B., Brotman, D. J., Emadi, A., Necochea, A. J., Samal, L., Wilson, L. M., Crim, M. T., & Bass, E. B. (2009). Outcomes of genetic testing in adults with a history of venous thromboembolism. *Evid Rep Technol Assess (Full Rep)*(180), 1-162. https://pubmed.ncbi.nlm.nih.gov/20629476/

SIGN. (2014). *Prevention and management of venous thromboembolism* https://www.sign.ac.uk/media/1060/sign122.pdf

Stevens, S. M., Woller, S. C., Bauer, K. A., Kasthuri, R., Cushman, M., Streiff, M., Lim, W., & Douketis, J. D. (2016). Guidance for the evaluation and treatment of hereditary and acquired thrombophilia. *J Thromb Thrombolysis*, *41*, 154-164. https://doi.org/10.1007/s11239-015-1316-1

SVM. (2013, 02/21/2013). *Don't do work up for clotting disorder (order hypercoagulable testing) for patients who develop first episode of deep vein thrombosis (DVT) in the setting of a known cause*. ABIM. https://www.aafp.org/pubs/afp/collections/choosing-wisely/32.html

Thompson, B. T., Kabrhel, Christopher. (2023, 12/2023). *Overview of acute pulmonary embolism in adults*. https://www.uptodate.com/contents/overview-of-acute-pulmonary-embolism-in-adults

Thrombosis Canada. (2023). Pulmonary Embolism (PE): Diagnosis. https://thrombosiscanada.ca/hcp/practice/clinical_guides?language=en-ca&guideID=PULMONARYEMBOLISMDIAGNOSISANDM

WHO. (2015). Medical eligibility criteria for contraceptive use. https://www.who.int/publications/i/item/9789241549158

Zhang, S., Taylor, A. K., Huang, X., Luo, B., Spector, E. B., Fang, P., & Richards, C. S. (2018). Venous thromboembolism laboratory testing (factor V Leiden and factor II c.*97G>A), 2018 update: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *20*(12), 1489-1498. https://doi.org/10.1038/s41436-018-0322-z

Whole Genome and Whole Exome Sequencing

Policy Number: AHS – M2032 – Whole Genome and Whole Exome Sequencing	Prior Policy Name and Number, as applicable: • AHS – M2032 Whole Genome Whole Exome Sequencing
Initial Policy Effective Date: 01/01/2026	

POLICY DESCRIPTION | RELATED POLICIES | INDICATIONS AND/OR LIMITATIONS OF COVERAGE | TABLE OF TERMINOLOGY | SCIENTIFIC BACKGROUND | GUIDELINES AND RECOMMENDATIONS | APPLICABLE STATE AND FEDERAL REGULATIONS | APPLICABLE CPT/HCPCS PROCEDURE CODES | EVIDENCE-BASED SCIENTIFIC REFERENCES

I. Policy Description

Whole genome sequencing (WGS) is the strategy of using next-generation technology to sequence the entire genome. Whole Exome Sequencing (WES) refers to sequencing of the exome, the component of the genome that predominantly encode proteins. Next generation sequencing (NGS) involves sequencing of multiple small fragments of DNA in parallel, producing fast, accurate sequencing results (Hulick, 2024).

This policy is applicable for undiagnosed rare germline disorders.

II. Related Policies

Policy Number	Policy Title
AHS-M2145	General Genetic Testing, Germline Disorders
AHS-M2146	General Genetic Testing, Somatic Disorders

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in the "Applicable State and Federal Regulations" section of this policy document.

1) For the evaluation of unexplained congenital or neurodevelopmental disorder in individuals less than 18 years of age, whole exome sequencing (WES) and comparator analysis (e.g., parents/siblings) WES **MEET COVERAGE CRITERIA** when **all** of the following criteria are met:

a) When the individual has been evaluated by an ABMGG board-certified medical geneticist or an ABGC board-certified genetic counselor (CGC) and has been counseled about the potential risks of genetic testing;

b) When the WES results will impact patient management and clinical outcome for the individual being tested;

c) When a genomic etiology is the most likely explanation for the phenotype;

d) When no other causative circumstances (e.g., environmental exposures, injury, infection) can explain the symptoms;

e) When the clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing (e.g., comparative genomic hybridization/chromosomal microarray analysis) is available;

f) When the differential diagnosis list and/or phenotype warrant testing of multiple genes and one of the following:

   i) WES is more practical than the separate single gene tests or panels that would be recommended based on the differential diagnosis.

   ii) WES results may preclude the need for multiple and/or invasive procedures, follow-up, or screening that would be recommended in the absence of testing.

2) For a fetus with ultrasound anomalies, WES and comparator analysis (e.g., parents/siblings) WES **MEETS COVERAGE CRITERIA** when **all** of the following criteria are met:

   a) When pre-test counseling has been provided by an ABMGG board-certified medical geneticist or an ABGC board-certified genetic counselor (CGC);

   b) When standard chromosomal microarray testing (CMA) and karyotype analysis have failed to yield a definitive diagnosis;

   c) When a genomic etiology is the most likely explanation for the phenotype;

   d) When no other causative circumstances (e.g., environmental exposures, injury, infection) can explain the symptoms;

   e) When clinical presentation does not fit a well-described syndrome for which single-gene or targeted panel testing is available. If a specific diagnosis is suspected, molecular testing for the suggested disorder (with single-gene test or gene panel) should be the initial test.

3) Reanalysis of exome sequencing (ES) data with WES and comparator analysis (e.g., parents/siblings) WES **MEETS COVERAGE CRITERIA** for **any** of the following situations:

   a) For individuals less than 18 years of age with initial negative ES results as an aid in clinical diagnosis when additional phenotypic findings are noted during a child's growth and development.

   b) For diagnostic results and results deemed to be possibly (but not definitively) associated with the fetal phenotype (new gene-disease associations might have been unknown at the time of initial diagnosis).

   c) For fetal ES with nondiagnostic or negative results, if a new phenotype develops in the proband after birth, a future pregnancy is planned, or a significant amount of time has passed (at least 12 months) since the initial testing was performed.

   d) If the original prenatal ES report does not account for the complete phenotype or if new/additional phenotypes develop over time.

4) When WES is unable to identify a causative variant and the clinical suspicion of a genomic etiology remains in situations where any of the above criteria are met in their entirety, whole genome sequencing (WGS) **MEETS COVERAGE CRITERIA.**

The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of an individual's illness.

5) If WES has been previously performed, further genetic tests involving only exome analyses **DO NOT MEET COVERAGE CRITERIA**.

6) Focused exome sequencing and targeted WGS **DO NOT MEET COVERAGE CRITERIA.**

7) For all other situations not described above, WES **DOES NOT MEET COVERAGE CRITERIA**.

8) For all other situations not described above, WGS **DOES NOT MEET COVERAGE CRITERIA**.

IV. Table of Terminology

Term	Definition
AAN	American Academy of Neurology
AANEM	American Association of Neuromuscular and Electrodiagnostic Medicine

AAP	American Academy of Pediatrics
ABGC	American Board of Genetic Counseling
ABMGG	American Board of Medical Genetics and Genomics
ACMG	American College of Medical Genetics and Genomics
ACOG	American College of Obstetricians and Gynecologists
ACTA2	*Actin alpha 2 smooth muscle*
ACTC1	*Actin alpha cardiac muscle 1*
AFF2	*AF4/FMR2 family member 2*
AMP	Association for Molecular Pathology
APC	*APC regulator of WNT signalling pathway*
APOB	*Apolipoprotein B*
AR	*Androgen receptor*
ASD	Autism spectrum disorder
ATN1	*Atrophin 1*
ATP7B	*ATPase copper transporting beta*
ATXN1	*Ataxin 1*
ATXN10	*Ataxin 10*
ATXN2	*Ataxin 2*
ATXN3	*Ataxin 3*
ATXN7	*Ataxin 7*
ATXN8OS	*Ataxin 8 opposite strand lncRNA*
AXL	*AXL receptor tyrosine kinase*
BMPR1A	*Bone morphogenetic protein receptor type 1A*
BRCA1	*BRCA1 DNA repair associated*
BRCA2	*BRCA2 DNA repair associated*
BTD	*Biotinidase*
C9orf72	C9orf72-SMCR8 complex subunit
CA	Congenital anomalies
CACNA1A	*Calcium voltage-gated channel subunit alpha1 A*
CACNA1S	*Calcium voltage-gated channel subunit alpha1 S*
CCDC141	*Coiled-coil domain containing 141*
CCDC88C	*Coiled-coil domain containing 88C*
CDON	*Cell adhesion associated; oncogene regulated*
CES	Clinical exome sequencing
CFES	Clinically focused exome sequencing
CGC	ABGC board-certified genetic counselor
CHD7	*Chromodomain helicase DNA binding protein 7*
CLIA '88	Clinical Laboratory Improvement Amendments of 1988
CMA	Chromosomal microarray analysis

CMS	Centers for Medicare and Medicaid Services
CNBP	*CCHC-type zinc finger nucleic acid binding protein*
COL3A1	*Collagen type III alpha 1 chain*
CSTB	*Cystatin B*
DCAF17	*DDB1 and CUL4 associated factor 17*
DCC	*DCC netrin 1 receptor*
DD	Developmental delay
DIP2B	*Disco interacting protein 2 homolog B*
DMPK	*DM1 protein kinase*
DMXL2	*Dmx like 2*
DNA	*Deoxyribonucleic acid*
DSC2	*Desmocollin 2*
DSG2	*Desmoglein 2*
DSP	*Desmoplakin*
EGF	Epidermal growth factor
EP	Expected pathogenic
ES	Exome sequencing
FBN1	*Fibrillin 1*
FGFR1	*Fibroblast growth factor receptor 1*
FMR1	*Fragile X messenger ribonucleoprotein 1*
FXN	*Frataxin*
GADL1	*Glutamate decarboxylase like 1*
GDD	Global developmental delay
Gdna	Genomic DNA
GLA	Galactosidase alpha
GnRH	Gonadotropin-releasing hormone
GS	Genome sequencing
HTT	*Huntingtin*
ID	Intellectual disability
IGSF10	*Immunoglobulin superfamily member 10*
IHH	Idiopathic hypogonadotropic hypogonadism
ISPD	International Society for Prenatal Diagnosis
JAMA	Journal of the American Medical Association
JPH3	*Junctophilin 3*
KCNH2	*Potassium voltage-gated channel subfamily H member 2*
KCNQ1	*Potassium voltage-gated channel subfamily Q member 1*
KP	Known pathogenic
LDLR	*Low density lipoprotein receptor*
LDT	Laboratory developed test

LMNA	*Lamin A/C*
MCC	*MCC regulator of WNT signalling pathway*
MEN1	*Menin 1*
MLH1	*MutL homolog 1*
MSH2	*MutS homolog 2*
MSH6	*MutS homolog 6*
MUTYH	*MutY DNA glycosylase*
MYBPC3	*Myosin binding protein C3*
MYH11	*Myosin heavy chain 11*
MYH7	*Myosin heavy chain 7*
MYL2	*Myosin light chain 2*
MYL3	*Myosin light chain 3*
NOA	Nonobstructive azoospermia
NDD	Neurodevelopmental disorders
NF2	*NF2, moesin-ezrin-radixin like (MERLIN) tumor suppressor*
NGS	Next-generation sequencing
NOP56	*NOP56 ribonucleoprotein*
NOTCH1	*Notch receptor 1*
NOTCH2NLC	*Notch 2 N-terminal like C*
OB-GYN	Obstetrician-gynecologist
OTC	Over the counter
PABPN1	*Poly(a) binding protein nuclear 1*
PCSK9	*Proprotein convertase subtilisin/kexin type 9*
PDE3A	*Phosphodiesterase 3A*
pES	Prenatal exome sequencing
PFS	Progression-free survival
PHOX2B	*Paired like homeobox 2B*
PICU	Pediatric intensive care unit
PKP2	*Plakophilin 2*
PMS2	*PMS1 homolog 2, mismatch repair system component*
PNPLA6	*Patatin like phospholipase domain containing 6*
POLR3A	*RNA polymerase III subunit A*
PPP2R2B	*Protein phosphatase 2 regulatory subunit Beta*
PQF	Perinatal Quality Foundation
PRKAG2	*Protein kinase AMP-activated non-catalytic subunit gamma 2*
PROKR2	*Prokineticin receptor 2*
PTEN	*Phosphatase and tensin homolog*
RB1	*RB transcriptional corepressor 1*
RELN	*Reelin*

RET	*Ret proto-oncogene*
rWGS	Rapid whole genome sequencing
RYR1	*Ryanodine receptor 1*
RYR2	*Ryanodine receptor 2*
SCN5A	*Sodium voltage-gated channel alpha subunit 5*
SDHAF2	*Succinate dehydrogenase complex assembly factor 2*
SDHB	*Succinate dehydrogenase complex iron sulfur subunit B*
SDHC	*Succinate dehydrogenase complex subunit C*
SDHD	*Succinate dehydrogenase complex subunit D*
SFM/SMFM	Society for Maternal and Fetal Medicine
SLIT2	*Slit guidance ligand 2*
SMAD3	*SMAD family member 3*
SMAD4	*SMAD family member 4*
SPRED3	*Sprouty related EVH1 domain containing 3*
STK11	*Serine/threonine kinase 11*
TCF4	*Transcription factor 4*
TGFBR1	*Transforming growth factor beta receptor 1*
TGFBR2	*Transforming growth factor beta receptor 2*
TMEM43	*Transmembrane protein 43*
TNNI3	*Troponin I3, cardiac type*
TNNT2	*Troponin T2, cardiac type*
TP53	*Tumor protein p53 gene*
TPM1	Tropomyosin 1
TRAPPC9	*Trafficking protein particle complex subunit 9*
TSC1	*TSC complex subunit 1*
TSC2	*TSC complex subunit 2*
VHL	*von Hippel-Lindau tumor suppressor gene*
VUS	Variants of unknown significance
WES	Whole exome sequencing
WGS	Whole genome sequencing
WT1	*WT1 transcription factor*

V. Scientific Background

DNA sequencing is a critical tool for the evaluation of many medical conditions. The two primary methods of DNA sequencing in the clinical setting are Sanger sequencing and next-generation sequencing or NGS. NGS is a technique that allows for the rapid sequencing of multiple strands of DNA. It is not limited to one specific type of test; rather it encompasses numerous technologies that produce swift and high-volume sequencing. NGS can be used to sequence larger sequences, such as the exome or the

entire genome. This is opposed to the traditional Sanger sequencing, which is more useful for sequencing a specific gene (Hulick, 2024).

The NGS procedure typically includes the following steps: first the patient's DNA is prepared to serve as a template, then DNA fragments are isolated (on solid surfaces such as small beads) where sequence data is generated. Then these results are compared against a reference genome. Any DNA sample may be used if the quality and quantity of that sample is sufficient, but the methods of library generation and data analysis often vary from panel to panel. Evaluating the results of a gene panel typically requires expertise in bioinformatics. Since NGS reports data on any variants found, great care must be taken to evaluate these gene variants, especially variants of unknown significance (VUS) and secondary findings (Hulick, 2024; Rehm et al., 2013).

Exome and genome sequencing are usually performed with NGS. The exome represents all the protein-encoding genes, and at least 85% of pathogenic mutations are found in the exome. Further, the exome only comprises approximately 1.5%-2% of the genome, thereby making it more cost effective to sequence than whole genome sequencing. The entire exome includes approximately 30 megabases compared to the genome's 3.3 gigabases. However, sequencing an entire genome may be useful as a pathogenic mutation may be in a noncoding region of the genome, such as gene regulation dysfunction. Most clinical NGS testing uses targeted panels or whole exome sequencing (WES), and whole genome sequencing (WGS) is only used in select cases. For instance, conditions such as nonsyndromic hearing loss (possible pathogenic variants in over 60 genes) may benefit from WES evaluation (Hulick, 2024).

Several companies have pivoted towards focused exome sequencing. These are panels tailored to individual phenotypes and target a maximum number of genes depending on the company. There is a >30% diagnostic yield, with freedom for clinicians to choose specific genes they are interested in, with reduced cost and options to reflex to WES for negative cases (GGC, 2022). In their study, Jia et al. (2023) retrospectively analyzed 372 pediatric patients who were referred to clinically focused exome sequencing (CFES), and concluded that CFES may be first-line for "diagnosing young children with suspected genetic conditions, as it validates the identification of molecular genetics alterations and facilitates comprehensive medical management. The patients that were more likely to receive diagnoses via CFES were those with "metabolism/homeostasis abnormalities, craniofacial /otolaryngology/ ophthalmologic abnormalities, and/or [abnormalities of] the integument" (Jia et al., 2023). Despite the novelty and expected benefits of focused exome sequencing, more clinical studies with larger sample sizes are necessary.

Proprietary Testing

Many proprietary technologies for WES and WGS are available. Companies such as Variantyx provide highly specialized genetic testing to patients and clinicians. The Genomic Unity® Exome Plus Analysis test sequences the exome, including intronic and regulatory variants, identifies disease causing deletions or duplications in the genome, and analyzes the mitochondrial genome with heteroplasmy (≥5%). This test may identify many genes for a variety of disorders including *AR, ATN1, ATXN1, ATXN2, ATXN3, ATXN7, ATXN8OS, ATXN10, C9ORF72, CACNA1A, CNBP, CSTB, DMPK, FMR1, FXN, HTT, JPH3, NOP56, NOTCH2NLC, PABPN1, PPP2R2B, TBP* (for adult-onset movement disorders), *AFF2, DIP2B, FMR1* (for early-onset intellectual disability disorders), and *PHOX2B, TCF4* (for other disorders) (variantyx, 2020). This test requires either a blood, saliva or gDNA (genomic DNA) sample and has an eight-week turnaround time.

Clinical Utility and Validity

Alfares et al. (2018) compared the cost-effectiveness and clinical utility of both WES and WGS. Data was analyzed from 108 participants; all 108 individuals had negative array comparative genomic hybridization (also known as chromosomal microarray) results and negative or inconclusive WES results before WGS was performed. Chromosomal microarray (CMA) is another common genetic testing method that can analyze many pieces of DNA simultaneously. The authors only pinpointed three positive cases where WGS identified a genetic or inherited disorder that WES did not recognize; further, it was noted that "30% of the positive cases identified by WGS could be identified by reanalyzing the WES raw data, and WGS achieved an only 7% higher detection rate. Therefore, until the cost of WGS approximates that of WES, reanalyzing WES raw data is recommended before performing WGS" (Alfares et al., 2018).

Yang et al. (2014) conducted a single-center observational study of 2000 patients with clinical whole exome sequencing performed for a suspected genetic disorder. A molecular diagnosis was reported for 504 patients (25.2%) with 58% of the diagnostic mutations not previously reported. The investigators concluded that "the yield of whole-exome sequencing may offer advantages over traditional molecular diagnostic approaches in certain patients" (Yang et al., 2014). Best et al. (2018) reviewed 31 different WES studies and noted that the diagnostic rates varied between 6.2% and 80%; however, the researchers state that the "differences in inclusion criteria and trio versus singleton approaches to sequencing largely account for the wide range of diagnostic rates."

Tammimies et al. (2015) evaluated the molecular diagnostic yield of CMA and WES in children with autism spectrum disorder (ASD). The patient cohort included 258 consecutively enrolled unrelated children with ASD, stratified into three groups based

on the presence of major congenital abnormalities and minor physical anomalies. All probands underwent CMA, with WES performed for 95 proband-parent trios. The molecular diagnostic yields of CMA and WES were comparable. Among the 95 patients undergoing WES, eight children (8.4%) received an ASD-related molecular diagnosis. Among the children who underwent both CMA and WES testing, the estimated proportion with an identifiable genetic etiology was 15.8%. The investigators concluded that "if replicated in additional populations, these findings may inform appropriate selection of molecular diagnostic testing for children affected by ASD" (Tammimies et al., 2015). A similar study was performed by Arteche-López et al. (2021) to validate WES as a "first-tier test for the genetic diagnosis of [ASD], when there is no suspicion of fragile X syndrome." Upon comparing the clinical utility of CMA, *FMR1* testing, and WES testing, the researchers "achieved a global diagnostic rate of 12.8% (44/343), the majority of them being characterised by WES (33/44; 75%) compared to CMA (9/44; 20.4%) or *FMR1* testing (2/44; 4.5%)," evidently demonstrating the "higher diagnostic power" of WES compared to CMA (Arteche-López et al., 2021).

Taylor et al. (2015) performed whole genome sequencing in 217 individuals across a broad spectrum of genetic disorders in whom previous screening had identified no pathogenic variants. Disease-causing variants were identified in 21% of cases, with the proportion increasing to 34% (23/68) for mendelian disorders and 57% (8/14) in family trios. The investigators concluded that the results "demonstrate the value of genome sequencing for routine clinical diagnosis but also highlight many outstanding challenges" (Taylor et al., 2015).

Miller et al. (2017) performed exome/whole genome sequencing to identify the genetic cause in patients with craniosynostosis, in whom prior clinically driven genetic testing had been negative. Out of the 40 patients' tests, associated mutations were identified in 15 patients (37.5%) involving 14 different genes. In five of the 15 positive cases, the molecular diagnosis had immediate, actionable consequences in patient management. The investigators concluded that "the benefits of exome/whole genome sequencing to identify causal mutations in craniosynostosis cases for which routine clinical testing has yielded negative results" (Miller et al., 2017).

Crowley et al. (2020) used WES in a single-center cohort study of 1005 pediatric IBD patients and found a 3% prevalence of damaging variants in genes linked to monogenic IBD, and that 1% of monogenic pediatric IBD patients have variants in genes associated with primary immunodeficiency that are potentially curable through allogeneic hematopoietic stem cell transplantation. As rare genetic variants could manifest in different phenotypes, the researchers believe that the "data supports the diagnosis of monogenic disease beyond the very early onset IBD population especially in children with a family history of autoimmune diseases and those with evidence of

extra-intestinal manifestations of IBD" and that WES will lend itself to provide definitive and personalized treatments in the future (Crowley et al., 2020).

Srivastava et al. (2014) conducted a retrospective cohort study on 78 children with neurodevelopmental disabilities and a prior unrevealing workup before WES. The overall presumptive diagnostic testing rate was 41% (32/78 patients). Results of WES affected patient management in all cases, most often related to reproductive planning (27/78). The investigators concluded that the high diagnostic yield of WES could lead to earlier diagnosis, impacting medical management, prognostication, and family planning (Srivastava et al., 2014).

https://ng.neurology.org/content/4/1/e212Haskell et al. (2018) studied the diagnostic utility of exome sequencing in the evaluation of neuromuscular disorders. A total of 93 undiagnosed patients with potential neuromuscular disorders participated in this study; the diagnostic yield of these 93 patients with exome sequencing was 12.9% "with one or more pathogenic or likely pathogenic variants identified in a causative gene associated with the patient's disorder" (Haskell et al., 2018). In this study, exome sequencing was able to provide or clarify a neuromuscular disorder diagnosis, but only in a small percentage of the population studied.

Involving WGS or WES as a supplemental level of evaluation has been able to effect change in medical care and treatment pathways. In the NIH-funded Undiagnosed Diseases Network, among 382 patients with complete evaluations, "28 (21%) of the patients who received a diagnosis, the diagnosis led to a recommendation regarding a change in therapy. In 49 (37%), the diagnosis led to a change in care other than therapy, such as the narrowing of diagnostic testing. In 48 (36%), the diagnosis led to variant-specific genetic counseling but did not lead to a change in the diagnostic or therapeutic strategy." The changes in therapy ranged from known drugs, vitamins, coenzyme supplementations, and transplant in one patient. This demonstrated evidence supporting usage of DNA sequencing for genetically determined conditions and a representative lens of how it can affect medical care (Splinter et al., 2018).

Muthaffar (2021) conducted a retrospective chart review for WES results between January 2018 to November 2019 for patients at a pediatric neurology clinic in Saudi Arabia to identify the utility of WES. It was found that "twenty-six children with undiagnosed neurological conditions were identified and underwent WES diagnosis. Nineteen patients (73.0%) of the cohort were diagnosed with pathogenic variants, likely pathogenic variants or variants of unknown significance (VUS)." The researcher also furthered the conclusions of the WES high diagnostic rate by proving direct implications on clinical management based on testing results. One patient who had a positive pathogenic *BTD* mutation, diagnosed at seven-years old with a biotinidase

deficiency, was started on biotin supplements after WES testing, and was able to breathe independently off a ventilator, regain motor capabilities with physical therapy, improve hearing, and eliminate convulsions (Muthaffar, 2021).

Using WES on eleven probands from ten Jordanian families who have been formerly diagnosed with limb-girdle dystrophy (LGMD) and Charcot-Marie-Tooth disease (CMT), Ababneh et al. (2021) identified a series of missense, nonsense, and deletion variants associated with neuromuscular disorders. Consequently, the researchers argue that "Utilization of WES is helpful to facilitate rapid and accurate NMDs diagnosis, complementing a thorough clinical evaluation", especially in a country where the risk of autosomal-recessive disorders is increased by consanguinity and the implementation of genetic diagnosis is limited and the results misunderstood (Ababneh et al., 2021).

Sanford et al. (2019) investigated the clinical utility of rWGS in children within the pediatric intensive care unit (PICU). They were able to make a molecular diagnosis by rWGS in 17 of 38 children, and in four of the 17 children diagnosed by rWGS (24%), "the genetic diagnoses led to a change in management while in the PICU, including genome-informed changes in pharmacotherapy and transition to palliative care… Eighty-two percent of diagnoses affected the clinical management of the patient and/or family after PICU discharge, including avoidance of biopsy, administration of factor replacement, and surveillance for disorder-related sequelae" (Sanford et al., 2019). In this retrospective analysis, benefits of rWGS were further elucidated in the setting of unknown or unclear clinical etiologies.

Reda et al. (2020) studied WES for metastatic solid cancer diagnoses in 506 patients. In this study, the somatic and germline exome analysis was restricted to 317 specific genes. Exome sequencing was successful in 386 tumor samples, and 342 patients received a therapeutic proposal based on their genetic results. However, only 79 patients were treated with an NGS matched therapy. While this study shows that WES is a possible tool to assist with metastatic solid cancer diagnoses and treatments, "no differences were observed between PFS [progression-free survival] ratios of patients treated with matched therapy versus standard therapy" (Reda et al., 2020).

Other studies have also yielded bifurcating results on the periodic revisiting of unsolved exome cases and for variants of unknown significance. Salfati et al. (2019) found that re-analysis of 101 WES cases one to seven years after initial analysis resulted in "the identification of additional diagnostic variants in 3 rare disease cases (5.9%) and 1 sudden unexplained death case (2%), which increased our molecular diagnostic yield to 31.4% and 12%, respectively." However, though they recognize the importance of any diagnostic yield to those families potentially affected, the authors

also acknowledge that "most of our cases remain unexplained after our re-analysis", which they attribute to an enduring lack of coverage of functional exonic variants, along with "the possibility of complicated oligogenic disease that is not easily dissected in small families, and the possibility of disease due to epigenetic, somatic, or other uninterrogated genomic aberrations." As such, "We [the authors] suggest that a 6-month cycle of automated re-analysis could improve the pace at which new findings are disseminated to patients. Periodic re-analysis by third party or other software not originally used to analyze cases is also potentially useful to uncover pathogenic variants that may be missed by the differences across genome interpretation platforms" (Salfati et al., 2019).

Parent-child Trio Testing

Parent-child trio testing is a strategy which helps to identify single pathogenic mutations among the many genomic variants in an individual. Specifically, the sequencing of both the parents and the patient allows for the variant to be identified easier and "filtered based on consistency or inconsistency according to the laws of Mendelian inheritance" (Sakai et al., 2013).

Lee et al. (2014) reported on the initial clinical indications for clinical exome sequencing (CES) referrals and molecular diagnostic rates for different indications and different test types. CES was performed on 814 patients with undiagnosed, suspected genetic conditions who underwent WES. CES was conducted using a trio-CES technique which involves both parents and their affected child sequenced simultaneously. Overall, a molecular diagnosis with a causative variant in a well-established clinical gene was provided for 213/814 (26%) cases. The trio-CES was associated with a higher molecular diagnostic yield (31%; 127/410 cases) than proband-CES or traditional molecular diagnostic methods. The investigators concluded that "additional studies designed to validate these findings and to explore the effect of this approach on clinical and economic outcomes are warranted" (Lee et al., 2014).

Soden et al. (2014) performed diagnostic WGS and/or WES in parent-child trios for 100 families with 119 children with neurodevelopmental disorders (NDD). A total of 45% of the families received molecular diagnoses of an established genetic disorder (53/119 affected children). An accelerated sequencing modality, rapid WGS, yielded diagnoses in 73% of families with acutely ill children (11/15). In this study, WES proved to be less costly than continued conventional diagnostic testing of children with NDD in whom initial testing failed to yield a diagnosis. The investigators concluded that "initial diagnostic evaluation of children with NDD should include trio WGS or WES, with extension of accelerated sequencing modalities to high-acuity patients" (Soden et al., 2014).

Another study compared fetal WES versus trio analysis WES on fetuses with sonographic abnormalities. The researchers found that trio analysis yielded a positive/definitive diagnosis in 30% (3/10) of the cases as compared to only 14.3% (2/14) of the singleton cases. They conclude, "In order to expedite interpretation of results, trio sequencing should be employed, but interpretation can still be compromised by incomplete coverage of relevant genes" (Drury et al., 2015). Similarly, these data are supported by another study of trio analysis of thirty different cases. A total of 10% of the cases were positive for a pathogenic finding, and 17% were de novo, inherited recessive, or X-linked variants. The authors conclude, "This study outlines the way for a substantial improvement in the diagnostic yield of prenatal genetic abnormalities through the application of next-generation sequencing" (Carss et al., 2014).

Yates et al. (2017) performed WES, including trio analysis, using samples obtained from deceased fetuses with ultrasound anomalies. They note that 20% of cases were positive overall with a definitive diagnosis with another 45% positive for possible pathogenic candidate variants. Comparing trio analysis to singleton analysis, 24% (n=11) of trio analysis resulted in a definitive diagnostic finding versus 14% (n=3) for singleton testing (Yates et al., 2017).

https://www.nature.com/articles/s41525-018-0053-8Clark et al. (2018) compared the diagnostic and clinical utility of WGS, WES and CMA in children with suspected genetic disorders. Trio analyses were also analyzed. Many studies were reviewed in this meta-analysis; the authors state that "In 37 studies, comprising 20,068 children, diagnostic utility of WGS (0.41, 95% CI 0.34-0.48, I2 = 44%) and WES (0.36, 95% CI 0.33-0.40, I2 = 83%) were qualitatively greater than CMA (0.10, 95% CI 0.08-0.12, I2 = 81%) (Clark et al., 2018). Further, a statistical difference was not found regarding the diagnostic utility of WES and WGS. Finally, "Subgroups with higher WGS/WES diagnostic utility were trios and those receiving hospital-based interpretation. WGS/WES should be considered a first-line genomic test for children with suspected genetic diseases" (Clark et al., 2018).

Zhang et al. (2021) performed WES and trio analysis on 18 unrelated men who have idiopathic hypogonadotropic hypogonadism (IHH), which is a rare genetic disorder that causes delayed or absent puberty as well as infertility due to gonadotropin-releasing hormone (GnRH) insufficiency/deficiency, and their parents. With this testing, "one reported and 10 novel variants in eight known IHH causative genes (*AXL, CCDC141, CHD7, DMXL2, FGFR1, PNPLA6, POLR3A,* and *PROKR2*), nine variants in nine recently reported candidate genes (*DCAF17, DCC, EGF, IGSF10, NOTCH1, PDE3A, RELN, SLIT2,* and *TRAPPC9*), and four variants in four novel candidate genes for IHH (*CCDC88C, CDON, GADL1,* and *SPRED3*) were identified in 77.8% (14/18) of IHH cases."

This analysis also supported oligogenic etiology for disease presentation, with 44.4% cases carrying at least two variants in IHH-related genes. They also found that the variants "tended to be maternally inherited (maternal with n = 17 vs paternal with n = 7; P = 0.028)," which was confirmed by their previous literature review, and due to the presence of female carriers, extends the notion that females may be more tolerant of "deleterious" IHH-related gene mutations. This study exemplifies the clinical utility of WES and trio analysis for reproductive genetic disorders and could be used to continue pedigree analyses for IHH (Zhang et al., 2021).

Malcher et al. (2022) investigated the use of whole-genome sequencing in identifying new candidate genes for nonobstructive azoospermia (NOA). The authors applied WGS for 39 patients with NOA to identify novel NOA-associated SNVs, yielding "8 potentially disease causing *[sic]* variants in 4 genes, followed by 30 variants in 20 genes that were previously linked to infertility, and 20 variants in 13 genes that have never been investigated with respect to male infertility but could be important in patients with NOA" in 29 of the 39 azoospermic individuals. Of these 58 variants, 16 were newly discovered and, as such, "highly recommended to examine their possible function and mechanism of participation in gametogenesis" (Malcher et al., 2022).

In their examination of whole exome and genome sequencing in a Mendelian disorder cohort, Ewans et al. (2022) determined that "WGS resulted in a diagnosis in one third (34%; 13/38 families) of undiagnosed families who had previously had WES." However, when adjusting for "factors such as improvements to gene-disease knowledge and genomic pipelines through contemporary WES reanalysis, the WGS diagnostic yield reduced to 19% (6/31 remaining families)", primarily due to "due to reduced WES coverage of critical regions that may be solved through an improved WES platform." Such results contribute to the debate about the "trade-off between the lower cost of WES and the higher diagnostic yield of WGS" and will ultimately be a function of "the clinical scenario and local resourcing and availability" (Ewans et al., 2022).

VI. Guidelines and Recommendations

American College of Medical Genetics (ACMG)

In 2012, the ACMG released a policy statement outlining points to consider in the clinical application of genomic sequencing to the detection of germ-line mutations. The ACMG recommended that WGS/WES should be considered in the clinical diagnostic assessment of a phenotypically affected individual when:

- "The phenotype or family history data strongly implicate a genetic etiology, but the phenotype does not correspond with a specific disorder for which a genetic test targeting a specific gene is available on a clinical basis."

- "A patient presents with a defined genetic disorder that demonstrates a high degree of genetic heterogeneity, making WES or WGS analysis of multiple genes simultaneously a more practical approach."
- "A patient presents with a likely genetic disorder, but specific genetic tests available for that phenotype have failed to arrive at a diagnosis."
- "A fetus with a likely genetic disorder in which specific genetic tests, including targeted sequencing tests, available for that phenotype have failed to arrive at a diagnosis."

ACMG stated that "WGS/WES may be considered in preconception carrier screening, using a strategy to focus on genetic variants known to be associated with significant phenotypes in homozygous or hemizygous progeny." ACMG further stated that WGS and WES should not be used at this time as an approach to prenatal screening or as a first-tier approach for newborn screening (ACMG, 2012).

ACMG released a guideline on informed consent for genome/exome sequencing. In that guideline, they noted that WGS/WES was not recommended "before the legal age of majority" unless for "phenotype-driven clinical diagnostic uses or circumstances in which early monitoring or interventions are available and effective" (ACMG, 2013).

In 2014 the ACMG published guidelines (Alford et al., 2014) for the clinical evaluation and etiologic diagnosis of hearing loss which state: "Pretest genetic counseling should be provided, and, with patient's informed consent, genetic testing, if available, should be ordered to confirm the diagnosis—this testing may include single-gene tests, hearing loss sequencing panels, whole-exome sequencing(WES), whole-genome sequencing (WGS), chromosome analysis, or microarray-based copy-number analysis, depending on clinical findings."

In 2020 the ACMG published guidelines on the use of fetal exome sequencing (ES) in prenatal diagnoses. These guidelines are below (Monaghan et al., 2020):

Pretest Considerations

- "Exome sequencing may be considered for a fetus with ultrasound anomalies when standard CMA and karyotype analysis have failed to yield a definitive diagnosis. If a specific diagnosis is suspected, molecular testing for the suggested disorder (with single-gene test or gene panel) should be the initial test. At the present time, there are no data supporting the clinical use for ES for other reproductive indications, such as the identification of sonographic markers suggestive of aneuploidy or a history of recurrent unexplained pregnancy loss.
- Trio analysis consisting of the proband and both biological parents is preferred to singleton (fetus only) or duo (fetus and one parent) analyses. Trio analysis

consistently shows higher diagnostic yields compared with nontrio analysis. It allows for the immediate identification of de novo variants, determination of phase for biallelic variants, and confirmation of carrier status in both parents when a homozygous variant is detected. For laboratories not requiring trio analysis for prenatal ES, all efforts should be made to determine inheritance of identified fetal variants with targeted testing of the biological parents. There may be circumstances where both biological parents are unable to submit specimens. In this scenario, variant segregation testing using the available parent or testing of other closely related family members should be considered.

- Pretest counseling is ideally provided by a genetics professional during which the types of variants that may be returned in a laboratory report for all tested family members would be reviewed.

Posttest Considerations

- Post-test counseling is recommended, regardless of the test result. It should be provided by individuals with relevant expertise, preferably a genetics professional.

Reanalysis Considerations

- For patients with initial negative ES results, reanalysis of exome sequencing data aids clinical diagnosis after 12 months. This outcome has been validated in the pediatric population as additional phenotypic findings might be noted during a child's growth and development. Continuous updates in database resources and new publications may provide further information for variant and gene classification.
- Due to the discovery of new gene–disease associations (that were unknown at the time of initial analysis), reanalysis can also be considered for diagnostic results and results deemed to be possibly (but not definitively) associated with the fetal phenotype.
- For fetal ES with nondiagnostic or negative results, reanalysis may be considered if a new phenotype develops in the proband after birth, a future pregnancy is planned, or a significant amount of time has passed (either at the discretion of the testing laboratory or at least 12 months) since the initial testing was performed.
- If the original prenatal ES report does not account for the complete phenotype or if new/additional phenotypes develop over time, a reanalysis could be considered" (Monaghan et al., 2020).

In 2020 the ACMG conducted a systematic evidence review to support guideline development for the use of exome and genome sequencing among patients with congenital anomalies, developmental delay, or intellectual disability (CA/DD/ID). From

their review, the ACMG concluded, "There is evidence that ES/GS for patients with CA/DD/ID informs clinical and reproductive decision-making, which could lead to improved outcomes for patients and their family members. Further research is needed to generate evidence regarding health outcomes to inform robust guidelines regarding ES/GS in the care of patients with CA/DD/ID" (Malinowski et al., 2020).

In 2021 the ACMG asserted that as the body of literature surrounding this continues to burgeon, it urges them to "strongly recommend ES and GS as a first-tier or second-tier test (guided by clinical judgment and often clinician–patient/family shared decision making after CMA or focused testing) for patients with one or more CAs prior to one year of age or for patients with DD/ID with onset prior to 18 years of age" (Manickam et al., 2021).

American College of Obstetricians and Gynecologists (ACOG) and Society for Maternal and Fetal Medicine (SFM)

In 2016 the ACOG and SFM published a joint committee opinion on "Microarrays and Next-Generation Sequencing Technology: The Use of Advanced Genetic Diagnostic Tools in Obstetrics and Gynecology", which states that "the routine use of whole-genome or whole-exome sequencing for prenatal diagnosis is not recommended outside of the context of clinical trials until sufficient peer reviewed data and validation studies are published" (ACOG & SFM, 2016).

However, ACOG and SFM note that WES may be considered when "specific genetic tests available for a phenotype, including targeted sequencing tests, have failed to determine a diagnosis in a fetus with multiple congenital anomalies suggestive of a genetic disorder." The guideline further clarifies that "in select circumstances (recurrent or lethal fetal anomalies in which other approaches have been noninformative), [WES] may be considered as a diagnostic tool, but only after other appropriate testing has been noninformative and after extensive counseling by an [OB-GYN[or other health care provider with genetics expertise who is familiar with these new technologies and their limitations" (ACOG & SFM, 2016). This committee opinion was reaffirmed in 2023.

Joint Position Statement from the International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM), and the Perinatal Quality Foundation (PQF)

Per the guideline, the word "sequencing" is used to refer to "whole exome sequencing, targeted analysis using clinical panels, and whole genome sequencing."

"The use of diagnostic sequencing is currently being introduced for evaluation of fetuses for whom standard diagnostic genetic testing, such as chromosomal

microarray analysis (CMA), has already been performed and is uninformative or is offered concurrently according to accepted practice guidelines, or for whom expert genetic opinion determines that standard genetic testing is less optimal than sequencing for the presenting fetal phenotype" (ISPD et al., 2018).

Routine use of prenatal sequencing as a diagnostic test cannot be supported due to "insufficient" validation and data about benefits and pitfalls (ISPD et al., 2018).

Within the section on recommendations for all diagnostic applications of genome-wide sequencing, concerning trio analysis, they state, "Diagnostic sequencing for fetal indications is best done as a trio analysis, where fetal and both parental samples are sequenced and analyzed together. The trio approach currently benefits timeliness of result interpretation and aids assignment of pathogenicity for detected sequence variants. If proband-only sequencing is performed, validation of diagnostic or potentially diagnostic findings best includes a determination of inheritance through targeted testing of samples from biological parents" (ISPD et al., 2018). However, the guideline could not recommend one sequencing method over another, nor was the guideline certain on the best way to interpret variants found in genome-wide sequencing.

The guideline provides three scenarios in which fetal sequencing may be "beneficial":

"A current pregnancy with a fetus with a single major anomaly or with multiple organ system anomalies that are suggestive of a possible genetic etiology, but no genetic diagnosis was found after CMA; or in select situations with no CMA result, following a multidisciplinary review and consensus, in which there is a fetus with a multiple anomaly 'pattern' that strongly suggests a single gene disorder."

"A personal (maternal or paternal) history of a prior undiagnosed fetus (or child) affected with a major single anomaly or multiple anomalies suggestive of a genetic etiology, and a recurrence of similar anomalies in the current pregnancy without a genetic diagnosis after karyotype or CMA. In addition, when such parents present for preconception counseling and no sample is available from the affected proband, or if a fetal sample cannot be obtained in an ongoing pregnancy, it is considered appropriate to offer sequencing for both biological parents to look for shared carrier status for autosomal recessive mutations that might explain the fetal phenotype. However, where possible, obtaining tissue from a previous abnormal fetus or child for exome sequencing is preferable."

"In families with a history of recurrent stillbirths of unknown etiology after karyotype and/or CMA, where the fetus in the current pregnancy has a recurrent pattern of anomalies (ISPD et al., 2018).

International Society for Prenatal Diagnosis (ISPD)

In 2022, the ISPD released an updated position statement on the use of genome-wide sequencing for prenatal diagnosis. Below are the pertinent recommendations:

- "Diagnostic sequencing for fetal indications is best done as a trio analysis, where fetal and both parental samples are sequenced and analyzed together."
- "Approaches to sequence analysis may vary from examination of genes known to be associated with fetal or neonatal phenotypes to a broader genome-wide strategy. It is also uncertain whether interpretation of variants found by genome-wide sequencing should follow the general guidelines for interpretation and reporting of results for children and adults, or whether a more restrictive approach, limited to those variants that explain the phenotype is preferable in the prenatal setting, or if a new approach restricting reporting to severe childhood conditions should be considered."
- "The current existing data support that prenatal sequencing is beneficial for the following indications:
    - A current pregnancy with a fetus having a major single anomaly or multiple organ anomalies:
        - For which no genetic diagnosis was found after CMA and a clinical genetic expert review considers the phenotype suggestive of a possible genetic etiology.
        - For which the multiple anomaly "pattern" strongly suggests a single gene disorder with no prior genetic testing. As pES is not currently validated to detect all CNVs, CMA should be run before or in parallel with pES in this scenario.
    - A personal (maternal or paternal) history of a prior undiagnosed fetus (or child) affected with a major single or multiple anomalies:
        - With a recurrence of similar anomalies in the current pregnancy without a genetic diagnosis after karyotype or CMA for the current or prior undiagnosed pregnancy.
        - When such parents present for preconception counseling and no sample is available from the affected proband, or if a fetal sample cannot be obtained in an ongoing pregnancy, it is considered appropriate to offer sequencing for both biological parents to look for shared carrier status for autosomal recessive mutations that might explain the fetal phenotype. However, where possible, obtaining tissue from a previous abnormal fetus or child for pES is preferable.
- There is currently no evidence that supports routine testing (including upon parental request) on fetal tissue obtained from an invasive prenatal procedure

(amniocentesis, CVS, cordocentesis, other) for indications other than fetal anomalies
    - There may be special settings when prenatal sequencing in the absence of a fetal phenotype visible on prenatal imaging can be considered, such as with a strong family history of a recurrent childhood-onset severe genetic condition with no prenatal phenotype in previous children for whom no genetic evaluation was done and is not possible. Such scenarios should be reviewed by an expert multidisciplinary team preferentially in the context of a research protocol. If sequencing is done for this indication, it must be done as trio sequencing, using an appropriate analytical approach" (Van den Veyver et al., 2022).

American Academy of Neurology (AAN) and American Association of Neuromuscular and Electrodiagnostic Medicine (AANEM)

The AAN/AANEM published guidelines (Kang et al., 2015) on the evaluation, diagnosis, and management of congenital muscular dystrophy (CMD) which state: "In individuals with CMD who either do not have a mutation identified in one of the commonly associated genes or have a phenotype whose genetic origins have not been well characterized, physicians might order whole-exome or whole-genome sequencing when those technologies become more accessible and affordable for routine clinical use (Level C)."

The AAN/AANEM published guidelines (Narayanaswami et al., 2014) on the diagnosis and treatment of limb-girdle and distal dystrophies which state: "In patients with suspected muscular dystrophy in whom initial clinically directed genetic testing does not provide a diagnosis, clinicians may obtain genetic consultation or perform parallel sequencing of targeted exomes, whole-exome sequencing, whole-genome screening, or next-generation sequencing to identify the genetic abnormality (Level C)."

Association for Molecular Pathology (AMP)

The AMP published a report on the spectrum of clinical utilities in molecular pathology testing procedures for inherited conditions and cancer. The background of this report states, "Whole genome sequencing is currently more expensive than WES, requires greater analysis, and generates more variants of uncertain significance. WES is a plausible approach when the clinical picture cannot be affirmed using a specific gene panel. As technologies and understanding of variants advance, whole genome sequencing might become the test of choice" (Joseph et al., 2016).

American Academy of Pediatrics (AAP)

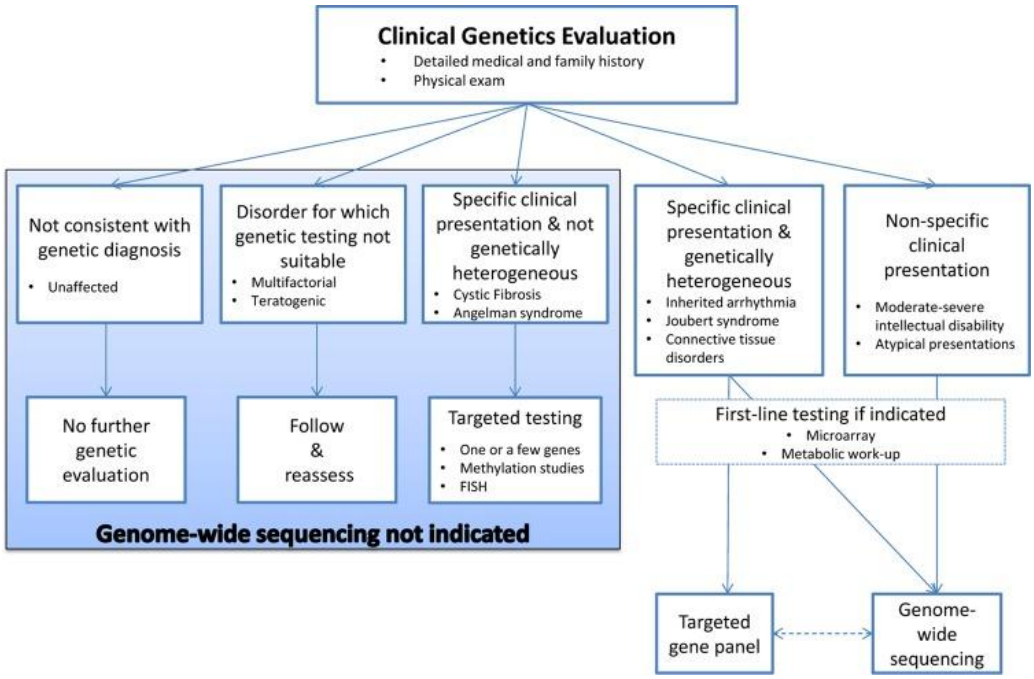
AAP published guidelines on the evaluation of children with autism spectrum disorder. According to the guidelines, CMA is recommended if the etiology for developmental disability is not known. Since Fragile X Syndrome increases the risk for autism spectrum disorder, DNA testing for Fragile X should be recommended in all children with ASD, especially for boys and children with a family history of intellectual disability. "The cytosine-guanine-guanine trinucleotide repeat expansion that is responsible for fragile X syndrome is not detected on CMA and must be ordered as a separate test. When the history and physical examination, CMA, and fragile X analysis do not identify an etiology, the next step at this time in the etiologic evaluation for [autism spectrum disorder] is whole-exome sequencing (WES)." AAP does not recommend the use of commercially marketed tests as they do not provide a molecular etiologic diagnosis (Hyman et al., 2020).

Canadian College of Medical Geneticists

In 2015, the Canadian College of Medical Geneticists published a position statement on genome-wide sequencing for monogenic diseases. Their relevant recommendations include the following:

- "Recommendations for diagnostic assessment:
  - Clinical exome sequencing, at this time, should only be used to interrogate the genome for nucleotide sequence variants in genes known to cause disease. Clinical WGS may be used to detect CNV and structural variation in addition to sequence variants, though it is not currently a first-tier test for such analyses.
  - Clinical genome-wide sequencing should be considered in the investigation of an affected individual when his/her phenotype or family history suggests a monogenic aetiology in whom the causal mutation(s) are unknown, and one or more of the following additional conditions apply:
    - the phenotype is associated with a high degree of genetic heterogeneity;
    - specific genetic tests have failed to arrive at a diagnosis and testing of other clinically relevant genes is appropriate;
    - genome-wide sequencing is a more cost-effective approach than available individual gene or gene panel testing."
- "Until the benefits of reporting incidental findings are established, we do not endorse the intentional clinical analysis of disease-associated genes other than those linked to the primary indication."

Below is a figure of a "decision aid to facilitate the diagnostic evaluation of patients with rare disease of suspected monogenic aetiology" (Boycott et al., 2015).



The Royal Australasian College of Physicians

In 2021, the Royal Austalasian College of Physicians provided guidelines on pediatric genetic testing in the context of intellectual disability (ID) and global developmental delay (GDD). For childhood syndromes or ID/GDD, the group recommends WES or WGS as tests of choice, since they offer "a broad, agnostic screen," but acknowledge that WES is more widely available and cost-effective at the time of publication. In terms of ordering singleton or trio testing, the group states that "the latter (trio) approach is highly recommended given it simplifies analysis… it is also a more streamlined clinical test as trio testing identifies fewer variants of uncertain significance than singleton testing" (Sachdev et al., 2021).

VII. Applicable State and Federal Regulations

DISCLAIMER: If there is a conflict between this Policy and any relevant, applicable government policy for a particular member [e.g., Local Coverage Determinations (LCDs) or National Coverage Determinations (NCDs) for Medicare and/or state coverage for Medicaid], then the government policy will be used to make the determination. For the most up-to-date Medicare policies and coverage, please visit the Medicare search website: https://www.cms.gov/medicare-coverage-database/search.aspx. For the most up-to-date Medicaid policies and coverage, visit the applicable state Medicaid website.

Food and Drug Administration (FDA)

Genotyping is considered a laboratory developed test (LDT); developed, validated, and performed by individual laboratories. Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). LDTs are not approved or cleared by the U. S. Food and Drug Administration; however, FDA clearance or approval is not currently required for clinical use.

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81415	Exome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis
81416	Exome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator exome (eg, parents, siblings) (List separately in addition to code for primary procedure)
81417	Exome (eg, unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained exome sequence (eg, updated knowledge or unrelated condition/syndrome)
81425	Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis
81426	Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator genome (eg, parents, siblings) (List separately in addition to code for primary procedure)
81427	Genome (eg, unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained genome sequence (eg, updated knowledge or unrelated condition/syndrome)
81479	Unlisted molecular pathology procedure
0209U	Cytogenomic constitutional (genome-wide) analysis, interrogation of genomic regions for copy number, structural changes and areas of homozygosity for chromosomal abnormalities
0214U	Rare diseases (constitutional/heritable disorders), whole exome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, proband Proprietary test: Genomic Unity® Exome Plus Analysis -Proband, Lab/Manufacturer: Variantyx Inc,

CPT	Code Description
0215U	Rare diseases (constitutional/heritable disorders), whole exome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, each comparator exome (eg, parent, sibling) Proprietary test: Genomic Unity® Exome Plus Analysis - Comparator, Lab/Manufacturer: Variantyx Inc, Variantyx Inc
0265U	Rare constitutional and other heritable disorders, whole genome and mitochondrial DNA sequence analysis, blood, frozen and formalin-fixed paraffin-embedded (FFPE) tissue, saliva, buccal swabs or cell lines, identification of single nucleotide and copy number variants
0335U	Rare diseases (constitutional/heritable disorders), whole genome sequence analysis, including small sequence changes, copy number variants, deletions, duplications, mobile element insertions, uniparental disomy (UPD), inversions, aneuploidy, mitochondrial genome sequence analysis with heteroplasmy and large deletions, short tandem repeat (STR) gene expansions, fetal sample, identification and categorization of genetic variants
0336U	Rare diseases (constitutional/heritable disorders), whole genome sequence analysis, including small sequence changes, copy number variants, deletions, duplications, mobile element insertions, uniparental disomy (UPD), inversions, aneuploidy, mitochondrial genome sequence analysis with heteroplasmy and large deletions, short tandem repeat (STR) gene expansions, blood or saliva, identification and categorization of genetic variants, each comparator genome (eg, parent)
0425U	Genome (eg, unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis, each comparator genome (eg, parents, siblings)
0426U	Genome (eg, unexplained constitutional or heritable disorder or syndrome), ultra-rapid sequence analysis

Current Procedural Terminology© American Medical Association. All Rights reserved.

Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.

IX. Evidence-based Scientific References

Ababneh, N. A., Ali, D., Al-Kurdi, B., Barham, R., Bsisu, I. K., Dababseh, D., Arafat, S., Khanfar, A. N., Makahleh, L., Ryalat, A. T., Sallam, M., El-Khateeb, M., Sharrack, B., &

Awidi, A. (2021). The utility of whole-exome sequencing in accurate diagnosis of neuromuscular disorders in consanguineous families in Jordan. *Clin Chim Acta*, *523*, 330-338. https://doi.org/10.1016/j.cca.2021.10.001

ACMG. (2012). Points to consider in the clinical application of genomic sequencing. *Genet Med*, *14*(8), 759-761. https://doi.org/10.1038/gim.2012.74

ACMG. (2013). Points to consider for informed consent for genome/exome sequencing [ACMG Policy Statement]. *Genetics In Medicine*, *15*, 748. https://doi.org/10.1038/gim.2013.94

ACOG, & SFM. (2016). Committee Opinion No.682: Microarrays and Next-Generation Sequencing Technology: The Use of Advanced Genetic Diagnostic Tools in Obstetrics and Gynecology. *Obstet Gynecol*, *128*(6), e262-e268. https://doi.org/10.1097/AOG.0000000000001817

Alfares, A., Aloraini, T., Subaie, L. A., Alissa, A., Qudsi, A. A., Alahmad, A., Mutairi, F. A., Alswaid, A., Alothaim, A., Eyaid, W., Albalwi, M., Alturki, S., & Alfadhel, M. (2018). Whole-genome sequencing offers additional but limited clinical utility compared with reanalysis of whole-exome sequencing. *Genet Med*, *20*(11), 1328-1333. https://doi.org/10.1038/gim.2018.41

Alford, R. L., Arnos, K. S., Fox, M., Lin, J. W., Palmer, C. G., Pandya, A., Rehm, H. L., Robin, N. H., Scott, D. A., & Yoshinaga-Itano, C. (2014). American College of Medical Genetics and Genomics guideline for the clinical evaluation and etiologic diagnosis of hearing loss. *Genet Med*, *16*(4), 347-355. https://doi.org/10.1038/gim.2014.2

Arteche-López, A., Gómez Rodríguez, M. J., Sánchez Calvin, M. T., Quesada-Espinosa, J. F., Lezana Rosales, J. M., Palma Milla, C., Gómez-Manjón, I., Hidalgo Mayoral, I., Pérez de la Fuente, R., Díaz de Bustamante, A., Darnaude, M. T., Gil-Fournier, B., Ramiro León, S., Ramos Gómez, P., Sierra Tomillo, O., Juárez Rufián, A., Arranz Cano, M. I., Villares Alonso, R., Morales-Pérez, P., . . . Alvarez-Mora, M. I. (2021). Towards a Change in the Diagnostic Algorithm of Autism Spectrum Disorders: Evidence Supporting Whole Exome Sequencing as a First-Tier Test. *Genes (Basel)*, *12*(4). https://doi.org/10.3390/genes12040560

Best, S., Wou, K., Vora, N., Van der Veyver, I. B., Wapner, R., & Chitty, L. S. (2018). Promises, pitfalls and practicalities of prenatal whole exome sequencing. *Prenat Diagn*, *38*(1), 10-19. https://doi.org/10.1002/pd.5102

Boycott, K., Hartley, T., Adam, S., Bernier, F., Chong, K., Fernandez, B. A., Friedman, J. M., Geraghty, M. T., Hume, S., Knoppers, B. M., Laberge, A. M., Majewski, J., Mendoza-Londono, R., Meyn, M. S., Michaud, J. L., Nelson, T. N., Richer, J., Sadikovic, B., Skidmore, D. L., . . . Armour, C. M. (2015). The clinical application of genome-wide sequencing for monogenic diseases in Canada: Position Statement of the Canadian College of Medical Geneticists. *J Med Genet*, *52*(7), 431-437. https://doi.org/10.1136/jmedgenet-2015-103144

Carss, K. J., Hillman, S. C., Parthiban, V., McMullan, D. J., Maher, E. R., Kilby, M. D., & Hurles, M. E. (2014). Exome sequencing improves genetic diagnosis of structural fetal abnormalities revealed by ultrasound. *Human molecular genetics*, *23*(12), 3269-3277. https://doi.org/10.1093/hmg/ddu038

Clark, M. M., Stark, Z., Farnaes, L., Tan, T. Y., White, S. M., Dimmock, D., & Kingsmore, S. F. (2018). Meta-analysis of the diagnostic and clinical utility of genome and exome sequencing and chromosomal microarray in children with suspected genetic diseases. *NPJ Genom Med*, *3*, 16. https://doi.org/10.1038/s41525-018-0053-8

Crowley, E., Warner, N., Pan, J., Khalouei, S., Elkadri, A., Fiedler, K., Foong, J., Turinsky, A. L., Bronte-Tinkew, D., Zhang, S., Hu, J., Tian, D., Li, D., Horowitz, J., Siddiqui, I., Upton, J., Roifman, C. M., Church, P. C., Wall, D. A., . . . Muise, A. M. (2020). Prevalence and Clinical Features of Inflammatory Bowel Diseases Associated With Monogenic Variants, Identified by Whole-Exome Sequencing in 1000 Children at a Single Center. *Gastroenterology*, *158*(8), 2208-2220. https://doi.org/10.1053/j.gastro.2020.02.023

Drury, S., Williams, H., Trump, N., Boustred, C., Lench, N., Scott, R. H., & Chitty, L. S. (2015). Exome sequencing for prenatal diagnosis of fetuses with sonographic abnormalities. *Prenat Diagn*, *35*(10), 1010-1017. https://doi.org/10.1002/pd.4675

Ewans, L. J., Minoche, A. E., Schofield, D., Shrestha, R., Puttick, C., Zhu, Y., Drew, A., Gayevskiy, V., Elakis, G., Walsh, C., Adès, L. C., Colley, A., Ellaway, C., Evans, C. A., Freckmann, M. L., Goodwin, L., Hackett, A., Kamien, B., Kirk, E. P., . . . Roscioli, T. (2022). Whole exome and genome sequencing in mendelian disorders: a diagnostic and health economic analysis. *Eur J Hum Genet*, *30*(10), 1121-1131. https://doi.org/10.1038/s41431-022-01162-2

GGC. (2022). *Focused NGS - Panel*. https://ggc.org/test-finder-item/focused-ngs-panel

Haskell, G. T., Adams, M. C., Fan, Z., Amin, K., Guzman Badillo, R. J., Zhou, L., Bizon, C., Chahin, N., Greenwood, R. S., Milko, L. V., Shiloh-Malawsky, Y., Crooks, K. R., Strande, N., Tennison, M., Tilley, C. R., Brandt, A., Wilhelmsen, K. C., Weck, K., Evans, J. P., & Berg, J. S. (2018). Diagnostic utility of exome sequencing in the evaluation of neuromuscular disorders. *Neurol Genet*, *4*(1), e212. https://doi.org/10.1212/nxg.0000000000000212

Hulick, P. (2024, February 7). *Next-generation DNA sequencing (NGS): Principles and clinical applications*. https://www.uptodate.com/contents/next-generation-dna-sequencing-ngs-principles-and-clinical-applications

Hyman, S. L., Levy, S. E., & Myers, S. M. (2020). Identification, Evaluation, and Management of Children With Autism Spectrum Disorder. *Pediatrics*, *145*(1). https://doi.org/10.1542/peds.2019-3447

ISPD, SMFM, & PQF. (2018). Joint Position Statement from the International Society for Prenatal Diagnosis (ISPD), the Society for Maternal Fetal Medicine (SMFM), and the Perinatal Quality Foundation (PQF) on the use of genome-wide sequencing for fetal diagnosis. *Prenat Diagn*, *38*(1), 6-9. https://doi.org/10.1002/pd.5195

Jia, A., Lei, Y., Liu, D. P., Pan, L., Guan, H. Z., & Yang, B. (2023). A Retrospective Analysis of Clinically Focused Exome Sequencing Results of 372 Infants with Suspected Monogenic Disorders in China. *Pharmgenomics Pers Med*, *16*, 81-97. https://doi.org/10.2147/pgpm.S387767

Joseph, L., Cankovic, M., Caughron, S., Chandra, P., Emmadi, R., Hagenkord, J., Hallam, S., Jewell, K. E., Klein, R. D., Pratt, V. M., Rothberg, P. G., Temple-Smolkin, R. L., & Lyon, E. (2016). The Spectrum of Clinical Utilities in Molecular Pathology Testing Procedures for Inherited Conditions and Cancer: A Report of the Association for Molecular Pathology. *J Mol Diagn*, *18*(5), 605-619. https://doi.org/10.1016/j.jmoldx.2016.05.007

Kang, P. B., Morrison, L., Iannaccone, S. T., Graham, R. J., Bonnemann, C. G., Rutkowski, A., Hornyak, J., Wang, C. H., North, K., Oskoui, M., Getchius, T. S., Cox, J. A., Hagen, E. E., Gronseth, G., & Griggs, R. C. (2015). Evidence-based guideline summary: evaluation, diagnosis, and management of congenital muscular dystrophy: Report of the Guideline Development Subcommittee of the American Academy of Neurology and the Practice Issues Review Panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. *Neurology*, *84*(13), 1369-1378. https://doi.org/10.1212/wnl.0000000000001416

Lee, H., Deignan, J. L., Dorrani, N., Strom, S. P., Kantarci, S., Quintero-Rivera, F., Das, K., Toy, T., Harry, B., Yourshaw, M., Fox, M., Fogel, B. L., Martinez-Agosto, J. A., Wong, D. A., Chang, V. Y., Shieh, P. B., Palmer, C. G., Dipple, K. M., Grody, W. W., . . . Nelson, S. F. (2014). Clinical exome sequencing for genetic identification of rare Mendelian disorders. *Jama*, *312*(18), 1880-1887. https://doi.org/10.1001/jama.2014.14604

Malcher, A., Stokowy, T., Berman, A., Olszewska, M., Jedrzejczak, P., Sielski, D., Nowakowski, A., Rozwadowska, N., Yatsenko, A. N., & Kurpisz, M. K. (2022). Whole-genome sequencing identifies new candidate genes for nonobstructive azoospermia. *Andrology*, *10*(8), 1605-1624. https://doi.org/10.1111/andr.13269

Malinowski, J., Miller, D. T., Demmer, L., Gannon, J., Pereira, E. M., Schroeder, M. C., Scheuner, M. T., Tsai, A. C., Hickey, S. E., & Shen, J. (2020). Systematic evidence-based review: outcomes from exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability. *Genet Med*, *22*(6), 986-1004. https://doi.org/10.1038/s41436-020-0771-z

Manickam, K., McClain, M. R., Demmer, L. A., Biswas, S., Kearney, H. M., Malinowski, J., Massingham, L. J., Miller, D., Yu, T. W., Hisama, F. M., & Directors, A. B. o. (2021). Exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability: an evidence-based clinical guideline of the American College of Medical Genetics and Genomics (ACMG). *Genetics In Medicine*, *23*(11), 2029-2037. https://doi.org/10.1038/s41436-021-01242-6

Miller, K. A., Twigg, S. R., McGowan, S. J., Phipps, J. M., Fenwick, A. L., Johnson, D., Wall, S. A., Noons, P., Rees, K. E., Tidey, E. A., Craft, J., Taylor, J., Taylor, J. C., Goos, J. A., Swagemakers, S. M., Mathijssen, I. M., van der Spek, P. J., Lord, H., Lester, T., . . .

Wilkie, A. O. (2017). Diagnostic value of exome and whole genome sequencing in craniosynostosis. *J Med Genet*, *54*(4), 260-268. https://doi.org/10.1136/jmedgenet-2016-104215

Monaghan, K. G., Leach, N. T., Pekarek, D., Prasad, P., & Rose, N. C. (2020). The use of fetal exome sequencing in prenatal diagnosis: a points to consider document of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*. https://doi.org/10.1038/s41436-019-0731-7

Muthaffar, O. Y. (2021). The Utility of Whole Exome Sequencing in Diagnosing Pediatric Neurological Disorders. *Balkan journal of medical genetics : BJMG*, *23*(2), 17-24. https://doi.org/10.2478/bjmg-2020-0028

Narayanaswami, P., Weiss, M., Selcen, D., David, W., Raynor, E., Carter, G., Wicklund, M., Barohn, R. J., Ensrud, E., Griggs, R. C., Gronseth, G., & Amato, A. A. (2014). Evidence-based guideline summary: diagnosis and treatment of limb-girdle and distal dystrophies: report of the guideline development subcommittee of the American Academy of Neurology and the practice issues review panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. *Neurology*, *83*(16), 1453-1463. https://doi.org/10.1212/wnl.0000000000000892

Reda, M., Richard, C., Bertaut, A., Niogret, J., Collot, T., Fumet, J. D., Blanc, J., Truntzer, C., Desmoulins, I., Ladoire, S., Hennequin, A., Favier, L., Bengrine, L., Vincent, J., Hervieu, A., Dusserre, J. G., Lepage, C., Foucher, P., Borg, C., . . . Ghiringhelli, F. (2020). Implementation and use of whole exome sequencing for metastatic solid cancer. *EBioMedicine*, *51*, 102624. https://doi.org/10.1016/j.ebiom.2019.102624

Rehm, H. L., Bale, S. J., Bayrak-Toydemir, P., Berg, J. S., Brown, K. K., Deignan, J. L., Friez, M. J., Funke, B. H., Hegde, M. R., & Lyon, E. (2013). ACMG clinical laboratory standards for next-generation sequencing. *Genet Med*, *15*(9), 733-747. https://doi.org/10.1038/gim.2013.92

Sachdev, R., Field, M., Baynam, G. S., Beilby, J., Berarducci, M., Berman, Y., Boughtwood, T., Cusack, M. B., Fitzgerald, V., Fletcher, J., Freckmann, M. L., Grainger, N., Kirk, E., Lundie, B., Lunke, S., McGregor, L., Mowat, D., Parasivam, G., Tyrell, V., . . . A, S. L. M. (2021). Paediatric genomic testing: Navigating medicare rebatable genomic testing. *J Paediatr Child Health*, *57*(4), 477-483. https://doi.org/10.1111/jpc.15382

Sakai, R., Sifrim, A., Vande Moere, A., & Aerts, J. (2013). TrioVis: a visualization approach for filtering genomic variants of parent-child trios. *Bioinformatics*, *29*(14), 1801-1802. https://doi.org/10.1093/bioinformatics/btt267

Salfati, E. L., Spencer, E. G., Topol, S. E., Muse, E. D., Rueda, M., Lucas, J. R., Wagner, G. N., Campman, S., Topol, E. J., & Torkamani, A. (2019). Re-analysis of whole-exome sequencing data uncovers novel diagnostic variants and improves molecular diagnostic yields for sudden death and idiopathic diseases. *Genome Medicine*, *11*(1), 83. https://doi.org/10.1186/s13073-019-0702-2

Sanford, E. F., Clark, M. M., Farnaes, L., Williams, M. R., Perry, J. C., Ingulli, E. G., Sweeney, N. M., Doshi, A., Gold, J. J., Briggs, B., Bainbridge, M. N., Feddock, M., Watkins, K., Chowdhury, S., Nahas, S. A., Dimmock, D. P., Kingsmore, S. F., Coufal, N. G., & Investigators, R. (2019). Rapid Whole Genome Sequencing Has Clinical Utility in Children in the PICU. *Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies*, *20*(11), 1007-1020. https://doi.org/10.1097/PCC.0000000000002056

Soden, S. E., Saunders, C. J., Willig, L. K., Farrow, E. G., Smith, L. D., Petrikin, J. E., LePichon, J. B., Miller, N. A., Thiffault, I., Dinwiddie, D. L., Twist, G., Noll, A., Heese, B. A., Zellmer, L., Atherton, A. M., Abdelmoity, A. T., Safina, N., Nyp, S. S., Zuccarelli, B., . . . Kingsmore, S. F. (2014). Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders. *Sci Transl Med*, *6*(265), 265ra168. https://doi.org/10.1126/scitranslmed.3010076

Splinter, K., Adams, D. R., Bacino, C. A., Bellen, H. J., Bernstein, J. A., Cheatle-Jarvela, A. M., Eng, C. M., Esteves, C., Gahl, W. A., Hamid, R., Jacob, H. J., Kikani, B., Koeller, D. M., Kohane, I. S., Lee, B. H., Loscalzo, J., Luo, X., McCray, A. T., Metz, T. O., . . . Ashley, E. A. (2018). Effect of Genetic Diagnosis on Patients with Previously Undiagnosed Disease. *N Engl J Med*, *379*(22), 2131-2139. https://doi.org/10.1056/NEJMoa1714458

Srivastava, S., Cohen, J. S., Vernon, H., Baranano, K., McClellan, R., Jamal, L., Naidu, S., & Fatemi, A. (2014). Clinical whole exome sequencing in child neurology practice. *Ann Neurol*, *76*(4), 473-483. https://doi.org/10.1002/ana.24251

Tammimies, K., Marshall, C. R., Walker, S., Kaur, G., Thiruvahindrapuram, B., Lionel, A. C., Yuen, R. K., Uddin, M., Roberts, W., Weksberg, R., Woodbury-Smith, M., Zwaigenbaum, L., Anagnostou, E., Wang, Z., Wei, J., Howe, J. L., Gazzellone, M. J., Lau, L., Sung, W. W., . . . Fernandez, B. A. (2015). Molecular Diagnostic Yield of Chromosomal Microarray Analysis and Whole-Exome Sequencing in Children With Autism Spectrum Disorder. *Jama*, *314*(9), 895-903. https://doi.org/10.1001/jama.2015.10078

Taylor, J. C., Martin, H. C., Lise, S., Broxholme, J., Cazier, J. B., Rimmer, A., Kanapin, A., Lunter, G., Fiddy, S., Allan, C., Aricescu, A. R., Attar, M., Babbs, C., Becq, J., Beeson, D., Bento, C., Bignell, P., Blair, E., Buckle, V. J., . . . McVean, G. (2015). Factors influencing success of clinical genome sequencing across a broad spectrum of disorders. *Nat Genet*, *47*(7), 717-726. https://doi.org/10.1038/ng.3304

Van den Veyver, I. B., Chandler, N., Wilkins-Haug, L. E., Wapner, R. J., & Chitty, L. S. (2022). International Society for Prenatal Diagnosis Updated Position Statement on the use of genome-wide sequencing for prenatal diagnosis. *Prenat Diagn*, *42*(6), 796-803. https://doi.org/10.1002/pd.6157

variantyx. (2020). *Genomic Unity® Exome Plus Analysis*. https://www.variantyx.com/products-services/rare-disorder-genetics/comprehensive-analyses/genomic-unity-exome-plus-analysis/

Yang, Y., Muzny, D. M., Xia, F., Niu, Z., Person, R., Ding, Y., Ward, P., Braxton, A., Wang, M., Buhay, C., Veeraraghavan, N., Hawes, A., Chiang, T., Leduc, M., Beuten, J., Zhang, J., He, W., Scull, J., Willis, A., . . . Eng, C. M. (2014). Molecular findings among patients referred for clinical whole-exome sequencing. *Jama*, *312*(18), 1870-1879. https://doi.org/10.1001/jama.2014.14601

Yates, C. L., Monaghan, K. G., Copenheaver, D., Retterer, K., Scuffins, J., Kucera, C. R., Friedman, B., Richard, G., & Juusola, J. (2017). Whole-exome sequencing on deceased fetuses with ultrasound anomalies: expanding our knowledge of genetic disease during fetal development. *Genet Med*, *19*(10), 1171-1178. https://doi.org/10.1038/gim.2017.31

Zhang, J., Tang, S.-Y., Zhu, X.-B., Li, P., Lu, J.-Q., Cong, J.-S., Wang, L.-B., Zhang, F., & Li, Z. (2021). Whole exome sequencing and trio analysis to broaden the variant spectrum of genes in idiopathic hypogonadotropic hypogonadism [Original Article]. *Asian Journal of Andrology*, *23*(3), 288-293. https://doi.org/10.4103/aja.aja_65_20