# Molecular Pathology/Molecular Diagnostics/ Genetic Testing

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Instructions for Use

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## Coverage Rationale

### Overview

A molecular diagnostic test (MDT) is any test that involves 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). The test may or may not include multiple components. A MDT may consist of a single mutation analysis/identification, and/or may or may not rely upon an algorithm or other form of data evaluation/derivation.

A laboratory developed test (LDT) is any test developed by a laboratory developed without Food and Drug Administration (FDA) approval or clearance.

### CMS National Coverage Determinations (NCDs)

For the tests in this policy that utilize the Next Generation Sequencing technology, Medicare does not have an NCD. NCD 90.2 *Next Generation Sequencing (NGS)* is applicable to diagnostic lab tests using NGS for somatic (acquired) and germline (inherited) cancer. For coverage guidelines of those tests, refer to NCD 90.2 Next Generation Sequencing (NGS). Medicare Administrative Contractors (MACs) may determine coverage of diagnostic lab tests using NGS for RNA sequencing and protein analysis.

### CMS Local Coverage Determinations (LCDs) and Articles

Local Coverage Determinations (LCDs)/Local Coverage Articles (LCAs) exist for tests in this policy that utilize the Next Generation Sequencing technology and compliance with these policies is required where applicable. LCDs/LCAs also exist for molecular tests in this policy that do not utilize the Next Generation Sequencing technology. For specific molecular testing LCDs/LCAs, refer to the table for Molecular Pathology/Molecular Diagnostics/Genetic Testing.

For coverage guidelines for states/territories with no LCDs/LCAs, refer to the covered and non-covered indications below.

## *Covered Indications*

### ABL1 (ABL Proto-Oncogene 1, Non-Receptor Tyrosine Kinase) Kinase Domain

*ABL1* gene analysis, variants in the kinase domain is considered reasonable and necessary in patients with acute lymphoblastic leukemia (ALL) and chronic myeloid leukemia (CML) to guide therapeutic decision making.

### ASXL1 (Additional Sex Combs Like 1, Transcriptional Regulator)

*ASXL1* gene analysis is considered reasonable and necessary for prognosing patients with acute myeloid leukemia, myeloproliferative disease [MPD - essential thrombocytosis (ET), myelofibrosis & polycythemia vera (PV)], and myelodysplastic syndrome (MDS).

### BDX-XL2 (Oncology Lung)

BDX-XL2 test (Biodesix, Seattle, WA) is reasonable and necessary for the management of a lung nodule, between 8 and 30 mm in diameter, in patients 40 years or older, and with a pre-test cancer risk (as assessed by the Mayo Clinic *Model for Solitary Pulmonary Nodules*) of 50% or less. The intended use of the test is to assist physicians in the management of lung nodules by identifying those lung nodules with a high probability of being benign. These lung nodules would then be candidates for non-invasive computed tomography (CT) surveillance instead of invasive procedures.

### DetermaRx™ (Oncology Lung)

Molecular classifiers for non-small cell lung cancer (NSCLC) are considered reasonable and necessary when members meet all of the following criteria:

- The patient has a non-squamous NSCLC with a tumor size < 5 cm, and there are no positive lymph nodes (i.e., American Joint Committee on Cancer (AJCC) Eighth Edition Stages I and IIa).
- The patient is sufficiently healthy to tolerate chemotherapy.
- Adjuvant platinum-containing chemotherapy is being considered for the patient.
- The test is ordered by a physician who is treating the patient for NSCLC (generally a medical oncologist, surgeon, or radiation oncologist) to help in the decision of whether or not to recommend adjuvant chemotherapy.

### Genomic Prostate Score® (GPS) Test (Previously Oncotype DX® Genomic Prostate Score)

The Genomic Prostate Score® (GPS) Test [previously Oncotype DX® Genomic Prostate Score (Genomic Health®)] is reasonable and necessary for use in very low risk, low risk, and favorable intermediate risk prostate cancer.

### JAK2 (Janus Kinase 2) and MPL (MPL Proto-Oncogene, Thrombopoietin Receptor) (Myeloproliferative Disorders)

Genetic testing of *JAK2* exon 12, performed to identify polycythemia vera (PV), is reasonable and necessary when the following criteria are met:

- Genetic testing impacts medical management; and
- Patient would meet WHO's diagnostic criteria for PV, if *JAK2* exon 12 testing were positive; and
- *JAK2 V617F* mutation analysis was previously completed and was negative.

Genetic testing of the *MPL* gene is reasonable and necessary when the following criteria are met:

- Genetic testing impacts medical management; and
- *JAK2 V617F* mutation analysis was previously completed and negative; and
- Patient would meet WHO's diagnostic criteria for MPD (i.e., ET, MPF) if a clonal marker were identified.

### KIT (V-Kit Hardy-Zuckerman 4 Feline Sarcoma Viral Oncogene Homolog)

*KIT* gene analysis is considered reasonable and necessary in patients who have gastrointestinal stromal tumor (GIST), acute myeloid leukemia (AML), melanoma, and myeloproliferative disease [MPD - essential thrombocytosis (ET), myelofibrosis & polycythemia vera (PV)] to guide therapeutic decision making.

### myPath® Melanoma

The purpose of this test is to assist dermatopathologists to arrive at the correct diagnosis of melanoma versus non-melanoma when examining skin biopsies. Molecular deoxyribonucleic acid (DNA)/ribonucleic acid (RNA) assays that aid in the diagnosis or exclusion of melanoma from a biopsy are reasonable and necessary when **all** of the following clinical conditions are met:

- The test is ordered by a board-certified or board-eligible dermatopathologist.
- The specimen is a primary (non-metastatic, non-re-excision specimen) cutaneous melanocytic neoplasm for which the diagnosis is equivocal/uncertain (i.e., clear distinction between benign or malignant cannot be achieved using clinical and/or histopathological features alone) despite the performance of standard-of-care test procedures and relevant ancillary tests (i.e., immunohistochemical stains).
- The specimen includes an area representative of the lesion or portion of the lesion that is suspicious for malignancy.
- The patient may be subjected to additional intervention, such as re-excision and/or sentinel lymph node biopsy, as a result of the diagnostic uncertainty.
- The patient has not been tested with the same or similar assay for the same clinical indication.
- The test is validated for use in the intended-use population and is performed according to its stated intended-use.

## Oncotype DX® Breast Cancer Assay (Oncology Breast mRNA)

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 is considered reasonable and necessary to guide therapeutic decision-making in patients with the following findings:

- Estrogen-receptor positive, node-negative carcinoma of the breast.
- Estrogen-receptor positive micrometastases of carcinoma of the breast.
- Estrogen-receptor positive breast carcinoma with 1-3 positive nodes.

## Oncotype DX® Breast DCIS Score™ Test (Ductal Carcinoma In Situ)

Oncotype DX® DCIS assay (Genomic Health, Inc., Redwood City, CA) is reasonable and necessary for women diagnosed with DCIS who are planning on having breast conserving surgery and considering adjuvant radiation therapy.

## PDGFRA (Platelet-Derived Growth Factor Receptor, Alpha Polypeptide)

*PDGFRA* gene analysis is considered reasonable and necessary in patients with *PDGFRA*-associated chronic eosinophilic leukemia or GIST caused by mutations in the *PDGFRA* gene to guide therapeutic decision making.

## Pigmented Lesion Assay (PLA) (Oncology Melanoma)

The PLA is reasonable and necessary for use on melanocytic skin lesions with 1 or more clinical or historical characteristics suggestive of melanoma, including 1 or more ABCDE criteria when a clinician trained in the clinical diagnosis of skin cancer is considering the need for biopsy to rule out melanoma. The PLA should not be used on clinically obvious melanoma. The PLA result is 1 element of the overall clinical assessment, and should be used in combination with clinical and historical signs of melanoma to obtain additional information prior to a decision to biopsy.

## PIK3CA (Phosphatidylinositol-4, 5-Biphosphate, 3-Kinase, Catalytic Subunit Alpha)

The U.S. FDA has approved Piqray (alpelisib) tablets, to be used in combination with the FDA-approved endocrine therapy fulvestrant, to treat postmenopausal women, and men, with hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, *PIK3CA*-mutated, advanced or metastatic breast cancer (as detected by an FDA-approved test) following progression on or after an endocrine-based regimen. *PIK3CA* testing is reasonable and necessary for this indication.

In addition to utilizing the coverage rationale referenced above in states/territories with no LCDs/LCAs, UnitedHealthcare also uses the criteria above to supplement the general Medicare criteria within the NGS jurisdiction regarding when *PIK3CA* testing is reasonable and necessary. UnitedHealthcare uses the criteria noted above in order to ensure consistency in reviewing the conditions to be met for coverage of *PIK3CA* testing. Use of these criteria to supplement the coverage criteria noted above provides clinical benefits by identifying *PIK3CA*-mutated breast cancer, which has shown a clinical benefit in individuals taking alpelisib with fulvestrant for treatment of HR +, HER2-, *PIK3CA*-mutant advanced breast cancer after CDK4/6i treatment. Specifically, there was a 7.9-month numeric improvement in median overall survival when alpelisib was added to fulvestrant treatment of individuals with *PIK3CA*-mutated, HR +, HER2- advanced breast cancer. The added criteria will also provide numerous clinical benefits by guiding the treatment plan and medication regimen for this specific type of breast cancer. The potential clinical harms of using these criteria may include denying claims in the NGS jurisdiction since there are no concrete clinical guidelines. However, with no specific clinical guidelines for this test, claims may be inappropriately allowed for indications other than the FDA approved indications for Piqray. The clinical benefits of using these criteria are highly likely to outweigh any clinical harms because the criteria will ensure this test is being used when reasonable and necessary, based on the clinical studies shown in this policy including a study of individuals with *PIK3CA*-mutant disease with prior CDK4/6i plus hormone therapy. In postmatching and unadjusted results, primary and secondary endpoints were in favor of treatment with alpelisib with fulvestrant over standard treatments.

For other indications such as colorectal cancer, prostate cancer, and borderline ovarian tumors, there is insufficient evidence to support *PIK3CA* testing.

### TERT (Telomerase Reverse Transcriptase)

*TERT* gene analysis is considered reasonable and necessary in patients with malignant neoplasm of the brain.

### Thyroseq®, ThyGeNEXT®, ThyraMIR™, and Afirma®

ThyroSeq® is a test utilized to better define the need for thyroid surgery and the type of such surgery. ThyraMIR™ is used as a companion test to ThyGeNEXT® when ThyGeNEXT® results are inconclusive. ThyroSeq®, ThyraMIR™, ThyGeNEXT® and Afirma® services are reasonable and necessary for patients with any of the following conditions:

- An indeterminate pathology on fine needle aspiration.
- Patients with one or more thyroid nodules with a history or characteristics suggesting malignancy such as:
  - Nodule growth over time.
  - Family history of thyroid cancer.
  - Hoarseness, difficulty swallowing or breathing.
  - History of exposure to ionizing radiation.
  - Hard nodule compared with rest of gland consistency.
  - Presence of cervical adenopathy.

### UroVysion™ Bladder Cancer Kit

UroVysion™ Bladder Cancer Kit is reasonable and necessary when performed on urine specimens from persons with hematuria suspected of having bladder cancer as an aid for initial diagnosis of bladder carcinoma and subsequent monitoring for tumor recurrence in patient previously diagnosed with bladder cancer. To date, UroVysion™ Bladder Cancer Kit is the only FDA approved assay that is designed to detect aneuploidy for chromosomes 3, 7, 17 and loss of the 9p21 locus via FISH.

## *Non-Covered Indications*

Molecular pathology tests for diseases or conditions that manifest severe signs or symptoms in newborns and in early childhood or that result in early death (e.g., Canavan disease) are not reasonable and necessary since these tests are generally not relevant to a Medicare member.

The following types of genetic tests are examples of services that are not relevant to a Medicare member, are not considered a Medicare benefit (statutorily excluded), and therefore will be denied as Medicare excluded tests:

- Tests considered screening in the absence of clinical signs and symptoms of disease that are not specifically identified by the law.
- Tests performed to determine carrier screening.
- Prenatal diagnostic testing.
- Tests performed on patients without signs or symptoms to determine risk for developing a disease or condition.
- Tests without diagnosis specific indications.

Screening services such as pre-symptomatic genetic tests and services used to detect an undiagnosed disease or disease predisposition are not a Medicare benefit and are not covered.

In accordance with the Code of Federal Regulations, Title 42, Subchapter B, Part 410, Section 410.32, the referring/ordering practitioner must have an established relationship with the patient, and the test results must be used by the ordering/referring practitioner in the management of the patient's specific medical problem.

Title XVIII of the Social Security Act, Section 1862(a)(1)(A) states "...no Medicare payment shall be made for items or services which are not reasonable and necessary for the diagnosis and treatment of illness or injury...".

Therefore, the following tests will be denied:

- **AFF2 [ALF transcription elongation factor 2 (FMR2)]** [e.g., fragile X intellectual disability 2 (FRAXE)]
- **AR (androgen receptor)** (e.g., spinal and bulbar muscular atrophy, Kennedy disease, X chromosome inactivation)
- **Ashkenazi Jewish Associated Disorders Carrier Screening Panel** (e.g., Bloom syndrome, Canavan disease, cystic fibrosis, familial dysautonomia, Fanconi anemia group C, Gaucher disease, Tay - Sachs disease)
- **ASPA (aspartoacylase)** (e.g., Canavan disease)
- **BCKDHB (branched - chain keto acid dehydrogenase E1, beta polypeptide)** (e.g., Maple syrup urine disease)
- **Cytogenomic (Genome-Wide) Analysis for Constitutional Chromosomal Abnormalities**

- **DMPK (DM1 protein kinase)** (e.g., myotonic dystrophy type 1)
- **F9 (coagulation factor IX)** (e.g., hemophilia B)
- **FANCC (Fanconi anemia, complementation group C)** (e.g., Fanconi anemia, type C)
- **Fetal Chromosomal Aneuploidy** (e.g., trisomy 21, 18, and 13, monosomy X)
- **Fetal Chromosomal Microdeletions** (e.g., DiGeorge syndrome, Cri - du - chat syndrome)
- **FMR1 (fragile X messenger ribonucleoprotein 1)** [e.g., fragile X syndrome, X-linked intellectual disability (XLID)]
- **FXN (frataxin)** (e.g., Friedreich ataxia)
- **G6PD (glucose-6-phosphate dehydrogenase)** (e.g., hemolytic anemia, jaundice)
- **Genetic Testing for Severe Inherited Conditions Carrier Screening Panel** [e.g., cystic fibrosis, Ashkenazi Jewish - associated disorders (e.g., Bloom syndrome, Canavan disease, Fanconi anemia type C, mucolipidosis type VI, Gaucher disease, Tay - Sachs disease), beta hemoglobinopathies, phenylketonuria, galactosemia]
- **Genome Sequencing** (e.g., unexplained constitutional or heritable disorder or syndrome)
- **HBA1/HBA2 (alpha globin 1 and alpha globin 2)** (e.g., alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease)
- **HBB (hemoglobin, subunit beta)** (e.g., sickle cell anemia, beta thalassemia, hemoglobinopathy)
- **Hearing Loss Panel** (e.g., non - syndromic hearing loss, Usher syndrome, Pendred syndrome)
- **Hereditary Peripheral Neuropathies Panel** (e.g., Charcot-Marie-Tooth, spastic paraplegia)
- **Hereditary Retinal Disorders Panel** (e.g., retinitis pigmentosa, Leber congenital amaurosis, cone-rod dystrophy)
- **HTT (huntingtin)** (e.g., Huntington disease)
- **IKBKAP (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein)** (e.g., familial dysautonomia)
- **MCOLN1 (mucolipin 1)** (e.g., Mucolipidosis, type IV)
- **MECP2 (methyl CpG binding protein 2)** (e.g., Rett syndrome)
- **Nuclear Encoded Mitochondrial Genes Panel** (e.g., neurologic or myopathic phenotypes)
- **PMP22 (peripheral myelin protein 22)** (e.g., Charcot - Marie - Tooth, hereditary neuropathy with liability to pressure palsies)
- **SMN1 (survival of motor neuron 1, telomeric)** (e.g., spinal muscular atrophy)
- **SMPD1 (sphingomyelin phosphodiesterase 1, acid lysosomal)** (e.g., Niemann - Pick disease, Type A)
- **SNRPN/UBE3A (small nuclear ribonucleoprotein polypeptide N and ubiquitin protein ligase E3A)** (e.g., Prader-Willi syndrome and/or Angelman syndrome)
- **Whole Mitochondrial Genome** [e.g., 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), Kearns-Sayre syndrome, chronic progressive external ophthalmoplegia]
- **X-linked Intellectual Disability (XLID) Panel** (e.g., syndromic and non - syndromic XLID)

**Note**: In the instance where the tests above are used for symptomatic adults, testing is unlikely to impact therapeutic decision-making in the clinical management of the patient and therefore not reasonable and necessary.

## DecisionDx®-SCC

Current molecular biomarker tests that risk stratify individuals with cutaneous squamous cell carcinoma (cSCC) are not reasonable and necessary.

Medicare does not have a National Coverage Determination (NCD) for DecisionDx®-SCC. LCDs/LCAs exist and compliance with these policies is required where applicable. For specific LCDs/LCAs, refer to the table for Molecular Pathology/Molecular Diagnostics/Genetic Testing.

For coverage guidelines for states/territories with no LCDs/LCAs or when the LCDs/LCAs are silent on coverage criteria, refer to the UnitedHealthcare Commercial Medical Policy titled Molecular Oncology Testing for Solid Tumor Cancer Diagnosis, Prognosis, and Treatment Decisions.

## MTHFR (5,10-Methylenetetrahydrofolate Reductase)

*MTHFR* genetic testing, which encodes the 5,10-methylenetetrahydrofolate reductase enzyme, for thrombophilia for all risk factors, signs, symptoms, diseases, or conditions, including cardiovascular risk assessment, is not reasonable and necessary. *MTHFR* is not considered to be clinically efficacious; therefore, testing is not reasonable and necessary.

### OVERA® and ROMA™

These multi marker serum tests related to ovarian cancer testing are not reasonable and necessary.

### Resolution ctDX Lung™

Resolution ctDX Lung™ is not reasonable and necessary for non-small cell lung cancer (NSCLC).

## Applicable Codes

The following list(s) of procedure and/or diagnosis codes is provided for reference purposes only and may not be all inclusive. Listing of a code in this policy does not imply that the service described by the code is a covered or non-covered health service; however, language may be included in the listing below to indicate if a code is non-covered. Benefit coverage for health services is determined by the member specific benefit plan document and applicable laws that may require coverage for a specific service. The inclusion of a code does not imply any right to reimbursement or guarantee claim payment. Other Policies and Guidelines may apply.

| CPT Code | Description |
|---|---|
| **Non-Covered** | |
| 0003U | Oncology (ovarian) biochemical assays of five proteins (apolipoprotein A - 1, CA 125 II, follicle stimulating hormone, human epididymis protein 4, transferrin), utilizing serum, algorithm reported as a likelihood score (Overa®) |
| 0179U | Oncology (non-small cell lung cancer), cell-free DNA, targeted sequence analysis of 23 genes (single nucleotide variations, insertions and deletions, fusions without prior knowledge of partner/breakpoint, copy number variations), with report of significant mutation(s) (Resolution ctDx Lung™) |
| 0315U | Oncology (cutaneous squamous cell carcinoma), mRNA gene expression profiling by RT-PCR of 40 genes (34 content and 6 housekeeping), utilizing formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as a categorical risk result (i.e., Class 1, Class 2A, Class 2B) (DecisionDx®-SCC) |
| 81171 | AFF2 (ALF transcription elongation factor 2 [FMR2]) (e.g., fragile X intellectual disability 2 [FRAXE]) gene analysis; evaluation to detect abnormal (e.g., expanded) alleles |
| 81172 | AFF2 (ALF transcription elongation factor 2 [FMR2]) (e.g., fragile X intellectual disability 2 [FRAXE]) gene analysis; characterization of alleles (e.g., expanded size and methylation status) |
| 81173 | AR (androgen receptor) (e.g., spinal and bulbar muscular atrophy, Kennedy disease, X chromosome inactivation) gene analysis; full gene sequence |
| 81174 | AR (androgen receptor) (e.g., spinal and bulbar muscular atrophy, Kennedy disease, X chromosome inactivation) gene analysis; known familial variant |
| 81200 | ASPA (aspartoacylase) (e.g., Canavan disease) gene analysis, common variants (e.g., E285A, Y231X) |
| 81204 | AR (androgen receptor) (e.g., spinal and bulbar muscular atrophy, Kennedy disease, X chromosome inactivation) gene analysis; characterization of alleles (e.g., expanded size or methylation status) |
| 81205 | BCKDHB (branched - chain keto acid dehydrogenase E1, beta polypeptide) (e.g., Maple syrup urine disease) gene analysis, common variants (e.g., R183P, G278S, E422X) |
| 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 |
| 81234 | DMPK (DM1 protein kinase) (e.g., myotonic dystrophy type 1) gene analysis; evaluation to detect abnormal (expanded) alleles |
| 81238 | F9 (coagulation factor IX) (e.g., hemophilia B), full gene sequence |
| 81239 | DMPK (DM1 protein kinase) (e.g., myotonic dystrophy type 1) gene analysis; characterization of alleles (e.g., expanded size) |

| CPT Code | Description |
|---|---|
| **Non-Covered** | |
| 81242 | FANCC (Fanconi anemia, complementation group C) (e.g., Fanconi anemia, type C) gene analysis, common variant (e.g., IVS4+4A>T) |
| 81243 | FMR1 (fragile X messenger ribonucleoprotein 1) (e.g., fragile X syndrome, X-linked intellectual disability [XLID]) gene analysis; evaluation to detect abnormal (e.g., expanded) alleles |
| 81244 | FMR1 (fragile X messenger ribonucleoprotein 1) (e.g., fragile X syndrome, X-linked intellectual disability [XLID]) gene analysis; characterization of alleles (e.g., expanded size and promoter methylation status) |
| 81248 | G6PD (glucose - 6 - phosphate dehydrogenase) (e.g., hemolytic anemia, jaundice), gene analysis; known familial variant(s) |
| 81249 | G6PD (glucose - 6 - phosphate dehydrogenase) (e.g., hemolytic anemia, jaundice), gene analysis; full gene sequence |
| 81257 | HBA1/HBA2 (alpha globin 1 and alpha globin 2) (e.g., alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis; common deletions or variant (e.g., Southeast Asian, Thai, Filipino, Mediterranean, alpha3.7, alpha4.2, alpha20.5, Constant Spring) |
| 81258 | HBA1/HBA2 (alpha globin 1 and alpha globin 2) (e.g., alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis; known familial variant |
| 81259 | HBA1/HBA2 (alpha globin 1 and alpha globin 2) (e.g., alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis; full gene sequence |
| 81260 | IKBKAP (inhibitor of kappa light polypeptide gene enhancer in B - cells, kinase complex - associated protein) (e.g., familial dysautonomia) gene analysis, common variants (e.g., 2507+6T>C, R696P) |
| 81271 | HTT (huntingtin) (e.g., Huntington disease) gene analysis; evaluation to detect abnormal (e.g., expanded) alleles |
| 81274 | HTT (huntingtin) (e.g., Huntington disease) gene analysis; characterization of alleles (e.g., expanded size) |
| 81284 | FXN (frataxin) (e.g., Friedreich ataxia) gene analysis; evaluation to detect abnormal (expanded) alleles |
| 81285 | FXN (frataxin) (e.g., Friedreich ataxia) gene analysis; characterization of alleles (e.g., expanded size) |
| 81286 | FXN (frataxin) (e.g., Friedreich ataxia) gene analysis; full gene sequence |
| 81289 | FXN (frataxin) (e.g., Friedreich ataxia) gene analysis; known familial variant(s) |
| 81290 | MCOLN1 (mucolipin 1) (e.g., Mucolipidosis, type IV) gene analysis, common variants (e.g., IVS3 - 2A>G, del6.4kb) |
| 81291 | MTHFR (5,10 - methylenetetrahydrofolate reductase) (e.g., hereditary hypercoagulability) gene analysis, common variants (e.g., 677T, 1298C) |
| 81302 | MECP2 (methyl CpG binding protein 2) (e.g., Rett syndrome) gene analysis; full sequence analysis |
| 81303 | MECP2 (methyl CpG binding protein 2) (e.g., Rett syndrome) gene analysis; known familial variant |
| 81304 | MECP2 (methyl CpG binding protein 2) (e.g., Rett syndrome) gene analysis; duplication/deletion variants |
| 81324 | PMP22 (peripheral myelin protein 22) (e.g., Charcot - Marie - Tooth, hereditary neuropathy with liability to pressure palsies) gene analysis; duplication/deletion analysis |
| 81325 | PMP22 (peripheral myelin protein 22) (e.g., Charcot - Marie - Tooth, hereditary neuropathy with liability to pressure palsies) gene analysis; full sequence analysis |
| 81326 | PMP22 (peripheral myelin protein 22) (e.g., Charcot - Marie - Tooth, hereditary neuropathy with liability to pressure palsies) gene analysis; known familial variant |
| 81329 | SMN1 (survival of motor neuron 1, telomeric) (e.g., spinal muscular atrophy) gene analysis; dosage/deletion analysis (e.g., carrier testing), includes SMN2 (survival of motor neuron 2, centromeric) analysis, if performed |
| 81330 | SMPD1(sphingomyelin phosphodiesterase 1, acid lysosomal) (e.g., Niemann - Pick disease, Type A) gene analysis, common variants (e.g., R496L, L302P, fsP330) |

| CPT Code | Description |
|---|---|
| **Non-Covered** | |
| 81331 | SNRPN/UBE3A (small nuclear ribonucleoprotein polypeptide N and ubiquitin protein ligase E3A) (e.g., Prader - Willi syndrome and/or Angelman syndrome), methylation analysis |
| 81336 | SMN1 (survival of motor neuron 1, telomeric) (e.g., spinal muscular atrophy) gene analysis; full gene sequence |
| 81337 | SMN1 (survival of motor neuron 1, telomeric) (e.g., spinal muscular atrophy) gene analysis; known familial sequence variant(s) |
| 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 |
| 81361 | HBB (hemoglobin, subunit beta) (e.g., sickle cell anemia, beta thalassemia, hemoglobinopathy); common variant(s) (e.g., HbS, HbC, HbE) |
| 81362 | HBB (hemoglobin, subunit beta) (e.g., sickle cell anemia, beta thalassemia, hemoglobinopathy); known familial variant(s) |
| 81363 | HBB (hemoglobin, subunit beta) (e.g., sickle cell anemia, beta thalassemia, hemoglobinopathy); duplication/deletion variant(s) |
| 81364 | HBB (hemoglobin, subunit beta) (e.g., sickle cell anemia, beta thalassemia, hemoglobinopathy); full gene sequence |
| 81412 | Ashkenazi Jewish associated disorders (e.g., 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 |
| 81420 | Fetal chromosomal aneuploidy (e.g., 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 (e.g., DiGeorge syndrome, Cri - du - chat syndrome), circulating cell - free fetal DNA in maternal blood |
| 81425 | Genome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis |
| 81426 | Genome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator genome (e.g., parents, siblings) (List separately in addition to code for primary procedure) |
| 81427 | Genome (e.g., unexplained constitutional or heritable disorder or syndrome); re - evaluation of previously obtained genome sequence (e.g., updated knowledge or unrelated condition/syndrome) |
| 81430 | Hearing loss (e.g., non - syndromic 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 (e.g., non - syndromic 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 |
| 81434 | Hereditary retinal disorders (e.g., 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 |
| 81440 | Nuclear encoded mitochondrial genes (e.g., 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 |

| CPT Code | Description |
|---|---|
| **Non-Covered** | |
| 81443 | Genetic testing for severe inherited conditions (e.g., cystic fibrosis, Ashkenazi Jewish - associated disorders [e.g., 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 (e.g., ACADM, ARSA, ASPA, ATP7B, BCKDHA, BCKDHB, BLM, CFTR, DHCR7, FANCC, G6PC, GAA, GALT, GBA, GBE1, HBB, HEXA, IKBKAP, MCOLN1, PAH) |
| 81448 | Hereditary peripheral neuropathies (e.g., Charcot-Marie-Tooth, spastic paraplegia), genomic sequence analysis panel, must include sequencing of at least 5 peripheral neuropathy-related genes (e.g., BSCL2, GJB1, MFN2, MPZ, REEP1, SPAST, SPG11, SPTLC1) |
| 81460 | Whole mitochondrial genome (e.g., 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 (e.g., Kearns - Sayre syndrome, chronic progressive external ophthalmoplegia), including heteroplasmy detection, if performed |
| 81470 | X - linked intellectual disability (XLID) (e.g., 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) (e.g., 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 |
| 81500 | Oncology (ovarian), biochemical assays of two proteins (CA - 125 and HE4), utilizing serum, with menopausal status, algorithm reported as a risk score (ROMA™) |
| 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 |
| **Provisional Coverage** | |
| 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 (ThyraMIR™) |
| 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") (Thyroseq®) |
| 0027U | JAK2 (Janus kinase 2) (e.g., myeloproliferative disorder) gene analysis, targeted sequence analysis exons 12-15 |
| 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 (Oncotype DX® Breast DCIS Score™) |
| 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 (Genomic Prostate Score® (GPS)) |
| 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 (BDX-XL2) |
| 0089U | Oncology (melanoma), gene expression profiling by RTqPCR, PRAME and LINC00518, superficial collection using adhesive patch(es) (Pigmented Lesion Assay (PLA)) |
| 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) (myPath® Melanoma) |

| CPT Code | Description |
|---|---|
| **Provisional Coverage** | |
| 0155U | Oncology (breast cancer), DNA, PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha) (e.g., breast cancer) gene analysis (i.e., 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 |
| 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 (ThyGeNEXT®) |
| 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) (ThyroSeq®) |
| 0288U | Oncology (lung), mRNA, quantitative PCR analysis of 11 genes (BAG1, BRCA1, CDC6, CDK2AP1, ERBB3, FUT3, IL11, LCK, RND3, SH3BGR, WNT3A) and 3 reference genes (ESD, TBP, YAP1), formalin-fixed paraffin-embedded (FFPE) tumor tissue, algorithmic interpretation reported as a recurrence risk score (DetermaRx™) |
| 81170 | ABL1 (ABL proto - oncogene 1, non - receptor tyrosine kinase) (e.g., acquired imatinib tyrosine kinase inhibitor resistance), gene analysis, variants in the kinase domain |
| 81175 | ASXL1 (additional sex combs like 1, transcriptional regulator) (e.g., myelodysplastic syndrome, myeloproliferative neoplasms, chronic myelomonocytic leukemia), gene analysis; full gene sequence |
| 81176 | ASXL1 (additional sex combs like 1, transcriptional regulator) (e.g., myelodysplastic syndrome, myeloproliferative neoplasms, chronic myelomonocytic leukemia), gene analysis; targeted sequence analysis (e.g., exon 12) |
| 81272 | KIT (v - kit Hardy - Zuckerman 4 feline sarcoma viral oncogene homolog) (e.g., gastrointestinal stromal tumor [GIST], acute myeloid leukemia, melanoma), gene analysis, targeted sequence analysis (e.g., exons 8, 11, 13, 17, 18) |
| 81279 | JAK2 (Janus kinase 2) (e.g., myeloproliferative disorder) targeted sequence analysis (e.g., exons 12 and 13) |
| 81309 | PIK3CA (phosphatidylinositol - 4, 5 - biphosphate 3 - kinase, catalytic subunit alpha) (e.g., colorectal and breast cancer) gene analysis, targeted sequence analysis (e.g., exons 7, 9, 20) |
| 81314 | PDGFRA (platelet - derived growth factor receptor, alpha polypeptide) (e.g., gastrointestinal stromal tumor [GIST]), gene analysis, targeted sequence analysis (e.g., exons 12, 18) |
| 81338 | MPL (MPL proto-oncogene, thrombopoietin receptor) (e.g., myeloproliferative disorder) gene analysis; common variants (e.g., W515A, W515K, W515L, W515R) |
| 81339 | MPL (MPL proto-oncogene, thrombopoietin receptor) (e.g., myeloproliferative disorder) gene analysis; sequence analysis, exon 10 |
| 81345 | TERT (telomerase reverse transcriptase) (e.g., thyroid carcinoma, glioblastoma multiforme) gene analysis, targeted sequence analysis (e.g., promoter region) |
| 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 (Oncotype DX® Breast Cancer Assay) |
| 81546 | Oncology (thyroid), mRNA, gene expression analysis of 10,196 genes, utilizing fine needle aspirate, algorithm reported as a categorical result (e.g., benign or suspicious) (Afirma®) |
| 88120 | Cytopathology, in situ hybridization (e.g., FISH), urinary tract specimen with morphometric analysis, 3 - 5 molecular probes, each specimen; manual (UroVysion™) |
| 88121 | Cytopathology, in situ hybridization (e.g., FISH), urinary tract specimen with morphometric analysis, 3 - 5 molecular probes, each specimen; using computer - assisted technology (UroVysion™) |

| Diagnosis Codes |
|---|
| Refer to Molecular Pathology/Molecular Diagnostics/Genetic Testing: Diagnosis Code List for diagnosis codes for CPT codes 0018U, 0026U, 0027U, 0045U, 0047U, 0080U, 0089U, 0090U, 0155U, 0245U, 0287U, 0288U, 81170, 81175, 81176, 81272, 81279, 81309, 81314, 81338, 81339, 81345, 81519, 81546, 88120, and 88121. |

# Centers for Medicare and Medicaid Services (CMS) Related Documents

After checking the table below and searching the Medicare Coverage Database, if no NCD, LCD, or LCA is found, refer to the criteria as noted in the Coverage Rationale section above.

## Molecular Pathology/Molecular Diagnostics/Genetic Testing

| NCD | LCD | LCA | Contractor Type | Contractor Name |
|---|---|---|---|---|
| **ABL1 Kinase Domain** | | | | |
| N/A | L35396 Biomarkers for Oncology | A52986 Billing and Coding: Biomarkers for Oncology | Part A and B MAC | Novitas |
| N/A | L36117 MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease | A54686 Billing and Coding: MolDX: BCR-ABL | Part A and B MAC | CGS |
| N/A | L36180 MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease | A55595 Billing and Coding: MolDX: BCR-ABL | Part A and B MAC | Noridian |
| N/A | L36186 MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease | A55600 Billing and Coding: MolDX: BCR-ABL | Part A and B MAC | Noridian |
| N/A | L36044 MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease | A53531 Billing and Coding: MolDX: BCR-ABL | Part A and B MAC | Palmetto |
| N/A | L36815 MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease | A55233 Billing and Coding: MolDx: BCR-ABL | Part A and B MAC | WPS |
| N/A | L35000 Molecular Pathology Procedures | A56199 Billing and Coding: Molecular Pathology Procedures | Part A and B MAC | NGS |
| **AFF2, AR, ASPA, BCKDHB, Cytogenomic (Genome-Wide) Analysis for Constitutional Chromosomal Abnormalities, DMPK, F9, FANCC, FMR1, FXN, G6PD, HBA1/HBA2, HBB, HTT, MCOLN1, PMP22, SMN1, SMPD1, SNRPN/UBE3A** | | | | |
| N/A | N/A | A58918 Billing and Coding: Molecular Pathology and Genetic Testing | Part A and B MAC | First Coast |
| N/A | L35062 Biomarkers Overview | A58917 Billing and Coding: Molecular Pathology and Genetic Testing | Part A and B MAC | Novitas |
| N/A | L34519 Molecular Pathology Procedures | A57451 Billing and Coding: Molecular Pathology Procedures | Part A and B MAC | First Coast |

| NCD | LCD | LCA | Contractor Type | Contractor Name |
|---|---|---|---|---|
| **Afirma®** | | | | |
| N/A | L35000 Molecular Pathology Procedures | A56199 Billing and Coding: Molecular Pathology Procedures | Part A and B MAC | NGS |
| N/A | L35396 Biomarkers for Oncology | A52986 Billing and Coding: Biomarkers for Oncology | Part A and B MAC | Novitas |
| N/A | L36021 MolDX: Molecular Diagnostic Tests (MDT) | A54185 Billing and Coding: MolDX: Afirma™ Assay by Veracyte Update | Part A and B MAC | CGS |
| N/A | L39650 MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules | A59474 Billing and Coding: MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules | Part A and B MAC | CGS |
| N/A | L39682 MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules | A59509 Billing and Coding: MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules | Part A and B MAC | Noridian |
| N/A | L39684 MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules | A59511 Billing and Coding: MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules | Part A and B MAC | Noridian |
| N/A | L39646 MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules | A59470 Billing and Coding: MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules | Part A and B MAC | Palmetto |
| N/A | L39720 MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules | A59560 Billing and Coding: MolDX: Molecular Testing for Risk Stratification of Thyroid Nodules | Part A and B MAC | WPS* |
| N/A | L38968 Thyroid Nodule Molecular Testing | A58656 Billing and Coding: Thyroid Nodule Molecular Testing | Part A and B MAC | NGS |
| **Ashkenazi Jewish Associated Disorders Carrier Screening Panel** | | | | |
| N/A | L36021 MolDX: Molecular Diagnostic Tests (MDT) | A54270 Billing and Coding: MolDX: IKBKAP Genetic Testing | Part A and B MAC | CGS |
| N/A | N/A | A58918 Billing and Coding: Molecular Pathology and Genetic Testing | Part A and B MAC | First Coast |
| N/A | L35062 Biomarkers Overview | A58917 Billing and Coding: Molecular Pathology and Genetic Testing | Part A and B MAC | Novitas |
| N/A | L35000 Molecular Pathology Procedures | A56199 Billing and Coding: Molecular Pathology Procedures | Part A and B MAC | NGS |

| NCD | LCD | LCA | Contractor Type | Contractor Name |
|---|---|---|---|---|
| **ASXL1, KIT, PDGFRA, TERT** | | | | |
| N/A | L35396 Biomarkers for Oncology | A52986 Billing and Coding: Biomarkers for Oncology | Part A and B MAC | Novitas |
| N/A | L35000 Molecular Pathology Procedures | A56199 Billing and Coding: Molecular Pathology Procedures | Part A and B MAC | NGS |
| **BDX-XL2** | | | | |
| N/A | L37054 BDX-XL2 | A57356 Billing and Coding: BDX-XL2 | Part A and B MAC | Noridian |
| N/A | L37062 BDX-XL2 | A57357 Billing and Coding: BDX-XL2 | Part A and B MAC | Noridian |
| N/A | L37031 BDX-XL2 | A56929 Billing and Coding: BDX-XL2 | Part A and B MAC | Palmetto |
| N/A | L37216 BDX-XL2 | A57558 Billing and Coding: BDX-XL2 | Part A and B MAC | WPS* |
| **DecisionDx®-SCC** | | | | |
| N/A | L39585 MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma | A59382 Billing and Coding: MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma | Part A and B MAC | CGS |
| N/A | L39589 MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma | A59386 Billing and Coding: MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma | Part A and B MAC | Noridian |
| N/A | L39594 MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma | A59401 Billing and Coding: MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma | Part A and B MAC | Noridian |
| N/A | L39583 MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma | A59380 Billing and Coding: MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma | Part A and B MAC | Palmetto |
| N/A | L39614 MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma | A59429 Billing and Coding: MolDX: Molecular Biomarker Testing for Risk Stratification of Cutaneous Squamous Cell Carcinoma | Part A and B MAC | WPS* |
| **DetermaRx™** | | | | |
| N/A | L38284 MolDX: Predictive Classifiers for Early Stage Non-small Cell Lung Cancer | A58038 Billing and Coding: MolDX: Predictive Classifiers for Early Stage Non-Small Cell Lung Cancer | Part A and B MAC | CGS |

| NCD | LCD | LCA | Contractor Type | Contractor Name |
|---|---|---|---|---|
| **DetermaRx™** | | | | |
| N/A | L38327 MolDX: Predictive Classifiers for Early Stage Non-Small Cell Lung Cancer | A57329 Billing and Coding: MolDX: Predictive Classifiers for Early Stage Non-Small Cell Lung Cancer | Part A and B MAC | Noridian |
| N/A | L38329 MolDX: Predictive Classifiers for Early Stage Non-Small Cell Lung Cancer | A57330 Billing and Coding: MolDX: Predictive Classifiers for Early Stage Non-Small Cell Lung Cancer | Part A and B MAC | Noridian |
| N/A | L38238 MolDX: Predictive Classifiers for Early Stage Non-Small Cell Lung Cancer | A58031 Billing and Coding: MolDX: Predictive Classifiers for Early Stage Non-Small Cell Lung Cancer | Part A and B MAC | Palmetto |
| N/A | L38443 MolDX: Predictive Classifiers for Early Stage Non-Small Cell Lung Cancer | A57112 Billing and Coding: MolDX: Predictive Classifiers for Early Stage Non-Small Cell Lung Cancer | Part A and B MAC | WPS* |
| **Fetal Chromosomal Aneuploidy and Microdeletions, Genome Sequencing (unexplained constitutional or heritable disorder or syndrome), Hearing loss Panel, Hereditary Retinal Disorders Panel, Hereditary Peripheral Neuropathies Panel, Nuclear Encoded Mitochondrial Genes Panel, Whole Mitochondrial Genome** | | | | |
| N/A | N/A | A58918 Billing and Coding: Molecular Pathology and Genetic Testing | Part A and B MAC | First Coast |
| N/A | L35062 Biomarkers Overview | A58917 Billing and Coding: Molecular Pathology and Genetic Testing | Part A and B MAC | Novitas |
| N/A | L35000 Molecular Pathology Procedures | A56199 Billing and Coding: Molecular Pathology Procedures | Part A and B MAC | NGS |
| **Genomic Prostate Score® (GPS) Test** | | | | |
| N/A | N/A | A56372 Billing and Coding: MolDX: Oncotype DX® Genomic Prostate Score | Part A and B MAC | Noridian |
| N/A | L38303 MolDX: Prostate Cancer Genomic Classifier Assay for Men with Localized Disease | A58371 Billing and Coding: MolDX: Prostate Cancer Genomic Classifier Assay for Men with Localized Disease | Part A and B MAC | CGS |
| N/A | L38341 MolDX: Prostate Cancer Genomic Classifier Assay for Men with Localized Disease | A57236 Billing and Coding: MolDX: Prostate Cancer Genomic Classifier Assay for Men with Localized Disease | Part A and B MAC | Noridian |

| NCD | LCD | LCA | Contractor Type | Contractor Name |
|---|---|---|---|---|
| **Genomic Prostate Score® (GPS) Test** | | | | |
| N/A | L38339 MolDX: Prostate Cancer Genomic Classifier Assay for Men with Localized Disease | A57372 Billing and Coding: MolDX: Prostate Cancer Genomic Classifier Assay for Men with Localized Disease | Part A and B MAC | Noridian |
| N/A | L38292 MolDX: Prostate Cancer Genomic Classifier Assay for Men with Localized Disease | A58343 Billing and Coding: MolDX: Prostate Cancer Genomic Classifier Assay for Men with Localized Disease | Part A and B MAC | Palmetto |
| N/A | L38433 MolDX: Prostate Cancer Genomic Classifier Assay for Men with Localized Disease | A57106 Billing and Coding: MolDX: Prostate Cancer Genomic Classifier Assay for Men with Localized Disease | Part A and B MAC | WPS* |
| **Genetic Testing for Severe Inherited Conditions Carrier Screening Panel, IKBKAP** | | | | |
| N/A | L36021 MolDX: Molecular Diagnostic Tests (MDT) | A54270 Billing and Coding: MolDX: IKBKAP Genetic Testing | Part A and B MAC | CGS |
| N/A | N/A | A58918 Billing and Coding: Molecular Pathology and Genetic Testing | Part A and B MAC | First Coast |
| N/A | L35062 Biomarkers Overview | A58917 Billing and Coding: Molecular Pathology and Genetic Testing | Part A and B MAC | Novitas |
| N/A | L34519 Molecular Pathology Procedures | A57451 Billing and Coding: Molecular Pathology Procedures | Part A and B MAC | First Coast |
| N/A | L35000 Molecular Pathology Procedures | A56199 Billing and Coding: Molecular Pathology Procedures | Part A and B MAC | NGS |
| **JAK2, MPL** | | | | |
| N/A | L36117 MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease | A56999 Billing and Coding: MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease | Part A and B MAC | CGS |
| N/A | L36180 MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease | A57421 Billing and Coding: MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease | Part A and B MAC | Noridian |
| N/A | L36186 MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease | A57422 Billing and Coding: MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease | Part A and B MAC | Noridian |

| NCD | LCD | LCA | Contractor Type | Contractor Name |
|---|---|---|---|---|
| **JAK2, MPL** | | | | |
| N/A | L36044 MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease | A56959 Billing and Coding: MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease | Part A and B MAC | Palmetto |
| N/A | L36815 MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease | A57570 Billing and Coding: MolDX: Genetic Testing for BCR-ABL Negative Myeloproliferative Disease | Part A and B MAC | WPS* |
| N/A | L35000 Molecular Pathology Procedures | A56199 Billing and Coding: Molecular Pathology Procedures | Part A and B MAC | NGS |
| **MECP2** | | | | |
| N/A | L36021 MolDX: Molecular Diagnostic Tests (MDT) | A54278 Billing and Coding: MolDX: MECP2 Genetic Testing | Part A and B MAC | CGS |
| N/A | N/A | A58918 Billing and Coding: Molecular Pathology and Genetic Testing | Part A and B MAC | First Coast |
| N/A | L35062 Biomarkers Overview | A58917 Billing and Coding: Molecular Pathology and Genetic Testing | Part A and B MAC | Novitas |
| N/A | L34519 Molecular Pathology Procedures | A57451 Billing and Coding: Molecular Pathology Procedures | Part A and B MAC | First Coast |
| N/A | L35000 Molecular Pathology Procedures | A56199 Billing and Coding: Molecular Pathology Procedures | Part A and B MAC | NGS |
| **MTHFR** | | | | |
| N/A | L35984 MolDX: Genetic Testing for Hypercoagulability / Thrombophilia (Factor V Leiden, Factor II Prothrombin, and MTHFR) | A56980 Billing and Coding: MolDX: Genetic Testing for Hypercoagulability / Thrombophilia (Factor V Leiden, Factor II Prothrombin, and MTHFR) | Part A and B MAC | CGS |
| N/A | L36155 MolDX: Genetic Testing for Hypercoagulability / Thrombophilia (Factor V Leiden, Factor II Prothrombin, and MTHFR) | A57423 Billing and Coding: MolDX: Genetic Testing for Hypercoagulability / Thrombophilia (Factor V Leiden, Factor II Prothrombin, and MTHFR) | Part A and B MAC | Noridian |
| N/A | L36159 MolDX: Genetic Testing for Hypercoagulability / Thrombophilia (Factor V Leiden, Factor II Prothrombin, and MTHFR) | A57424 Billing and Coding: MolDX: Genetic Testing for Hypercoagulability / Thrombophilia (Factor V Leiden, Factor II Prothrombin, and MTHFR) | Part A and B MAC | Noridian |

| NCD | LCD | LCA | Contractor Type | Contractor Name |
|---|---|---|---|---|
| **MTHFR** | | | | |
| N/A | L36089 MolDX: Genetic Testing for Hypercoagulability/Thrombophilia (Factor V Leiden, Factor II Prothrombin, and MTHFR) | A56899 Billing and Coding: MolDX: Genetic Testing for Hypercoagulability/Thrombophilia (Factor V Leiden, Factor II Prothrombin, and MTHFR) | Part A and B MAC | Palmetto |
| N/A | L36400 MolDX: Genetic Testing for Hypercoagulability/Thrombophilia (Factor V Leiden, Factor II Prothrombin, and MTHFR) | A57571 Billing and Coding: MolDX: Genetic Testing for Hypercoagulability/Thrombophilia (Factor V Leiden, Factor II Prothrombin, and MTHFR) | Part A and B MAC | WPS* |
| N/A | L35000 Molecular Pathology Procedures | A56199 Billing and Coding: Molecular Pathology Procedures | Part A and B MAC | NGS |
| **myPath® Melanoma** | | | | |
| N/A | L39389 MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma | A59163 Billing and Coding: MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma | Part A and B MAC | CGS |
| N/A | L39373 MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma | A59179 Billing and Coding: MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma | Part A and B MAC | Noridian |
| N/A | L39375 MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma | A59181 Billing and Coding: MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma | Part A and B MAC | Noridian |
| N/A | L39345 MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma | A59109 Billing and Coding: MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma | Part A and B MAC | Palmetto |
| N/A | L39479 MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma | A59261 Billing and Coding: MolDX: Molecular Assays for the Diagnosis of Cutaneous Melanoma | Part A and B MAC | WPS* |
| **Oncotype DX® Breast Cancer Assay** | | | | |
| N/A | L36021 MolDX: Molecular Diagnostic Tests (MDT) | A54195 Billing and Coding: MolDX: Oncotype DX® Breast Cancer Assay | Part A and B MAC | CGS |
| N/A | L35160 MolDX: Molecular Diagnostic Tests (MDT) | A54480 Billing and Coding: MolDX: Oncotype DX® Breast Cancer Assay | Part A and B MAC | Noridian |
| N/A | L36256 MolDX: Molecular Diagnostic Tests (MDT) | A54482 Billing and Coding: MolDX: Oncotype DX® Breast Cancer Assay | Part A and B MAC | Noridian |
| N/A | L35025 MolDX: Molecular Diagnostic Tests (MDT) | A53105 Billing and Coding: MolDX: Oncotype DX® Breast Cancer Assay | Part A and B MAC | Palmetto |

| NCD | LCD | LCA | Contractor Type | Contractor Name |
|---|---|---|---|---|
| **Oncotype DX® Breast Cancer Assay** | | | | |
| N/A | L36807 MolDX: Molecular Diagnostic Tests (MDT) | A55230 Billing and Coding: MolDX: Oncotype DX® Breast Cancer Assay | Part A and B MAC | WPS* |
| N/A | L35000 Molecular Pathology Procedures | A56199 Billing and Coding: Molecular Pathology Procedures | Part A and B MAC | NGS |
| N/A | L36951 MolDX: Oncotype DX® Breast Cancer for DCIS (Genomic Health™) | A56887 Billing and Coding: MolDX: Oncotype DX® Breast Cancer for DCIS (Genomic Health™) | Part A and B MAC | CGS |
| N/A | L36941 MolDX: Oncotype DX® Breast Cancer for DCIS (Genomic Health™) | A57619 Billing and Coding: MolDX: Oncotype DX® Breast Cancer for DCIS (Genomic Health™) | Part A and B MAC | Noridian |
| **Oncotype DX® Breast DCIS Score™ Test** | | | | |
| N/A | L36947 MolDX: Oncotype DX® Breast Cancer for DCIS (Genomic Health™) | A57620 Billing and Coding: MolDX: Oncotype DX® Breast Cancer for DCIS (Genomic Health™) | Part A and B MAC | Noridian |
| N/A | L36912 MolDX: Oncotype DX® Breast Cancer for DCIS (Genomic Health™) | A56870 Billing and Coding: MolDX: Oncotype DX® Breast Cancer for DCIS (Genomic Health™) | Part A and B MAC | Palmetto |
| N/A | L37199 MolDX: Oncotype DX® Breast Cancer for DCIS (Genomic Health™) | A57583 Billing and Coding: MolDX: Oncotype DX® Breast Cancer for DCIS (Genomic Health™) | Part A and B MAC | WPS* |
| **OVERA®** | | | | |
| N/A | L38371 Multimarker Serum Tests Related to Ovarian Cancer Testing | A57020 Billing and Coding: Multimarker Serum Tests Related to Ovarian Cancer Testing | Part A and B MAC | NGS |
| **Pigmented Lesion Assay (PLA)** | | | | |
| N/A | L38111 MolDX: Pigmented Lesion Assay | A57915 Billing and Coding: MolDX: Pigmented Lesion Assay | Part A and B MAC | CGS |
| N/A | L38151 MolDX: Pigmented Lesion Assay | A58052 Billing and Coding: MolDX: Pigmented Lesion Assay | Part A and B MAC | Noridian |
| N/A | L38153 MolDX: Pigmented Lesion Assay | A58053 Billing and Coding: MolDX: Pigmented Lesion Assay | Part A and B MAC | Noridian |
| N/A | L38051 MolDX: Pigmented Lesion Assay | A57868 Billing and Coding: MolDX: Pigmented Lesion Assay | Part A and B MAC | Palmetto |
| N/A | L38178 MolDX: Pigmented Lesion Assay | A57983 Billing and Coding: MolDX: Pigmented Lesion Assay | Part A and B MAC | WPS* |
| **PIK3CA** | | | | |
| N/A | L36021 MolDX: Molecular Diagnostic Tests (MDT) | A54295 Billing and Coding: MolDX: PIK3CA Gene Tests | Part A and B MAC | CGS |

| NCD | LCD | LCA | Contractor Type | Contractor Name |
|---|---|---|---|---|
| **PIK3CA** | | | | |
| N/A | N/A | A55597 Billing and Coding: MolDX: PIK3CA Gene Tests | Part A and B MAC | Noridian |
| N/A | N/A | A55602 Billing and Coding: MolDX: PIK3CA Gene Tests | Part A and B MAC | Noridian |
| N/A | L35025 MolDX: Molecular Diagnostic Tests (MDT) | A53558 Billing and Coding: MolDX: PIK3CA Gene Tests | Part A and B MAC | Palmetto |
| N/A | L36807 MolDX: Molecular Diagnostic Tests (MDT) | A55200 Billing and Coding: MolDX: PIK3CA Gene Tests | Part A and B MAC | WPS* |
| N/A | L35000 Molecular Pathology Procedures | A56199 Billing and Coding: Molecular Pathology Procedures | Part A and B MAC | NGS |
| **Resolution ctDx Lung™** | | | | |
| N/A | L38065 MolDX: Plasma-Based Genomic Profiling in Solid Tumors | A57917 Billing and Coding: MolDX: Plasma-Based Genomic Profiling in Solid Tumors | Part A and B MAC | CGS |
| N/A | L39230 MolDX: Plasma-Based Genomic Profiling in Solid Tumors | A58973 Billing and Coding: MolDX: Plasma-Based Genomic Profiling in Solid Tumors | Part A and B MAC | Noridian |
| N/A | L39232 MolDX: Plasma-Based Genomic Profiling in Solid Tumors | A58975 Billing and Coding: MolDX: Plasma-Based Genomic Profiling in Solid Tumors | Part A and B MAC | Noridian |
| N/A | L38043 MolDX: Plasma-Based Genomic Profiling in Solid Tumors | A57867 Billing and Coding: MolDX: Plasma-Based Genomic Profiling in Solid Tumors | Part A and B MAC | Palmetto |
| N/A | L38168 MolDX: Plasma-Based Genomic Profiling in Solid Tumors | A57936 Billing and Coding: MolDX: Plasma-Based Genomic Profiling in Solid Tumors | Part A and B MAC | WPS* |
| **ROMA™** | | | | |
| N/A | L35000 Molecular Pathology Procedures | A56199 Billing and Coding: Molecular Pathology Procedures | Part A and B MAC | NGS |
| N/A | L38371 Multimarker Serum Tests Related to Ovarian Cancer Testing | A57020 Billing and Coding: Multimarker Serum Tests Related to Ovarian Cancer Testing | Part A and B MAC | NGS |
| **ThyraMIR™, ThyroSeq®** | | | | |
| N/A | L35396 Biomarkers for Oncology | A52986 Billing and Coding: Biomarkers for Oncology | Part A and B MAC | Novitas |
| N/A | L38968 Thyroid Nodule Molecular Testing | A58656 Billing and Coding: Thyroid Nodule Molecular Testing | Part A and B MAC | NGS |

| NCD | LCD | LCA | Contractor Type | Contractor Name |
|---|---|---|---|---|
| **ThyGeNEXT®** | | | | |
| N/A | L35396 Biomarkers for Oncology | A52986 Billing and Coding: Biomarkers for Oncology | Part A and B MAC | Novitas |
| **UroVysion™** | | | | |
| N/A | L35396 Biomarkers for Oncology | A52986 Billing and Coding: Biomarkers for Oncology | Part A and B MAC | Novitas |
| N/A | L36975 Bladder/Urothelial Tumor Markers | A56471 Billing and Coding: Bladder/Urothelial Tumor Markers | Part A and B MAC | CGS |
| N/A | L36678 Lab: Bladder/Urothelial Tumor Markers | A55028 Billing and Coding: Lab: Bladder/Urothelial Tumor Markers | Part A and B MAC | Noridian |
| N/A | L36680 Lab: Bladder/Urothelial Tumor Markers | A55029 Billing and Coding: Lab: Bladder/Urothelial Tumor Markers | Part A and B MAC | Noridian |
| N/A | L33420 Lab: Bladder/Urothelial Tumor Markers | A53095 Billing and Coding: Lab: Bladder/Urothelial Tumor Markers | Part A and B MAC | Palmetto |
| **X-Linked intellectual disability (XLID) Panel** | | | | |
| N/A | L36021 MolDX: Molecular Diagnostic Tests (MDT) | A54274 Billing and Coding: MolDX: L1CAM Gene Sequencing Guidelines | Part A and B MAC | CGS |
| N/A | L36021 MolDX: Molecular Diagnostic Tests (MDT) | A54278 Billing and Coding: MolDX: MECP2 Genetic Testing | Part A and B MAC | CGS |
| N/A | N/A | A58918 Billing and Coding: Molecular Pathology and Genetic Testing | Part A and B MAC | First Coast |
| N/A | L35062 Biomarkers Overview | A58917 Billing and Coding: Molecular Pathology and Genetic Testing | Part A and B MAC | Novitas |
| N/A | L35000 Molecular Pathology Procedures | A56199 Billing and Coding: Molecular Pathology Procedures | Part A and B MAC | NGS |

| Medicare Administrative Contractor (MAC) With Corresponding States/Territories | |
|---|---|
| **MAC Name (Abbreviation)** | **States/Territories** |
| CGS Administrators, LLC (CGS) | KY, OH |
| First Coast Service Options, Inc. (First Coast) | FL, PR, VI |
| National Government Services, Inc. (NGS) | CT, IL, ME, MA, MN, NH, NY, RI, VT, WI |
| Noridian Healthcare Solutions, LLC (Noridian) | AS, AK, AZ, CA, GU, HI, ID, MT, NV, ND, Northern Mariana Islands, OR, SD, UT, WA, WY |
| Novitas Solutions, Inc. (Novitas) | AR, CO, DC, DE, LA, MD, MS, NJ, NM, OK, PA, TX, VA** |

| | |
|---|---|
| Palmetto GBA (Palmetto) | AL, GA, NC, SC, TN, VA**, WV |
| Wisconsin Physicians Service Insurance Corporation (WPS)* | IA, IN, KS, MI, MO, NE |
| **Notes** | |
| *Wisconsin Physicians Service Insurance Corporation: Contract Number 05901 applies only to WPS Legacy Mutual of Omaha MAC A Providers.<br>**For the state of Virginia: Part B services for the city of Alexandria and the counties of Arlington and Fairfax are excluded for the Palmetto GBA jurisdiction and included within the Novitas Solutions, Inc. jurisdiction. | |

## CMS Benefit Policy Manual

Chapter 15; § 80.1–80.1.3 Clinical Laboratory Services

## CMS Claims Processing Manual

Chapter 12; § 60 Payment for Pathology Services
Chapter 16, § 10.2 General Explanation of Payment; § 20 Calculation of Payment Rates - Clinical Laboratory Test Fee Schedules; § 40 Billing for Clinical Laboratory Tests

## CMS Transmittal(s)

Transmittal 2439, Change Request 11655, Dated 02/21/2020 (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determination (NCDs)--July 2020 Update)
Transmittal 4481, Change Request 11574, Dated 12/20/2019 (Internet Only Manual Update to Pub 100-04, Chapter 16, Section 40.8 – Laboratory Date of Service Policy)
Transmittal 10092, Change Request 11749, Dated 05/01/2020 (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determination (NCDs)--October 2020 Update)
Transmittal 10193, Change Request 11655, Dated 06/19/2020 (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determination (NCDs)--July 2020 Update)
Transmittal 10261, Change Request 11905, Dated 07/31/2020 (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determination (NCDs)--January 2021 Update)
Transmittal 10346, Change Request 11837, Dated 09/11/2020 (National Coverage Determination (NCD 90.2): Next Generation Sequencing (NGS) for Medicare Beneficiaries with Germline (Inherited) Cancer)
Transmittal 10624, Change Request 12124, Dated 03/23/2021 (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determination (NCDs)--July 2021)
Transmittal 11055, Change Request 12483, Dated 10/21/2021 (National Coverage Determination (NCD) 90.2, Next Generation Sequencing (NGS))
Transmittal 11400, Change Request 12705, Dated 05/04/2022 (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determination (NCDs)--October 2022 Update)
Transmittal 11460, Change Request 12705, Dated 06/17/2022 (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determination (NCDs)--October 2022 Update)
Transmittal 11461, Change Request 12483, Dated 06/21/2022 (National Coverage Determination (NCD) 90.2, Next Generation Sequencing (NGS))
Transmittal 12184, Change Request 13278, Dated 08/03/2023, (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determinations (NCDs)--January 2024 Update)
Transmittal 12319, Change Request 13391, Dated 10/19/2023 (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determinations (NCDs)--April 2024 Update--CR 2 of 2
Transmittal 12350, Change Request 13391, Dated 11/03/2023, (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determinations (NCDs)-- April 2024 Update--CR 2 of 2)
Transmittal 12355, Change Request 13278, Dated 11/09/2023, (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determinations (NCDs)-- January 2024 Update)
Transmittal 12440, Change Request 13391, Dated 01/03/2024 (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determinations (NCDs)--April 2024 Update--CR 2 of 2)
Transmittal 12444, Change Request 13278, Dated 01/04/2024 (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determinations (NCDs)--January 2024 Update)
Transmittal 12626, Change Request 13596, Dated 05/09/2024 (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determinations (NCDs)--October 2024)

## Others

L35062 Biomarkers Overview

A56541 Billing and Coding: Biomarkers Overview
A58917 Billing and Coding: Molecular Pathology and Genetic Testing
A58918 Billing and Coding: Molecular Pathology and Genetic Testing
L36021 MolDX: Molecular Diagnostic Tests (MDT)
A56973 Billing and Coding: MolDX: Molecular Diagnostic Tests (MDT)
L35160 MolDX: Molecular Diagnostic Tests (MDT)
A57526 Billing and Coding: MolDX: Molecular Diagnostic Tests (MDT)
L36256 MolDX: Molecular Diagnostic Tests (MDT)
A57527 Billing and Coding: MolDX: Molecular Diagnostic Tests (MDT)
L35025 MolDX: Molecular Diagnostic Tests (MDT)
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L34519 Molecular Pathology Procedures
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L38288 MolDX: Repeat Germline Testing
A57141 Billing and Coding: MolDX: Repeat Germline Testing
L38351 MolDX: Repeat Germline Testing
A57331 Billing and Coding: MolDX: Repeat Germline Testing
L38353 MolDX: Repeat Germline Testing
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A59641 Billing and Coding: MolDX: Proteomics Testing
L36256 MolDX: Proteomics Testing
A59642 Billing and Coding: MolDX: Proteomics Testing
L35025 MolDX: Proteomics Testing
A59636 Billing and Coding: MolDX: Proteomics Testing
L36807 MolDX: Proteomics Testing
A59649 Billing and Coding: MolDX: Proteomics Testing
CMS Clinical Laboratory Fee Schedule, CMS Website
Code of Federal Regulations, Title 42 §410.32 Diagnostic x-ray tests, diagnostic laboratory tests, and other diagnostic tests: Conditions
Palmetto GBA MolDx Website
Palmetto GBA MolDx Manual, Palmetto GBA MolDx Website
Social Security Act, Title XVIII Section 1862(a)(1)(A)

# Clinical Evidence

## ABL1 (ABL Proto-Oncogene 1, Non-Receptor Tyrosine Kinase) Kinase Domain

The *ABL1* gene provides directions for making a protein involved in various functions in cells throughout the body. The ABL1 protein operates as a kinase, which is an enzyme that changes the activity of other proteins by adding a cluster of oxygen and phosphorus atoms (a phosphate group) at specific positions. The ABL1 kinase is normally turned off (inactive) and must be turned on (activated) to perform its tasks. The ABL1 kinase can be activated by a several various triggers and can add a phosphate group to many different proteins (also called substrates). This diversity allows *ABL1* to be involved in a wide variety of cellular processes, including cell growth and division (proliferation), maturation (differentiation), and movement (migration). It can either aid in cell survival or trigger-controlled cell death (apoptosis), depending on cellular conditions. The *ABL1* gene is a part of a class of genes known as oncogenes. When mutated,

oncogenes have the potential to cause normal cells to become cancerous. A genetic rearrangement (translocation) involving the *ABL1* gene induces a type of cancer of blood-forming cells called chronic myeloid leukemia. This slow-growing cancer leads to an overproduction of abnormal white blood cells (MedlinePlus, 2016a).

Branford et al. (2018) performed a clinical trial to study the genomic analysis of cancer-associated mutations at diagnosis of chronic myeloid leukemia (CML) patients with high-risk disease. 46 patients were included in the study. Cancer gene variants were found in 15 (56%) of 27 patients with subsequent blast crisis (BC) or poor outcome and in 3 (16%) of 19 optimal responders. 39 patients were tested at BC. All of them had cancer gene variants, including *ABL1* kinase domain mutations in 58%. However, *ABL1* mutations occurred simultaneously with other mutated cancer genes in 89% of cases, and these predated *ABL1* mutations in 62% of evaluable patients. The authors concluded that their genomic analysis findings discovered many relevant variants at diagnosis in patients with poor outcome and all patients at BC. Future refined biomarker testing of specific variants will likely offer prognostic information to aid in a risk-adapted therapeutic approach.

Liu et al. (2013) performed a study to determine if decreased microRNA-30a levels are correlated with enhanced ABL1 and BCR-ABL1 expression in chronic myeloid leukemia (CML). 16 patients with CML and 10 control subjects were included in the study. CML is correlated with overexpression of BCR-ABL1. MiR-30a targeted BCR-ABL1 and was under-expressed in bone marrow from CML patients. In K562 leukemia cells, overexpression of miR-30a decreased ABL1 and BCR-ABL1 protein expression, reduced proliferation, and arrested cell cycle progression between G1 and S. The authors concluded that the findings greatly suggest that miR-30a acts as a tumor suppressor by downregulating ABL1 and BCR-ABL1 expression. Upregulation of miR-30a in hematopoietic cells could have therapeutic efficacy against CML.

Chiaretti et al. (2007) performed a study to assess whether T-cell acute lymphoblastic leukemia (T-ALL) patients with overexpression of ABL demonstrated an abnormal gene expression pattern and were characterized by having specific rearrangements. The expression profile of 128 adults with ALL were previously evaluated by oligonucleotide arrays: 33 had T-ALL. In the current study, the expression levels of ABL1 in T-ALL cases were assessed and identified 3 patients who had ABL1 levels comparable to those detected in BCR/ABL + cases and 1 who had a notable greater level of ABL1 expression. In order to establish the incidence of ABL1 overexpression in TALL, 7 additional patients were tested by quantitative (Q)-polymerase chain reaction (PCR) and reverse transcription (RT)-PCR. The 3 cases with ABL1 expression levels comparable to those found in BCR/ABL + cases had a distinct signature characterized by a high expression of genes involved in regulation of transcription. The fourth case, with the greatest levels of ABL1, harbored the NUP214-ABL1 rearrangement, which was validated by fluorescence in situ hybridization (FISH). 3 out of the 4 patients were refractory to induction chemotherapy. Out of the 17 additional patients evaluated by Q-PCR and RT-PCR, none demonstrated ABL1 overexpression. The authors concluded that overall, overexpression of ABL1 was found in 8% of T-ALL cases. These results emphasize the value of microarray analyses in order to identify specific signatures associated with ABL1 overexpression, as well as rearrangements, e.g. NUP214-ABL1, in adult T-ALL.

## *Clinical Practice Guidelines*

### National Comprehensive Cancer Network (NCCN)

NCCN Guidelines for Chronic Myeloid Leukemia (v1.2025) make the following recommendations for the initial diagnosis and workup of Chronic Myeloid Leukemia:

- History & Physical, including palpation of the spleen.
- CBC with differential.
- Chemistry profile.
- Bone marrow aspirate and biopsy for morphologic and cytogenetic evaluation.
- Quantitative RT-PCR (qPCR) to establish the presence of quantifiable *BCR:ABL1.*
- Hepatitis B panel.

NCCN also provides the following treatment recommendations based on *BCR:ABL1* mutation profile:

- Patients with disease resistant to primary treatment with imatinib should be treated with a 2G TKI (bosutinib, dasatinib, or nilotinib) in the second-line setting, taking into account *BCR:ABL1* kinase domain mutation status.
- Patients with disease resistant to primary treatment with bosutinib, dasatinib, or nilotinib can be treated with an alternate TKI (other than imatinib), taking into account *BCR:ABL1* kinase domain mutation status. Subsequent therapy with an alternate 2G TKI would be effective only in patients with identifiable *BCR:ABL1* mutations that confer resistance to TKI therapy. Ponatinib is preferred for patients with no identifiable BCR*:ABL1* mutations.
    - Ponatinib is the preferred treatment option for patients with a *T315I* mutation in any phase. It is also a treatment option for CP-CML with resistance or intolerance to at least two prior TKIs or for patients with AP-CML or BP-CML for whom no other TKI is indicated.

- Asciminib is a treatment option for CP-CML patients with the *T315I* mutation and/or CP-CML with resistance or intolerance to at least two prior TKIs.

## AFF2 [ALF Transcription Elongation Factor 2 (FMR2)] [e.g., Fragile X Intellectual Disability 2 (FRAXE)]

Fragile XE syndrome is a genetic disorder caused by mutations in the *AFF2* gene. It impairs cognitive functioning and thinking ability. Most affected individuals have mild intellectual disabilities. Some have borderline cognitive function, meaning that it is below average but not low enough to be classified as an intellectual disability. Individuals with two X chromosomes (typical for females) are seldomly diagnosed likely due to very mild signs and symptoms, if present at all. The most common sign of impaired cognitive function is learning disabilities, which are probably a result of behavioral and communication problems, which include delayed speech, hyperactivity, poor writing skills, and a short attention span. Some individuals show autistic behaviors like repetitive behaviors, hand flapping, and intense interest in a particular subject. Cognitive functioning remains steady and does not get worse with age (MedlinePlus, 2024a). It is also used for carrier screening (NIH, 2023).
There is no cure for fragile XE syndrome. Early intervention and support services can help. Special education classes, occupational therapy, speech therapy, and behavioral therapies may be beneficial to children (Weissman, 2024).

Molecular pathology tests for diseases or conditions that manifest severe signs or symptoms in newborns and in early childhood or that result in early death are generally not relevant to a Medicare member. Carrier screening is not a Medicare benefit.

## AR (Androgen Receptor) (e.g., Spinal and Bulbar Muscular Atrophy, Kennedy Disease, X Chromosome Inactivation)

The *AR* gene provides instructions for making a protein called an androgen receptor. Androgens are hormones (such as testosterone) that are important for normal male sexual development before birth and during puberty. By turning the genes on or off as necessary, the androgen receptor complex helps direct the development of male sex characteristics. Androgens and androgen receptors also have other important functions in both males and females, such as regulating hair growth and sex drive. Hundreds of different mutations in the *AR* gene have been identified in people with androgen insensitivity syndrome, spinal and bulbar muscular atrophy (Kennedy's disease), androgenic alopecia (also known as male pattern baldness, and in women it is known as female pattern hair loss), polycystic ovarian syndrome and prostate cancer (MedlinePlus, 2020a).

Kennedy disease is a rare, slowly progressive neuromuscular disorder in which symptoms present mainly between the ages of 20 and 50. It affects approximately 1 in 200,000 people worldwide and mostly occurs in males. Treatment is for symptoms and life expectancy is normal, though a small percentage of patients may succumb to the disease in their 60's or 70's due to swallowing complications. The diagnosis is suspected based on physical signs and symptoms, and sometimes family history and there is currently no known treatment or cure (NORD, 2023).

Studies of the number of CAG repeats in *AR* alleles in males with Kennedy disease have established a correlation between number of CAG repeats and disease severity. Males whose alleles have a larger number of CAG repeats tend to have earlier disease onset and more rapid progression. Clinical trials of anti-androgen drugs did not consistently reveal significant efficacy, but leuprorelin was efficacious as a treatment for dysphagia in a follow-up clinical trial in Japan, leading to its approval in Japan but not elsewhere. Supportive care involving physical, occupational and speech/language therapy to improve quality of life, maximize function, and reduce complications is recommended (La Spada, 2022).

Hayes Molecular Test Assessment of Spinal and Bulbar Muscular Atrophy (SBMA; Kennedy Disease) (2010, updated 2014) states that SBMA is caused by the expansion of a CAG trinucleotide repeat in the androgen receptor (*AR*) gene, which is located on the X chromosome at bands q11 to q12. Analytical validity studies of SBMA testing are rare. However, the available studies suggest that the analysis is both accurate and reproducible. For the diagnosis of SBMA in patients with symptoms of lower motor neuron disease, bulbar dysfunction, and/or signs of androgen insensitivity, Hayes assigns a rating of C (potential but unproven benefit). For the diagnosis of SBMA in patients with suspected ALS, Hayes assigns a rating of D2 (insufficient evidence). For predictive testing in asymptomatic adults with an established family history of SBMA, Hayes assigns a rating of C. For carrier testing in the female relatives of known SBMA patients or carriers, Hayes assigns a rating of C. For the prenatal or preimplantation genetic diagnosis of SBMA, Hayes assigns a rating of D2.

Screening services such as pre-symptomatic genetic tests and services used to detect an undiagnosed disease or disease predisposition, prenatal diagnostic testing, and carrier screening are not a Medicare benefit.

## Ashkenazi Jewish Associated Disorders Carrier Screening Panel (e.g., Bloom Syndrome, Canavan Disease, Cystic Fibrosis, Familial Dysautonomia, Fanconi Anemia Group C, Gaucher Disease, Tay-Sachs Disease)

Genetic testing is performed for carrier screening of conditions in people of Ashkenazi Jewish descent (ACOG, 2017, reaffirmed 2023).

Carrier screening is not a Medicare benefit.

## ASPA (Aspartoacylase) (e.g., Canavan Disease)

Genetic testing is used to diagnose Canavan Disease in newborns who typically present symptoms in the first few months of life with delayed motor milestones (e.g., head control and sitting). They will manifest macrocephaly, hypotonia, and intellectual disability. Life expectancy is variable, but many individuals die in childhood or adolescence. It is also used for carrier screening (ACOG, 2017, reaffirmed 2023).

Molecular pathology tests for diseases or conditions that manifest severe signs or symptoms in newborns and in early childhood or that result in early death are generally not relevant to a Medicare member. Carrier screening is not a Medicare benefit.

## ASXL1 (Additional Sex Combs Like 1, Transcriptional Regulator)

In 2022, Hu and Wang conducted a meta-analysis to explore the effect of gene mutations on overall response rate (ORR) and overall survival (OS) in myelodysplastic syndrome (MDS) treated with hypomethylating agents (HMAs). The results of the meta-analysis demonstrated a pooled odds ratio (OR) and 95% confidence interval (CI) for ORR, and the pooled hazard ratio (HR) and 95%CI for OS were chosen to estimate the effect. The pooled OR of *TET2* was 0.73 (95%CI: 0.59-0.91, $p$ = 0.005), and the pooled OR of *ASXL1* was 1.38 (95%CI: 1.12-1.71, $p$ = 0.003). As for prognosis, the pooled HR of *RUNX1* was 1.45 (95%CI: 1.15-1.85, $p$ = 0.002). The pooled HR of *TP53* was 2.30 (95%CI: 1.83-2.90, $p$ < 0.001), and the pooled HR of *U2AF1* was 1.41 (95%CI: 1.15-1.74, $p$ = 0.001). There was no statistical difference shown in other genes. Therefore, the authors concluded that *TET2* mutation and *ASXL1* wild type were the predictors of better response to HMAs. *TP53*, *RUNX1*, and *U2AF1* mutations were associated with poor prognosis in MDS.

In the 2022 systematic review and meta-analysis by Sutandyo et al., the authors aimed to explore adults with MDS to elucidate the role of these genes in acute myeloid leukemia (AML) transformation risk. The outcome of this review was measured using the hazard ratio (HR). The results of the exploration were no statistically significant difference in AML transformation risk between *U2AF1* mutant and *U2AF1* wildtype MDS patients (HR: 1.41; 95% CI: 0.95-2.07, p = 0.08, I2 = 0%). Pooled HR showed that patients with *SRSF2* mutation had a higher risk of AML transformation (HR 2.62; 95% CI: 1.54-4.45; p = .0004; I2 = 55%). The pooled HR for *SF3B1* was 0.48 (95% CI: 0.22-1.06, p = 0.07, I2 = 55%). *TET2*, *ASXL1*, and *EZH2* mutations were not associated with AML transformation. Meanwhile, *DNMT3A* mutations were associated with AML transformation with pooled HR of 2.73 (95% CI: 1.43-5.21; p = 0.08; I2: 67%). The pooled HR for *IDH* genes was smaller (HR: 2.92; 95%CI: 1.21-7.06; p = 0.02; I2:65%). Patients with *RUNX1* mutation were associated with AML transformation (HR: 1.85; 95%CI: 1.11-3.09; p = 0.02; I2:38%). The authors concluded that individuals with mutations of *SRSF2*, *DNMT3A*, *IDH*, and *RUNX1* have a higher HR for AML transformation.

Zhao et al. (2022) created a meta-analysis that evaluated the prognostic efficacy of *ASXL1* and *TET2* mutations in the chronic myelomonocytic leukemia (CMML) population. The results of the meta-analysis demonstrated that The total HR of OS was 0.74, 95% CI = 0.61 - 0.91, $P$ = 0.005, compared with CMML patients without *TET2* mutations (*TET2*MT), and the total HR of OS was 1.56, 95% CI = 1.34 - 1.80, $P$ = 0.000, compared with CMML patients without *ASXL1* mutation (*ASXL1*WT), indicating that *TET2*MT *and ASXL1*WT *were* favorable for prognosis of CMML. Whether the gene is mutated or not, the acute transformation rate of disease and mortality rate were further considered for assessment. Compared with the CMML patients with *TET2*MT *and ASXL1*WT, the HR of patients within both *TET2*MT *and ASXL1*MT *was* 1.51 (95% CI = 1.14 - 1.99; $P$ = 0.004), the HR of patients with neither *TET2*MT nor *ASXL1*MT *was* 1.49 (95%CI = 1.12 - 1.98; $P$ = 0.007), and the HR of *TET2*WT and *ASXL1*MT *patients* was 1.88 (95%CI = 1.21 - 2.94; $P$ = 0.005).The authors concluded that the presence of *TET2*MT and *ASXL1*WT genotype was the most beneficial for the survival of individuals with CMML.

Wang et al. (2021) performed a meta-analysis to evaluate the prognostic value and clinical characteristics of *ASXL1* mutations in patients with primary myelofibrosis (PMF). The results showed that *ASXL1* mutations may predict a shorter overall survival (HR = 2.30, 95% CI: 1.79–2.94) and a higher likelihood of transforming into acute leukemia (LFS: HR = 1.77, 95% CI: 1.30–2.42; the rate of acute leukemia transformation: OR = 2.06, 95% CI: 1.50–2.83). *ASXL1* mutations were also correlated with men over 65 years old, lower platelet counts levels, and a greater risk of the international prognostic score system. The authors concluded that *ASXL1* mutations have a substantial adverse effect on the

prognosis of PMF patients and may aid in prognostic assessment and risk stratification for patients with PMF. Study limitations included that all articles were retrospective observational studies and that the studies were all published in English which potentially allow for some publication bias.

Guglielmelli et al. (2021) performed a multi-center study to evaluate specific gene mutations and thrombosis in essential thrombocythemia (ET). 502 patients with World Health Organization (WHO)-defined ET and an average age of 55 years old were included in the study. The most frequent mutation, other than *JAK2/CALR/MPL*, was *ASXL1* (7–20%). Leukocytosis (≥ 11 × 109/L) was noted in 22% of patients, abnormal karyotype in 9%, and extreme thrombocytosis (≥ 1000 × 109/L) in 27%. Treatment plan included antiplatelet therapy for low-risk disease and cytoreductive drugs for high-risk disease. The authors concluded that there was favorable influence of harboring *ASXL1*/*RUNX1*/*EZH2* mutations on arterial thrombosis in the context of arterial events that occurred both before and after diagnosis.

Andréasson et al. (2020) performed a cohort study to identify gene mutations that could be used together with clinical data as prognostic markers to guide treatment decisions in patients with polycythemia vera (PV). 85 patients were included in the study with a mean age of 71. An increased number of mutations found by the selected gene panel was substantially linked to inferior overall survival. Only mutations in the *ASXL1* gene (found in 8.2% of the patients, n = 7) had a significant impact on survival, when correlating individual mutations to overall survival. The projected 5-year survival for *ASXL1* -mutated patients was 43%, whereas the projected 5-year survival was 74% for non-mutated patients. The authors concluded that *ASXL1* is a marker for poor prognosis and targeted testing of a few clinically significant genes such as the *ASXL1* gene should be done and interpreted together with fundamental clinical data. The study was limited by the sample size.

In 2020, Wan and Han conducted a meta-analysis to obtain the myeloid tumors in myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN) 's mutational profiles and explore possible similarities and differences. Fifty-three articles were eligible for the meta-analysis; at most, 9,809 cases were involved for any gene. The top mutant genes and their pooled mutation rates were as follows: *SF3B1* (20.2% [95% CI 11.6-30.5%]) in MDS, *TET2* (39.2% [95% CI 21.7-52.0%]) in MDS/MPN, and *JAK2* (67.9% [95% CI 64.1-71.6%]) in MPN. Subgroup analysis revealed that leukemic transformation-related genes were more commonly mutated in high-risk MDS (MDS with multilineage dysplasia and MDS with excess blasts) than in other MDS entities. Thirteen genes, including *ASXL1, U2AF1, SRSF2, SF3B1*, and *ZRSR2*, had significantly higher mutation frequencies in primary myelofibrosis (PMF) compared with essential thrombocythemia and polycythemia vera this difference distinguished PMF from MPN and likened it to MDS. Chronic myelomonocytic leukemia and atypical chronic myeloid leukemia were similar entities but showed several mutational differences. A heat map demonstrated that juvenile myelomonocytic leukemia and MDS/MPN with ring sideroblasts and thrombocytosis were distinct entities, whereas MDS/MPN-unclassifiable was closest to high-risk MDS. The authors concluded that such genetic closeness or difference reflected features in these conditions' pathogenesis, diagnosis, treatment, and progression and could inspire future genetic studies.

Ohgami et al. (2015) conducted a cohort study to evaluate the frequency and clinicopathologic significance of 19 genes currently identified as substantially mutated in myeloid neoplasms, *ASXL1, CBL, CEBPA, CSF3R, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, NPM1, NRAS, RUNX1, SETBP1, SF3B1, SRSF2, TET2, TP53,* and *U2AF1*, in 93 cases of acute myeloid leukemia (AML) using capture target enrichment and next-generation sequencing. Among these cases, 79% demonstrated at least 1 nonsynonymous mutation, and cases of AML with recurrent genetic abnormalities demonstrated a lower frequency of mutations versus AML with myelodysplasia-related changes. Mutational analysis further showed that *TP53* mutations are associated with complex karyotype AML, whereas *ASXL1* and *U2AF1* mutations are associated with AML with myelodysplasia-related changes. *U2AF1* mutations were associated with trilineage morphologic dysplasia. Univariate analysis showed that *U2AF1* and *TP53* mutations are associated with poor overall survival (OS), absence of clinical remission, and poor disease-free survival. *TET2* and *ASXL1* mutations are associated with poor OS. In multivariate analysis, *TP53* and *U2AF1* mutations retained independent prognostic significance in DFS and OS, respectively. The authors concluded that findings showed unique relationships between mutations in clinicopathologic prognosis, AML, morphologic dysplasia, and subtype categorization.

Schnittger et al. (2013) conducted a comparative study aimed to evaluate *ASXL1*mut in 740 AML with intermediate risk karyotype for association with other mutations, frequency, and impact on outcome. 553 cases had a normal karyotype (NK) and 187 had intermediate risk aberrant cytogenetics. Overall, *ASXL1*mut were found in 127/740 patients (17.2%). *ASXL1*mut were more often found in males than in females (23.5% vs 9.9%). They were associated with a history of preceding myelodysplastic syndromes, older age (median: 71.8 vs 61.8), and with a more immature immunophenotype in comparison to patients with wild-type *ASXL1* (*ASXL1wt*). *ASXL1*mut were more often found in patients with aberrant karyotype (58/187; 31.0%), particularly in cases with trisomy 8 (39/74; 52.7%), than in those with NK (69/553; 12.5%). *ASXL1*mut were observed more often in *RUNX1*mut and less often in *NPM1*mut, *FLT3*-internal tandem duplication (ITD), *FLT3*-TKD and *DNMT3A*mut. Patients with *ASXL1*mut had a shorter event free survival and overall survival (OS)

compared to *ASXL1*wt. In multivariable analysis, *ASXL1*mut was an independent adverse factor for OS (relative risk: 1.70). The authors concluded that *ASXL1*mut are the most frequent mutations in the intermediate risk group acute myeloid leukemia (AML). Due to their strong and independent dismal prognostic impact, the authors suggest adding them into the diagnostic work-up of AML.

## BCKDHB (Branched-Chain Keto Acid Dehydrogenase E1, Beta Polypeptide) (e.g., Maple Syrup Urine Disease)

Genetic testing is used to diagnose maple syrup urine disease (MSUD) in infants whose urine has a distinctive sweet odor. Affected individuals manifest poor feeding, lethargy, and developmental delays. It is also used for carrier screening (ACOG, 2017, reaffirmed 2023).

Molecular pathology tests for diseases or conditions that manifest severe signs or symptoms in newborns and in early childhood or that result in early death are generally not relevant to a Medicare member. Carrier screening is not a Medicare benefit.

## BDX-XL2 (Oncology Lung)

Lung nodules are rounded densities, under 30mm in diameter, detected by chest radiograph (CXR) or CT scan. Nodules are mostly surrounded by lung tissue and are also called coin lesions, solitary pulmonary nodules or lesions, or a “spot” on the lung. The edges of a nodule can be described as smooth or irregular (stellate or spiculated) with irregular edges somewhat more indicative for cancer. Heavily calcified nodules with smooth edges are generally benign and solid nodules that have not shown growth over time are considered benign. For a comprehensive review of nodules and their evaluation see the 2-part series by Patel et al. (2013a and 2013b).

Lung nodules are detected incidentally or through lung cancer screening. Lung cancer screening now has Medicare coverage in the United States (US). As of April 26, 2016, there were 806 sites registered for screening (ACR, 2022). The estimated number of new lung nodules incidentally detected annually in the US is 1.57 million whereas it is anticipated that another 1.5 million are detected by screening annually in the US (Gould et al. 2015).

Early detection of lung nodules is an opportunity to reduce lung cancer mortality but it comes with significant risks. These risks are both for patients and health care delivery networks. For patients, a major problem is the risk of unnecessary invasive procedures to find the minority of nodules that are cancer. For health care delivery, the risks are both costs and overloading the health care system.

Of the expected 3 million nodules per year found incidentally or by lung cancer screening, the majority will be Medicare age. Four recent studies underscore the importance of lung cancer evaluations to the Medicare population:

- The mean age of 377 eligible patients in an 18-site retrospective chart review study was 65 (Tanner et al. 2015).
- A prospective study across 12 sites and 475 patients found 62.5% of patients were 65 years of age or older (Vachani et al. 2015b).
- A recent study reported that between Jan 2009 and Dec 2011, 8,979 Medicare patients from a random sampling of 5% of Medicare claims, underwent lung cancer evaluations because of an abnormal chest CT scan (Lokhandwala et al. 2016).
- In the National Lung Screening Trial (NLST), over 57% of enrollees were over 65 years of age (Aberle et al. 2011). Also, the rates of nodule detection increased dramatically with age. Medicare enrollees are more likely to meet lung cancer screening criteria and have more nodules detected.

A set of guidelines for lung nodule management is published and updated by the American College of Chest Physicians (ACCP). The ACCP guidelines for lung nodules, updated in 2013, is the primary reference used by pulmonologists in the US (Gould et al. 2013). The ACCP Guidelines state: “Although clinical and radiographic [CT scans] characteristics cannot reliably distinguish between benign and malignant nodules in most individuals, it is nevertheless important to estimate the clinical probability of malignancy before ordering imaging tests or biopsy procedures”. The pretest probability of malignancy (pCA) is estimated by using clinical judgment or with a quantitative risk model (Gould et al. 2007 and Swensen et al. 1997). Establishing a pCA creates 3 risk stratification groups, namely, Low, Intermediate, and High probability, with Low risk having pCA below 5% and High risk having pCA above 65%. The general concept is that Low risk patients will be observed with CT surveillance to watch for growth if a nodule is malignant. Conversely, the guidelines suggest those patients in the High-risk group go directly to surgery. The logic is that the probability of cancer is high enough that a negative biopsy will not change the care pathway. The Intermediate risk group (5-65% pCA) are recommended to enter the diagnostic odyssey that often includes positron emission tomography (PET) scanning as the next step. A negative PET suggests a benign nodule, so the patient is followed with CT scans. A positive PET scan goes on to surgery or biopsy. This is the overall concept, but PET has sensitivity and specificity challenges. In particular,

current estimates of PET sensitivity from 72% to 94% are reviewed in the 2013 ACCP Guidelines in section 4.2.3 (Gould et al. 2013). False positive PET scans for nodules are an additional problem with estimates of the false positive PET scan rate of 39% (Tanner et al. 2015).

A pulmonary community practice observational chart review of 18 practices and 377 patients found a wide variation in management of nodules (Tanner et al. 2015). The surgery rate for benign nodules was 35%, and the rate of surgery was the same for Low, Intermediate, and High-risk patients. The risk categories were calculated by the study and despite a Low risk, 28% had biopsies and 17% had surgery. The rates of surgery for benign nodules range between 10% and 55%. A survey of 196 pulmonologists supports the potential of a non-invasive biomarker to positively improve lung nodule management decisions (Vachani et al. 2014).

Biopsies can be obtained through a bronchoscope or a needle passed through the chest wall with CT image guidance. A community practice chart review found 38% of patients had a form of biopsy (Tanner et al. 2015). Complications with biopsies or surgery are increased with age, smoking history, and other lung disease. Biopsy through the bronchoscope has the lowest risk with a 2-4% risk of bleeding or pneumothorax (Gould et al. 2013). A disadvantage of this procedure is inaccurate sampling of the nodule. Correct sampling averages about 50% (Gould et al. 2013). The correct sampling rate may improve with modern navigation techniques that are being adopted. Bronchoscopic biopsy use for nodules is currently about 20% of nodules (Lokhandwala et al. 2016). Needle biopsies are done in about 15% of patients with nodules with a 1% risk of bleeding, and a 15-19% risk of pneumothorax (Weiner et al. 2011). About half (7%) of patients with a pneumothorax require chest tube placement with a significant period of hospitalization. Most needle biopsies are diagnostic, but the risk of a non-diagnostic result with a malignant nodule is about 20% (Gould et al. 2013). Biopsies (combined bronchoscopy and needle) are performed in about 25% of nodules (200,000), and the procedures are for benign nodules in 42-62% (104,000). Complications from biopsies result in hospitalization in 2-7% of cases (Lokhandwala et al. 2016 and Weiner et al. 2011). That translates into 4,680 excess hospitalizations per year that are potentially avoidable.

Eventually, most malignant nodules go to surgery for resection and about 15-25% of patients have biopsy attempts before surgery. The overall surgery rate is about 34% (270,000 per year) for benign and malignant nodules in the nodule population (Tanner et al. 2015). Complications include death (2% in Medicare population) (Iniguez et al. 2016), prolonged lung air leak (3-5%), and pneumonia (1-8%) (Gould et al. 2013). Published rates for surgery for benign nodules range from 31-44% (Tanner et al. 2015, Aberle et al. 2011, Li et al. 2013). This translates into an estimated 102,000 surgeries and 2,052 deaths per year that are avoidable for patients that do not have lung cancer.

## *Test Description and Intended Use*

BDX-XL2 is a proteomic risk predictor that integrates the expression levels of 2 proteins with 5 clinical risk factors. The BDX-XL2 assay is performed on fresh-frozen EDTA plasma samples using mass spectrometry (Li et al. 2015, Vachani et al. 2015a). Results are reported as “Likely Benign” when the post-test probability that a lung nodule is benign is 90% or higher. Otherwise, the test reports “Indeterminate” when the post-test probability is less than 90%. “Likely Benign” test reports also include the post-test probability that the lung nodule is benign, ranging from 90% to 98%. This is further detailed in Table 1 below, along with the performance of the test at each post-test probability.

The intended use of the test is to assist physicians in the management of lung nodules by identifying those lung nodules with a high post-test probability of being benign. These lung nodules would then be candidates for non-invasive CT surveillance instead of invasive diagnostic procedures, such as biopsy or surgery.

## *Clinical Validation*

The discovery, clinical validation, and analytical performance of earlier versions of the assay were previously reported (Li et al. 2015, Vachani et al. 2015a, Li et al. 2013). The current version of (BDX-XL2) is a refinement that incorporates clinical risk factors (nodule size, age, smoking history, nodule location and nodule spiculation). BDX-XL2 was retrospectively validated on the prospective observational PANOPTIC study (NCT01752114) of lung nodule management. Silvestri et al. (2018) analyzed the results of the PANOPTIC trial which enrolled 685 subjects across 33 sites in the US and Canada. Validation of BDX-XL2 followed the National Academy of Medicine’s guidelines for rigorous test development (Micheel et al. 2012). In the PANOPTIC study, 178 subjects met the intended use population of BDX-XL2. This consisted of 149 benign lung nodules (as determined by histopathology after biopsy or surgery, or by stable CT surveillance of a lung nodule after at least 1 year) and 29 malignant lung nodules (as determined by histopathology after biopsy or surgery), yielding a cancer prevalence of 16.3%. Per protocol, this cohort of patients was split into separate verification (n = 69) and validation (n = 109) subsets, with pre-specified interim and final analyses on each subset, respectively, to determine the performance characteristics of the test. Since the test system and clinical endpoints were

unchanged between the interim and final analyses, all analyses were blinded, and the 2 patient cohorts were mutually exclusive, the 69 and 109 patients are combined below.

For these 178 subjects, BDX-XL2 yielded a sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of 97% (CI: 82%-100%), 44% (CI: 36%-52%), 98% (CI: 92%-100%), and 25% (CI: 17%-34%), respectively. It was noted that the post-test probability of BDX-XL2 is equivalent to its NPV. Procedure use and clinical factors were collected in the PANOPTIC study permitting the comparison of BDX-XL2 performance to (1) current practice, as characterized by physician pre-test risk assessment, denoted as pCA, which is based on the physician's clinical assessment of risk using a clinical risk model and/or clinical judgement; (2) PET; and (3) clinical risk factor models including the 'Mayo', 'VA', 'Brock' and 'Herder' models. Using area under the curve (AUC) as a general performance measure, the respective AUCs for BDX-XL2, pCA, PET, and these 4 clinical factor models were: 76% (CI: 69%-82%), 69% (CI: 62% - 76%), 58% (CI: 46%-69%), 69% (CI: 62%-76%), 60% (CI: 53%-67%), 71% (CI: 63%-77%), and 67% (CI: 56%-78%). BDX-XL2 had statistically significant improved performance over all above diagnostic modalities with p-values 2.1xE-11 (pCA), 0.001 (PET), 0.0009 (Mayo), 2.7xE-7 (VA), 0.005 (Brock) and 0.02 (Herder).

## *Clinical Utility*

Current practice has been previously characterized (Tanner et al. 2015, Vachani et al. 2014), as well as, the potential clinical utility of an earlier version (Vachani et al. 2015b). The clinical utility of BDX-XL2 is measured in terms of its potential to reduce unnecessary invasive procedures, such as biopsies and surgeries, on benign lung nodules while not significantly increasing the number of malignant lung nodules routed to CT surveillance, thereby delaying surgical resection. Assuming strict adherence to management recommendations based on assay results (i.e., active surveillance if "likely benign"), an earlier version of Xpresys® Lung demonstrated a potential 32% reduction in invasive procedures on benign lung nodules without increasing the number of malignant nodules routed to CT surveillance (Vachani et al. 2015b) based on a retrospective analysis of a prospective observational study of lung nodule management (NCT01752101) (Vachani et al. 2015b). Similarly, in the PANOPTIC study, the potential clinical utility of the current version of (BDX-XL2) was assessed retrospectively. Specifically, if BDX-XL2 were used to guide lung management (and assuming a post-test probability of 98%), then invasive procedures on benign lung nodules would have been reduced 36% (CI: 22%-52%) with only 3% (CI: 0%-18%) of malignant nodules routed to CT surveillance (compared to 45% with current practice in the PANOPTIC study).

## *Summary of Analytical and Clinical Performance*

### General

- **Intended Use**: BDX-XL2 is intended for the evaluation of 8-30 mm lung nodules in patients 40 years or older with a pre-test cancer risk (as assessed by the Mayo Clinic Model for Solitary Pulmonary Nodules) of 50% or less.
- **Validated Specimen Type(s)**: Plasma from K2 EDTA vacutainer tubes.

### Analytical Performance

| Description | Results (with 95% confidence ntervals, if applicable) |
|---|---|
| **Repeatability (within run precision)**<br>8 samples tested 3 times within a run, 1 instrument, 2 operators, 3 runs, 3 non-consecutive days, 1 manufacturing reagent lot for critical reagents. Qualitative results represent only 2 possible final results (i.e., indeterminate and likely benign) with post-test probabilities for the latter from 90 to 98%. | Analytes (analytical repeatability):<br>• ARRLG3BP CV = 7.0% (3.6%-10.3%)<br>• ARRC163A CV = 5.9% (3.6%-8.2%)<br><br>Score (analytical repeatability): Score CV = 9.0% (3.4%-14.6%)<br><br>Qualitative (clinical concordance): 62.5% (5/8; 24.5%-91.5%)<br><br>ARR is defined as the ratio between the peak area for the endogenous quantifier peak and the peak area of the corresponding SIS peptide, multiplied by a calibration factor that is specific for each SIS lot.<br><br>Score is defined as Log2[ARRLG3BP/ ARRC163A] |

| Description | Results (with 95% confidence ntervals, if applicable) |
|---|---|
| **Intermediate precision (between run precision)**<br>7 samples tested once in 3 different runs, 1 instrument, 2 operators, 3 runs, 3 non-consecutive days, 1 manufacturing reagent lot for critical reagents. Qualitative results represent only 2 possible final results (i.e., indeterminate and likely benign) with post-test probabilities for the latter from 90 to 98%. | Analytes (analytical repeatability):<br>• ARRLG3BP CV = 14.5% (10.1%-18.9%)<br>• ARRC163A CV = 12.8% (9.1%-16.6%)<br><br>Score (analytical repeatability): Score CV = 6.0% (3.7%-8.4%)<br><br>Qualitative (clinical concordance): 85.7% (6/7; 42.1%-99.6%) |
| Reproducibility (between sites) | Not applicable |
| Minimum input quantity | 20 µL plasma |
| Limit of blank (LOB) | Defined as the upper 99.5% confidence interval (CI) for the response ratio (ARR) observed in negative controls. |
| Limit of detection (LOD) | The lower limit of response for each analyte in samples and positive controls is defined as the lower limit of quantification. |
| Limits of quantitation (LOQ) | The lower limit of quantification (LLOQ) is defined as the lowest response ratio within the linear range where the coefficient of variation (CV) was equal to or below 0.20, where linearity was established using 5 replicate measures at each concentration.<br><br>The upper limit of quantification (ULOQ) is defined as the highest response ratio within the linear range where the coefficient of variation (CV) was equal to or below 0.20, where linearity was established using 5 replicate measures at each concentration.<br><br>LG3BP:<br>• ULOQ = 42<br>• LLOQ = 0.043<br><br>C163A:<br>• ULOQ = 71<br>• LLOQ = 0.053 |
| Linearity | Not applicable for qualitative interpretation (i.e., likely benign or indeterminate). For individual analytes, the linear response range was established between the LLOQ and ULOQ. |
| Interfering substances | Visual inspection to detect hemolysis (ge. 100 mg/dL of hemoglobin rejected).<br><br>MRM-MS chromatograms for each analyte in every sample are inspected for interference, with any interference resulting in QC failure. |
| Specimen stability, primary (EDTA whole blood) | 3 hours at 2-8 °C based on validation study (manuscript in preparation). |
| Specimen stability, intermediate (plasma) | 24 months at -70 °C based on validation study (manuscript in preparation). |
| Reagent closed/shelf-life stability | 24 months at -70 °C for 2 critical reagents (i.e., Human Plasma Samples (HPS) and SIS peptides). ARR for 623 samples evaluated with slope not significantly different from zero (p = 0.77).<br><br>Non-critical (general purpose) reagents are stored and expired per manufacturer recommendations. |
| Reagent open/in use stability | Critical reagents: Not applicable since single use aliquots. Non-critical reagents are stored and expired per manufacturer recommendations. |

## Clinical Performance: Validity

BDX-XL2 integrates the relative abundance of 2 plasma proteins (LG3BP and C163A) with 5 clinical risk factors (age, smoking status, nodule diameter, nodule spiculation status and nodule location). From these 7 markers, a numerical value, XL_2(k), for a patient k, is calculated.

XL_2(k) ranges between 0 and 1 and its value is used to index the post-test probability (i.e., NPV) validated in the PANOPTIC study (see Table 1 below).

| XL_2(k) Value | Post-Test Benign Probability (i.e., NPV) (95% CI) | Sensitivity (95% CI) | Specificity (95% CI) | PPV (95% CI) | Test Report |
|---|---|---|---|---|---|
| 0 to 0.131 | 98% (92-100%) | 97% (82-100%) | 44% (36-52%) | 25% (17% - 34%) | Likely Benign |
| > 0.131 to 0.1613 | 97% (91-100%) | 93% (77-99%) | 49% (41-57%) | 26%(18-36%) | Likely Benign |
| 0.1613 to 0.172 | 96% (90-99%) | 90% (73-98%) | 54% (45-62%) | 27% (19-37%) | Likely Benign |
| > 0.172 to 0.176 | 95% (89-99%) | 86% (68-96%) | 55% (47-63%) | 27% (18-37%) | Likely Benign |
| > 0.176 to 0.1785 | 94% (87-98%) | 83% (64-94%) | 56% (47-64%) | 27% (18-37%) | Likely Benign |
| > 0.1785 to 0.193 | 93% (86-98%) | 79% (60-92%) | 57% (44-65%) | 26% (18-37%) | Likely Benign |
| > 0.193 to 0.195 | 92% (85-96%) | 76% (56-90%) | 58% (49-66%) | 26% (17-37%) | Likely Benign |
| > 0.195 to 0.2306 | 91% (84-96%) | 69% (49-85%) | 64% (56-72%) | 27% (18-39%) | Likely Benign |
| > 0.2306 to 0.354 | 90% (84-95%) | 55% (36-74%) | 83% (75-88%) | 38% (24-54%) | Likely Benign |
| > 0.354 | - | - | - | - | Indeterminate |

## Clinical Performance: Utility

PANOPTIC was a non-interventional study; however, the potential clinical utility of BDX-XL2 can be estimated by evaluating how many benign (benefit) and malignant (harm) nodules would have been routed away from invasive procedures into CT surveillance if BDX-XL2 had been used to guide patient management in the study (and assuming complete compliance). Table 2 summarizes the potential clinical utility of BDX-XL2 at each post-test probability.

| Probability of Being Benign | Of Benign Nodules (95% CI) | CT Surveillance (95% CI) |
|---|---|---|
| 98% | 15/42 = 36% (22% - 52%) | 1/29 = 3% (0% -18%) |
| 97% | 17/42 = 40% (26% - 57%) | 2/29 = 7% (1% -23%) |
| 96% | 19/42 = 45% (30% - 61%) | 2/29 = 7% (1% -23%) |
| 95% | 20/42 = 48% (32% - 64%) | 2/29 = 7% (1% -23%) |
| 94% | 20/42 = 48% (32% - 64%) | 2/29 = 7% (1% -23%) |
| 93% | 22/42 = 52% (36% - 68%) | 3/29 = 10% (2% -27%) |
| 92% | 22/42 = 52% (36% - 68%) | 4/29 = 14% (4% -32%) |
| 91% | 25/42 = 60% (43% - 74%) | 4/29 = 14% (4% -32%) |
| 90% | 32/42 = 76% (61% - 88%) | 7/29 = 24% (10% -44%) |

### *Table 2: Potential Clinical Utility of BDX-XL2*

In the PANOPTIC study, there were 178 intended use subjects (when the verification and validation sets are combined) of whom 29 had malignant lung nodules and 149 had benign lung nodules. Of the 149 benign lung nodules, 42 had at least 1 invasive procedure. Hence the denominator of “42” in column 2 of Table 2. Of the 29 malignant lung nodules, 13 were routed to CT surveillance. That is, 13/29 = 45% of malignant nodules were routed to CT surveillance in PANOPTIC. This is substantially larger than the largest corresponding value in Table 2 (i.e., 24%).

In summary, the BDX-XL2 assay is reasonable and necessary to assist physicians in the management of lung nodules by identifying those lung nodules with a high probability of being benign. This assay is only covered when the following conditions are met:

- Patient is at least 40 years of age and has a lung nodule of diameter 8 to 30 mm, and
- The pre-test risk of cancer as determined by the Mayo risk prediction algorithm is 50% or less.

**Note**: The BDX-XL2 test should not be ordered if a physician does not intend to act upon the test result. It is expected that physicians will advise nodule surveillance for at least 80% of patients with a post-test probability of 98% or higher.

It is recognized that evidence of clinical utility for the BDX-XL2 assay for ≥ 40-year-old patients with an 8 to 30 mm lung nodules and a pre-test cancer risk (as assessed by the Mayo Clinic Model for Solitary Pulmonary Nodules) of ≤ 50% is promising at the current time. Clinical studies underway at this time are expected to demonstrate clinical utility. These studies are designed to show a statistically significant reduction in the number of benign lung nodules experiencing invasive procedures between a prospective group of patients managed by BDX-XL2 and a contemporaneous group not managed by BDX-XL2. A secondary end-point will show that the management of lung nodules by BDX-XL2 does not (i.e., is 1 statistically non-inferior to) the number of malignant nodules routed to CT surveillance (determined at 1 year interval) as compared to current practice without BDX-XL2. Continued coverage for BDX-XL2 testing will be dependent on annual review of prospective data and peer-reviewed studies.

Data collected by Biodesix through ongoing studies will support utility including:
- All clinical risk factors to calculate the Mayo, VA, and Brock cancer risk predictors.
- PET result (if used).
- Physician-assessed pre-test cancer risk assessment.
- Physician post-test lung nodule management recommendation.
- Any subsequent procedures (non-invasive or invasive).
- Clinical diagnosis based on those procedures (i.e., benign or malignant).

## Cytogenomic (Genome-Wide) Analysis for Constitutional Chromosomal Abnormalities

Routine chromosome analysis has been used historically to identify chromosome abnormalities during pregnancy when risk factors are present, such as advanced maternal age and chromosome abnormalities. Chromosome microarray analysis (CMA) does not require cell culture or dividing cells, so it provides an advantage in turn-around time for time sensitive analysis, as is often the case during pregnancy. In addition, CMA can identify smaller chromosomal abnormalities than a routine chromosome analysis and is able to identify chromosomal breakpoints that are unbalanced but may appear balanced on a conventional karyotype. CMA does have limitations; it cannot detect totally balanced chromosomal material or low-level mosaicism. Some arrays may not detect triploidy. Clinicians may use CMA as a first line test, or only when fetal abnormalities are identified (Society for Maternal-Fetal Medicine (SMFM), 2016).

Screening services such as pre-symptomatic genetic tests and services used to detect an undiagnosed disease or disease predisposition and prenatal diagnostic testing are not a Medicare benefit.

## DetermaRx™ (Oncology Lung)

Non-Small Cell Lung Cancer (NSCLC) is a deadly cancer with estimated incidence of 41.2 per 100,000 people and an incidence in those 65 years of age and older of 238.1 per 100,000 people (Noone et al. 2018). While mortality rates have improved since 1975, the 5-year relative survival is estimated to be only 24.2% for all stages of disease (SEER Summary Stages) and only 60.1% for localized disease, the least advanced stage of disease (Noone et al. 2018).

While clear clinical and pathologic staging approaches have been developed to risk stratify patients and clear treatment guidelines-based risk strata have been published to guide management based on risk strata, disease recurrence is common. Kelsey et al. (2009) conducted a large retrospective study of patterns of disease recurrence in early-stage patients undertaken at a single large academic center. The study reviewed the records of patients who underwent surgery for T1 and T2 and N0 and N1 NSCLC patients and looked for evidence of recurrence in the medical record. Records of 975 patients were reviewed, the majority of whom had Stage IA (45%) or Stage IB (39%) disease. Nearly all patients (96%) had negative surgical margins. Adjuvant chemotherapy was used in 7% and radiation treatment was used in 3%. The rate of local recurrence in this cohort was 23%, and the rate of distant recurrence was 34%, suggesting that disease recurrence is common even among patients with localized margin-negative disease classified as low risk based on available clinical and pathologic data. More recent data from the Surveillance, Epidemiology, and End Results database, shows that even among those patients with localized disease, 5-year survival rates are at around 60% (Noone et al. 2018).

### *Molecular Risk Stratification*

The frequent recurrence of NSCLC following resection in patients classified as low risk based on clinical and pathologic data motivated the development of a molecular classifier that might be able to more accurately identify which patients are likely to have disease recurrence or metastatic disease.

The Razor 14-Gene Lung Cancer Assay is quantitative PCR analysis designed to be used on formalin-fixed, paraffin embedded lung cancer tissue. The test relies on an algorithmic interpretation of the quantitative PCR data on RNA from 11 cancer-related target genes (*BAG1*, *BRCA1*, *CDC6*, *CDK2AP1*, *ERBB3*, *FUT3*, *IL11*, *LCK*, *RND3*, *SH3BGR*, *WNT3A*) and 3 reference genes (*ESD*, *TBP*, *YAP1*).

Of note, Oncocyte acquired the rights to develop and market Razor's treatment stratification test in September 2019. Therefore, the Razor 14-Gene Lung Cancer Assay is now called DetermaRx™ (Oncocyte, 2021).

A clinical validation study by Kratz et al. (2012a) briefly describes the development of the test and describes clinical validation of the test's predictive ability in two additional cohorts. The test and algorithmic interpretation were developed using a training cohort of 361 non-squamous resected samples at a single academic medical center. The test was then validated in samples from 433 patients with Stage I disease from hospitals in the Kaiser Permanente Northern California system by Kaiser Permanente Division of Research (KPDOR). Another large-scale validation was done on a cohort of 1006 Chinese patients treated at institutions participating in the China Clinical Trials Consortium (CCTC). In the validation components the biopsy samples were sent to a laboratory and data was analyzed by researchers who were blinded to patient outcomes. A risk class of low, intermediate, or high risk was assigned to each biopsy prior to outcome data being released. The investigators studied overall survival in relation to risk score as the primary outcome, and lung-cancer specific mortality in relation to the risk score as a secondary outcome. In the KPDOR Cohort the mean age was 66.6 years with a median of 106 months of survivor follow-up. The 5-year mortality rate (time post-resection) was 43%. The majority of cases (77%) were adenocarcinoma. The KPDOR cohort was strictly Stage I cancers, of which 68% were Stage IA. In the CCTC cohort the mean age was 58.3 years with a median of 53.4 months of survivor follow-up. The 5-year mortality rate (time post-resection) was 42%. The majority of cases (88%) were adenocarcinoma. This cohort contained patients with Stages I, II, and III disease; 47% had stage I disease (24% stage IA and 23% Stage IIA), 22% had stage II disease (7% had Stage IIA disease and 15% had Stage IIB disease), and 26% had stage III disease. In summary, this initial clinical validation study showed that there were significant differences in overall and cancer-specific survival between molecular classifier risk strata, even among AJCC Stage I patients in the KPDOR cohort. The estimated 5 overall year survival based on molecular risk group for all patients in the KPDOR cohort was 71.4% in the low-risk group, 58.3% in the intermediate-risk group, and 49.2% in the high-risk group. The estimated 5-year lung-cancer specific survival based on molecular risk group for all patients in the KPDOR cohort was 84.6% in the low-risk group, 70.3% in the intermediate-risk group, and 63.3% in the high-risk group. The estimated 5 overall year survival based on molecular risk group for all patients in the CCTC cohort was 74.1% in the low-risk group, 57.4% in the intermediate-risk group, and 44.6% in the high-risk group.

A subsequent study by Woodard et al. (2018) evaluated the ability of the Razor 14-Gene Lung Cancer Assay to identify high risk disease particularly in small node-negative disease from the above cohorts. In 2012b, (prior to the publication of the new AJCC staging information), Kratz et al. addressed the issue of whether the assay accurately risk stratifies patients with small tumors, including a subset analysis of tumors < 1 cm. While this group was only 26 patients, they found that risk stratification based on the assay was associated with statistically significant differences in 5-year survival.

Following the development of the assay and retrospective validation studies, the ability of the test to differentiate early recurrence was prospectively studied by Woodard et al. (2014) of 52 patients with non-squamous NSCLC. The average age was 62 years, with a mean tumor size of 3.23 cm. The study was mostly Stage I patients; 25 Stage IA, 15 Stage IB, 7 Stage IIA, 2 Stage IIB, and 2 Stage IIIA. The median disease-free interval was 10.3 months, and the median lung-cancer specific survival was 10.3 months. Overall mortality was 8%. No recurrences or lung-cancer specific deaths were observed in the low or intermediate risk groups. The recurrence rate was 29% in the high-risk group with a lung-cancer specific mortality of 14% in this group.

A more recent observational prospective study by Woodard et al. (2018) using the Razor 14-Gene Lung Cancer Assay in a slightly larger single institution cohort has been published. In this study, 100 consecutive patients with stages IA, IB, and IIA disease treated with a surgical resection between 2011 and 2015 received molecular testing for risk stratification. The sample had a median age of 67.5 years and was composed of 58 Stage IA patients, 32 Stage IB patients, and 10 Stage IIA patients. The treating clinicians were made aware of the results of the molecular classification results, though the decision of whether or not a patient received adjuvant chemotherapy was individualized to the patient. There were 52 patients stratified as molecular low risk, and 48 stratified as molecular high risk, which for this study included both intermediate and high-risk classifications. No patients with molecular low risk disease were given adjuvant treatment. The 5-year disease free survival was 93.8% among those with molecular low risk disease and 91.7% among those with molecular high-risk disease treated with adjuvant chemotherapy. For those with molecular high-risk disease not treated, 5-year disease-free survival was 48.9%.

## DMPK (DM1 Protein Kinase) (e.g., Myotonic Dystrophy Type 1)

The *DMPK* gene provides instructions for making a protein called myotonic dystrophy protein kinase. This protein appears to play an important role in muscle, heart, and brain cells. The protein may be involved in communication within cells. It also appears to regulate the production and function of important structures inside muscle cells by interacting with other proteins. For example, myotonic dystrophy protein kinase has been shown to turn off part of a muscle protein called myosin phosphatase. Myosin phosphatase is an enzyme that plays a role in muscle contraction and relaxation. Mutations in the *DMPK* gene cause a form of myotonic dystrophy known as myotonic dystrophy type 1 (MedlinePlus, 2020b). Myotonic dystrophy type 1 is the most common form of muscular dystrophy. The diagnosis is based on a patient's medical history, family history, physical examination findings, and genetic studies; the diagnostic standard is genetic testing to identify CTG repeat expansions. Treatments may focus on specific symptoms such as muscle weakness and/or atrophy, cardiac arrhythmias, fatigue, and myotonia. Currently, there are no cures for myotonic dystrophy type 1. (Hartman et al. 2024).

Hayes Molecular Test Assessment for Myotonic Dystrophy Types 1 and 2 (2009, updated 2013) states that myotonic dystrophy (DM) is a neuromuscular condition which manifests as progressive weakness, muscle abnormalities, and a multisystemic phenotype, with involvement of cardiac, endocrine, respiratory, and other systems. Myotonic dystrophy type 1 (DM1) and the milder, but phenotypically similar, myotonic dystrophy type 2 (DM2) are caused by genetic variants in 2 separate genes. Both genes display an autosomal dominant mode of inheritance. A severe form of DM1, congenital myotonic dystrophy (CDM1), can occur in the neonatal period with early mortality, as well as various physical and mental disabilities. The cause of DM1 is an expansion of a trinucleotide (CTG) repeat in the dystrophia myotonica protein kinase (*DMPK*) gene. The cause of DM2 is an expansion of a tetranucleotide repeat (CCTG) in the zinc finger protein 9 (*ZFP9*) gene. There is no treatment to prevent DM1 or DM2, or to change prognosis. For confirmation of a diagnosis of DM1 or DM2 in symptomatic patients in the absence of classical symptoms or a family history of the disorder, Hayes assigns a rating of C (potential but unproven benefit). For confirmation of a diagnosis of DM1 or DM2 in symptomatic patients who have classic symptoms and/or a family history of the disorder, Hayes assigns a rating of D2 (insufficient evidence). For diagnosis of DM1 or DM2 in asymptomatic adults who are at an increased risk of DM1 or DM2 through a positive family history, Hayes assigns a rating of D2. For diagnosis of DM1 in asymptomatic adults who are at an increased risk of DM1 through a positive family history, and are considering reproductive options, Hayes assigns a rating of C. For diagnosis of DM2 in asymptomatic adults who are at an increased risk of DM2 through a positive family history, and are considering reproductive options, Hayes assigns a rating of D2. For prenatal diagnosis or PGD of DM1 in couples in which 1 or both members has been confirmed to be affected with, or is a presymptomatic carrier of, DM1 through genetic testing, Hayes assigns a rating of B (some proven benefit). For prenatal diagnosis or PGD of DM2, Hayes assigns a rating of D2.

Screening services such as pre-symptomatic genetic tests and services used to detect an undiagnosed disease or disease predisposition, prenatal diagnostic testing, and carrier screening are not a Medicare benefit.

## F9 (Coagulation Factor IX) (e.g., Hemophilia B)

Hemophilia B is caused by deficiency in factor IX clotting activity which causes prolonged oozing after surgery, injuries, or tooth extractions, and recurrent or delayed bleeding before a wound is completely healed. The age of diagnosis and frequency of bleeding episodes depends on the level of factor IX clotting activity. In any individual, bleeding episodes can be more frequent in children and adolescents than in adults. Severe hemophilia B is often diagnosed by age 2 and can have on average, 2-5 spontaneous bleeding episodes every month. Moderate hemophilia B rarely has spontaneous bleeding, although this depends on the individual, with frequency of bleeding episodes varying from once/month to once/year from prolonged or delayed oozing after relatively minor trauma. Moderate hemophilia B is diagnosed before 5-6 years old. Mild hemophilia B does not have spontaneous bleeding episodes with frequency of bleeding ranging from once/year to once/decade. Individuals with mild hemophilia B are usually not diagnosed until later in life when they undergo surgery, tooth extractions, or there is major trauma. Hemophilia B diagnosis is established by low factor IX clotting activity. *F9* molecular testing confirms the diagnosis. Depending on the *F9* sequencing method used, single-exon, multi-exon, or whole-gene deletions/duplications may not be detected. It is also used for prenatal/preimplantation testing and carrier screening (Konkle and Nakaya Fletcher, 2024).

Tests to diagnose hemophilia B include partial thromboplastin time, prothrombin time, and serum factor IX activity (MedlinePlus, 2024b).

Molecular pathology tests for diseases or conditions that manifest severe signs or symptoms in newborns and in early childhood or that result in early death are generally not relevant to a Medicare member. Prenatal diagnostic testing and carrier screening are not a Medicare benefit.

## FANCC (Fanconi Anemia, Complementation Group C) (e.g., Fanconi Anemia, Type C)

Genetic testing is used to diagnose Fanconi anemia (FA) in individuals with prenatal and/or postnatal short stature, skeletal malformations, abnormal skin pigmentations, microcephaly, ophthalmic anomalies, and genitourinary tract anomalies with macrocytosis, increased fetal hemoglobin, and cytopenia. It is also used for prenatal testing, preimplantation genetic testing, and carrier screening (Mehta and Ebens, 2021).

Molecular pathology tests for diseases or conditions that manifest severe signs or symptoms in newborns and in early childhood or that result in early death are generally not relevant to a Medicare member. Prenatal diagnostic testing and carrier screening are not a Medicare benefit.

## Fetal Chromosomal Aneuploidy (e.g., Trisomy 21, 18, and 13, Monosomy X)

Cell-free DNA testing is used for noninvasive prenatal testing that mainly looks for Down syndrome (trisomy 21, 18, 13, and extra or missing copies of the X and Y chromosome) (MedlinePlus, 2021b).

Prenatal diagnostic testing is not a Medicare benefit.

## Fetal Chromosomal Microdeletions (e.g., Digeorge Syndrome, Cri-Du-Chat Syndrome)

Noninvasive prenatal testing (NIPT) is used in the clinical detection of fetal autosomal deletions (Yin et al. 2019).

Prenatal diagnostic testing is not a Medicare benefit.

## FMR1 (Fragile X Messenger Ribonucleoprotein 1) [e.g., Fragile X Syndrome, X-Linked Intellectual Disability (XLID)]

In a Hayes Clinical Utility Evaluation on Fragile X Syndrome, it is noted that genetic testing is used to diagnose Fragile X Syndrome (FXS), the most common cause of inherited intellectual disability (ID) and is caused by changes in the *FMR1* gene. There is no cure for FXS. For use of FXS genetic testing to diagnose individuals with intellectual disability (ID) and/or autism spectrum disorder (ASD) of unknown cause, and/or other features of FXS, Hayes assigns a rating of C (potential but unproven benefit). For use of FXS genetic testing for carrier screening in women to be used for family planning/reproductive decision making related to having a pregnancy/child with FXS, Hayes a assigns a rating of C. For use of FXS genetic testing for prenatal diagnosis in an at-risk pregnancy, Hayes assigns a rating of C. For use of FXS genetic testing for newborn screening, Hayes assigns a rating of D2 (insufficient evidence) (Hayes Clinical Utility Evaluation Genetic Testing For Fragile X Syndrome, 2017, updated 2021).

The average age of FXS diagnosis is 42 months for girls and 35-37 months for boys (CDC, 2024).

Molecular pathology tests for diseases or conditions that manifest severe signs or symptoms in newborns and in early childhood or that result in early death are generally not relevant to a Medicare member. Screening services such as pre-symptomatic genetic tests and services used to detect an undiagnosed disease or disease predisposition, prenatal diagnostic testing, and carrier screening are not a Medicare benefit.

## FXN (Frataxin)

Genetic testing is used to diagnose Friedreich ataxia which presents as muscle weakness, dysarthria, spasticity particularly in the lower limbs, bladder dysfunction, scoliosis, absent lower-limb reflexes, and loss of position and vibration sense. Onset is usually at 10-15 years of age. It is also used for prenatal testing, preimplantation genetic testing, and carrier testing (Bidichandani, 2017).

In a Hayes Molecular Test Assessment for Friedreich Ataxia (FRDA), it is noted that FRDA is caused by expansion of a GAA trinucleotide repeat in the frataxin (*FXN*) gene. *FXN* gene testing can be considered in adults or children with symptoms consistent with FRDA, Friedreich ataxia with retained reflexes (FARR), late-onset Friedreich ataxia (LOFA), or in apparently recessive or sporadic ataxia patients. Carrier testing can be performed in first-degree relatives of individuals with genetically confirmed FRDA. Prenatal or preimplantation genetic diagnosis can be performed in families where both parents carry *FXN* repeat expansions. Genetic testing for confirmation of diagnosis of FRDA in patients with symptoms compatible with FRDA earned a Hayes rating D1 (no proven benefit and/or not safe). Hayes rating D2 (insufficient evidence) was assigned for confirmation or exclusion of diagnosis of FRDA in patients with idiopathic or apparently recessive ataxia. Hayes rating C (potential but unproven benefit) was assigned for confirmation or exclusion of diagnosis of FRDA in patients with idiopathic or apparently recessive ataxia to provide information to relatives at risk. Hayes rating D2 was assigned for carrier testing in relatives of individuals with genetically confirmed FRDA to facilitate reproductive

decision making. Hayes rating D2 was assigned for prenatal or preimplantation genetic diagnosis in families with genetically confirmed FRDA (Hayes Molecular Test Assessment Friedreich Ataxia (FRDA), 2011, updated 2014).

Molecular pathology tests for diseases or conditions that manifest severe signs or symptoms in newborns and in early childhood or that result in early death are generally not relevant to a Medicare member. Prenatal diagnostic testing and carrier screening are not a Medicare benefit.

## G6PD (Glucose-6-Phosphate Dehydrogenase)

The *G6PD* gene provides instructions for making an enzyme called glucose-6-phosphate dehydrogenase. More than 200 mutations in this gene cause Glucose-6-phosphate dehydrogenase (G6PD) deficiency, a disorder that affects red blood cells, which carry oxygen from the lungs to tissues throughout the body and is most common in males. The most common medical problem associated with glucose-6-phosphate dehydrogenase deficiency is hemolytic anemia. An estimated 400 million people worldwide have glucose-6-phosphate dehydrogenase deficiency. This condition occurs most frequently in certain parts of Africa, Asia, the Mediterranean, and the Middle East. It affects about 1 in 10 African American males in the United States (MedlinePlus, 2023a, 2023b).

The diagnosis of G6PD deficiency is made by a quantitative spectrophotometric analysis which is a rapid fluorescent spot test. If the blood spot fails to fluoresce under ultraviolet light the diagnosis is positive. Genetic tests may be used for population screening, family studies, or prenatal diagnosis (Frank, 2005). The presence of acute hemolytic anemia (AHA) will prompt testing for G6PD deficiency which is done via the standard spectrophotometric assay which is highly reliable. In most cases, the result will be clear cut and below the normal range (Luzzatto, 2016).

Screening services such as pre-symptomatic genetic tests and services used to detect an undiagnosed disease or disease predisposition, prenatal diagnostic testing, and carrier screening are not a Medicare benefit.

## Genetic Testing for Severe Inherited Conditions Carrier Screening Panel [e.g., Cystic Fibrosis, Ashkenazi Jewish-Associated Disorders (e.g., Bloom Syndrome, Canavan Disease, Fanconi Anemia Type C, Mucolipidosis Type VI, Gaucher Disease, Tay-Sachs Disease), Beta Hemoglobinopathies, Phenylketonuria, Galactosemia]

Carrier screening is not a Medicare benefit.

## Genome Sequencing (Unexplained Constitutional or Heritable Disorder Syndrome)

Hayes published a Clinical Utility Evaluation (2023) addressing the use of genetic testing, including WES and WGS, for individuals with clinically diagnosed autism spectrum disorder (ASD). Overall, Hayes found evidence from few very poor-quality studies supporting the use of genetic testing for in individuals with this disorder. Although limited evidence indicates that results of genetic testing may lead to additional testing and treatment recommendations in a portion of individuals tested, it is not clear if there are improved outcomes or any benefit in comparison with standard evaluation protocols.

In a 2022 (updated 2023) Clinical Utility Evaluation, Hayes found insufficient evidence for use of WES or WGS to assist with clinical decision-making and improve overall outcomes in adults with suspected neuromuscular disease or movement disorders. Limited, very low-quality evidence was found for WES; larger prospective studies investigating impact on clinical management and outcomes are required. For WGS, no studies investigating use in adults suspected to have neuromuscular or movement disorders were identified. Studies evaluating WGS data and its relationship to management and outcomes in individuals with these disorders are needed.

A Hayes Clinical Utility Evaluation (2021a, updated 2022) indicates uncertain clinical utility for WES and insufficient clinical utility for WGS when these technologies are used to inform clinical action and/or improve outcomes in children 18 years or younger with neurological phenotypes for whom a diagnosis has not been determined after standard diagnostic tests. In the case of WES, included studies (n = 12) documented changes in treatment and improved outcomes in a small portion of individuals tested (2-22%). For WGS, outcomes are from a small and narrowly defined population group focused on infants with neurological phenotypes; Hayes notes that additional studies evaluating both larger numbers and a broader range of children with neurological symptoms are required.

An additional Clinical Utility Evaluation (Hayes, 2021b, updated 2023) found insufficient evidence for utility of WES and WGS to guide clinical care in individuals with a primary phenotype of ID alone. This evaluation did does not address ID in individuals with other disorders including NDD or global DD, which are discussed in separate Hayes reports. No peer-reviewed studies were found that assessed clinical utility for individuals with a primary phenotype of ID.

In a 2021 publication, Krantz et al. reported the results of their investigation of the effect of WGS on the impact of clinical management of infants admitted to an intensive care unit (ICU) from 5 US children's hospitals. Their multicenter randomized trial incorporated a time-delayed study design and focused on selection of children whose providers suspected genetic disorder. Usual care was continued through the study, capturing variation in management, and helping with the assessment of real-world clinical situations. A total of 354 infants were enrolled from September 2017 to April 2019, with observation through July 2019. Infants between 0 and 120 days old were included (mean age = 15 days). The infants were randomized to receive WGS results either 15 days (early) or 60 days (delayed) after study enrollment. Infants were racially and ethnically diverse with a geographically distributed population in the US. The researchers indicated that twice as many infants in the early group vs the delayed group received a change in management (COM) (34 of 161 vs. 17 of 165) and molecular diagnosis (55 of 176 vs 27 of 178) at 60 days. COM and diagnostic efficacy doubled in the delayed group at 90 days (to 45 of 161 and 56/178, respectively). The study, however, showed no measurable difference in length of stay or survival. The authors concluded that comprehensive genomic testing of acute care infants can impact clinical management and that WGS specifically positively impacts patient care and should be considered for critically ill infants with suspected genetic disease as a primary tool. Of note, this study was industry sponsored and conflicts of interest were present which could have impacted choice of methods (in particular, outcomes), or the validity of the interpretation of the findings. In addition, the findings may not be generalizable to ICUs outside of tertiary referral centers, which may have a lower incidence of genetic disease. The relevance of study findings on clinical outcomes is unclear and was not examined in this study.

In a 2021 preliminary report, Smedley et al. shared results of their pilot study investigating the role of genome sequencing in individuals with undiagnosed rare diseases. The study included 2,183 families with a total of 4,660 participants who were recruited after having been identified by health care providers and researchers as having rare diseases that had not yet been diagnosed after receipt of standard care (including no diagnostic testing or approved diagnostic tests which did not include genome sequencing) in the UK National Health Service. Among the participants, 161 disorders including a broad array of rare diseases, was present. Data was collected on clinical features, genome sequencing was performed, and new pathogenic variants were identified through the analysis. The disease categories of participants being evaluated for rare genetic conditions included: cardiovascular disorder, ciliopathy, dermatologic disorder, dysmorphic or congenital abnormality, endocrine disorder, gastroenterological disorder, growth disorder, hematologic or immunologic disorder, hearing or ear disorder, metabolic disorder, intellectual disability, neurologic or neurodevelopmental disorder, ophthalmologic disorder, renal and urinary tract disorder, respiratory disorder, rheumatologic disorder, skeletal disorder, or tumor syndrome. The report indicates that diagnostic yields were highest in families with larger pedigrees and were higher for disorders likely to have a monogenic cause (35%) than for disorders with a complex cause (11%). Fourteen percent of diagnoses were made using a combination of automated approaches and research which was especially important for cases with etiologic noncoding, structural, and mitochondrial genome variants as well as variants which were not well covered by ES. In the course of the study, 3 new disease genes and 19 new associations were discovered. Ultimately, 25% of diagnoses that were made had immediate implications for clinical decision-making for affected individuals and their families. The researchers concluded that study showed an increase in diagnostic yield for rare diseases when genome sequencing was used and supports the case for using genomic sequencing when diagnosing certain specific rare diseases. However, the study did not include a comparison group and the relevance of the study findings on clinical outcome is only documented in the publication with anecdotal reports.

Malinowski et al. (2020) reported on the outcome of an American College of Medical Genetics and Genomics (ACMG) systematic review performed to assist with creation of an evidence-based guideline addressing the use of ES and GS. This ACMG practice guideline is included in the Clinical Practice Guidelines section of this policy. Primary literature including health, clinical, reproductive, and psychosocial outcomes resulting from ES/GS in individuals with CA/DD/ID was identified. Ultimately, 167 articles were included; these were largely case reports or small case series and of note, all but one study lacked a comparison group. Changes to clinical management or reproductive decision-making were the most frequently reported outcomes and were observed in nearly all included studies. Further, a significant number of the articles reported clinical impact on family members of the affected individual or an impact on reproductive outcomes. The authors concluded that for individuals with CA/DD/ID, ES and GS assists with clinical and reproductive decision-making, potentially improving outcomes for affected individuals and family members. However, there were some noted conflicts of interest and the relevance of these findings on clinical outcomes is not clear.

While following the ACMG guidelines to assess variant pathogenicity, Hou et al. (2020) conducted a prospective cohort study combining deep phenotyping with WGS. Participants were adults (n = 1,190) who consented to WGS and agreed to participate in metabolomics, clinical laboratory testing, advanced imaging and provide family/medical history. Phenotypic results were, subsequently, integrated with genomic results. Positive pathogenic findings suggesting a genetic risk predisposition, were found in 17.3% of adults. When genetic results were incorporated with deep phenotyping, 11% had observed genotype/phenotype correlations. Greater than 75% of these correlations included risk for dyslipidemia (n = 24), cardiomyopathy, arrhythmia/other cardiac conditions (n = 42) and endocrine/diabetic conditions (n = 17). Approximately

6% of participants with pathogenic variants did not have a genotype/phenotype correlation. Hou et al. concluded that results of this study and future studies can provide beneficial information to aid in precision medical practice. The authors indicated that this study did not measure health outcomes or benefits. Repeat evaluation of these individuals is required to characterize the clinical significance of the findings.

In order to analyze the application of WES and WGS as a routine diagnostic tool for patients, Smith et al. (2019) undertook a scoping review of the literature, following the Preferred Reporting Items for Systematic review and Meta-analysis (PRISMA) method of reporting observational studies. The timeframe from which they drew from the literature was 2009 to 2017, and they focused on diagnostic WES or WGS for infant and pediatric patients. A total of 171 articles were found, of which 131 were case reports, 40 were aggregate analysis and 4 were focused on a cost-effectiveness objective. The only metric consistently reported across all studies was diagnostic yield, and that varied broadly by clinical category and test type. In aggregate it was 33.2%. The authors concluded that multi-disciplinary research that focuses on consistency in outcome measurement is needed to demonstrate clinical utility.

Clark et al. (2018) conducted a meta-analysis comparing the diagnostic and clinical utility of WGS, WES and chromosome microarray (CMA) in children suspected of having genetic disease. Analysis of the literature from January 2011 to August 2017 was conducted following the Preferred Reporting Items for Systematic review and Meta-analysis (PRISMA) and Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines. Thirty-seven studies of 20,068 children were included. Overall, the diagnostic utility of WES and WGS was greater than CMA. In studies from only 2017, the diagnostic utility of WGS was greater than CMA. Among studies featuring in cohort comparisons, the diagnostic utility of WES was greater than CMA. The diagnostic utility between WGS and WES was not significantly different. In studies with in-cohort comparisons of WGS and WES, there was a greater chance of achieving a diagnosis when a trio was available than singleton testing, and with in-hospital interpretation versus a reference lab interpretation. In this study, clinical utility was defined as a change in clinical management. Cases where the only change was reproductive planning or a change in genetic counseling were excluded. The clinical utility of WES was greater, but not statistically significant, than CMA. However, WGS was higher for clinical utility than CMA, and met statistical significance ($p < 0.0001$). The authors identified several limitations with the meta-analysis, such as the heterogeneity of the pooled data, taking diagnostic rates at face value, and that only one study met the highest level of evidence criteria for clinical interventions. Overall, they concluded that more randomized, well designed and controlled clinical studies are needed but WES and WGS could be considered over CMA for a first-tier test in a child suspected of having a genetic diagnosis.

## *Prenatal Genetic Diagnosis or Screening*

A 2023 systematic review and meta-analysis by Shreeve et al. sought to determine the incremental yield of WGS over WES and/or CMA in fetuses and infants with an anomaly that either was or could have been detected via ultrasound in the prenatal period. Secondary outcomes included the assessment of turnaround time and quantity of DNA required for these tests. A total of 18 studies comprising 1,284 individual cases met inclusion criteria for the study. Eight studies (754 cases) were prenatal cohorts and the remaining ten studies included postmortem, neonatal, or infants demonstrating congenital structural abnormalities. The incremental yield of WGS over WES (1%) was not significant (95% CI 0%-4%, I2 = 4 7%). Yield of WGS over quantitative fluorescence-polymerase chain reaction (QF-PCR)/CMA was 26% for all (95% CI 18-36%, I2 = 86%), 16% for prenatal (9-24%, I2 = 85%), and 39% (95%CI 27-51%, I2 = 53%)for postnatal cases. Pooled median turnaround time for WGS was 18 days; only one study documented turnaround time for CMA/WES, so no comparison could be made. The study found a significant incremental yield with use of WGS compared to CMA for the genetic evaluation of congenital anomalies, but no significant increase in incremental diagnostic yield of WGS over WES. The authors note that there is currently insufficient evidence to promote the use of WGS over CMA and WES, but the use of WGS over standard pathways of testing uses less DNA and has the potential for faster turnaround times. Additional studies are recommended.

In a study assessing the diagnostic yield of prenatal genetic testing using trio WES and WGS compared to standard CMA, Miceikaite et al. (2023) found a 25% increase in diagnostic yield when trio WES/WGS was performed in pregnancies where CMA had been negative. Testing took place between the 12$^{th}$ and 21$^{st}$ week of gestation, and all pregnancies included (n = 40) had documented fetal anomalies or increased nuchal translucency (≥ 5 mm). For each pregnancy, trio WES or WGS and standard CMA were performed. Of the 40 total pregnancies, 16 were found to have a genetic sequence variation, CNV or aneuploidy which corresponded with the fetal phenotype; the overall diagnostic yield of WES/WGS was 40%. A total of six chromosomal abnormalities were detected via CMA and each of these was also identified by WES/WGS. An important finding was that WES testing yielded more consistent identification of mosaic sequence variations than WGS, related to the ability of WES to sequence more deeply. The researchers assert that although this study is limited by small sample size, the results bolster the existing evidence supporting higher diagnostic yield of WES/WGS over CMA and speculate that WES/WGS testing has promise for use as valuable, standalone testing for prenatal diagnostic use.

A 2020 (updated 2023) Hayes Clinical Utility Evaluation found that the evidence supporting WES and WGS related to improvement of diagnosis and assistance with pregnancy and post-pregnancy management when abnormalities are detected by ultrasound or other testing is lacking. Large studies including outcome data and impact on clinical management are required to support clinical utility for the use of WES and WGS in the prenatal setting.

Molecular pathology tests for diseases or conditions that manifest severe signs or symptoms in newborns and in early childhood or that result in early death are generally not relevant to a Medicare member. Screening services such as pre-symptomatic genetic tests and services used to detect an undiagnosed disease or disease predisposition and prenatal diagnostic testing are not a Medicare benefit.

## Genomic Prostate Score Assay (Previously Oncotype DX® Genomic Prostate Score)

In a retrospective study, Janes et al. (2023), evaluated whether the Oncotype Dx Genomic Prostate Score (GPS) is related to time to biochemical failure (BCF), distant metastasis (DM), and prostate cancer related death (PCD) in 238 individuals (69% Black) with localized PCa (any NCCN risk group) undergoing treatment with external beam radiation therapy (EBRT). The researchers aimed to gather data that would provide more information regarding whether the assessment of PCa progression risk could guide decisions regarding EBRT treatment intensity. Also evaluated was whether these associations were altered dependent on race. Study outcomes were time to BCF (per Phoenix criteria), DM, and PCD; median follow-up time for individuals who did not experience BCF was 7.6 years. Univariable analysis showed GPS results per 20-unit increase had a significant association with BCF (HR, 3.62; 95% CI, 2.59-5.02), DM (HR, 4.48; 95% CI, 2.75-7.38), and PCD (HR, 5.36; 95% CI, 3.06-9.76). In multivariable models that underwent adjustment for baseline clinical and pathological factors, GPS results were persistently significant with HRs similar to those in the univariable analysis. No significant association between GPS results and race were identified (p = .923) with HRs for BCF in Black individuals comparable to those in non-Black individuals (HR, 3.88; 95% CI, 2.40-6.24 and HR, 4.01; 95% CI, 2.42-6.45, respectively). The authors indicate that the results of this study support the GPS assay as a strong and independent predictor of time to BCF, DM, and PCD in individuals with PCa treated with EBRT and could help identify higher-risk individuals who should receive treatment intensification or deintensification. Limitations included the retrospective, nonrandomized study design and the incorporation of only a single institution. In addition, data from this study is most applicable to individuals at higher risk of adverse outcomes. Results of ongoing studies investigating the association of the GPS test with long-term outcomes in individuals who have undergone treatment with EBRT are needed before clinical utility can be established in this setting.

Helfand and colleagues (2022) sought to assess the association of the Oncotype DX GPS results with time to biochemical recurrence after prostatectomy in a group of participants with NCCN intermediate (n = 109) and higher (n = 32) risk PCa. A total of 141 individuals were included, all of whom had undergone radical prostatectomy. Univariable and multivariable Cox proportional hazards models were used to analyze the association of GPS results with time to biochemical recurrence in 120 of the participants. The median follow-up time was 28 months (20-38). The researchers found a significant relationship between GPS results and time to biochemical recurrence as both a continuous and dichotomous variable in univariable (HR per 20 GPS units 2.36, 95% CI 1.45–3.80, p < 0.001; HR for GPS result 41–100 vs 0–40 3.28, 95% CI 1.61–7.19, p < 0.001) and multivariable models accounting for NCCN risk group (HR per 20 GPS units 2.14, 95% CI 1.31–3.46, p = 0.003; HR for GPS result 41–100 vs 0–40 3.00, 95% CI 1.43–6.72, p = 0.003) or biopsy Gleason Score and diagnostic PSA or PSA density. This led the authors to conclude that the BPS assay was a strong prognostic indicator of biochemical recurrence after radical prostatectomy in this group of individuals with unfavorable intermediate and higher risk PCa and has potential for use in further stratification of individuals with unfavorable intermediate and/or high-risk disease. This information could, in turn, assist with clinical management decisions such as consideration of more aggressive treatments or de-escalation of therapy based on GPS results. Although the results of this study (funded by the manufacturer of the Oncotype DX GPS assay) are promising, the study was limited by its single-institution, retrospective design, and the initial treatment of all participants with radical prostatectomy which reduces the utility of the results with respect to other therapies. Further, high-quality studies which evaluate the GPS's relationship with outcomes after radiation therapy, with or without hormone treatment, and the clinical impact of mono versus multimodal treatment in individuals whose GPS results show higher risk are needed.

To further evaluate the association between the Oncotype DX Genomic Prostate Score (GPS) and final pathology (including extraprostatic extension [EPE], positive surgical margin [PSM] and seminal vesicle invasion [SVI]), a retrospective analysis of 749 individuals who had undergone Oncotype DX testing was performed by Covas Moschovas et al. (2022). After testing, the participants had robotic RP performed by the same surgeon. In odds ratio assessment with multivariable analyses per 20 point GPS change, GPS was an independent predictor of EPE (OR 1.8, 95% CI 1.4-2.3) and SVI (OR 2.1, 95% CI 1.3-3.4). Furthermore, percentage of cases with EPE and SVI increased with GPS quartile when they were grouped by quartile. Based on these results, the authors assert that the Oncotype DX GPS is significantly associated with adverse pathology after RP, noting that the risk of EPE and SVI will increase with the GPS, and contend

that the use of Oncotype DX GPS may help providers improve preoperative counseling and implement surgical plans for individuals with greater risk of EPE or other negative pathology.

In a 2021 publication (included in Hayes, Oncotype DX GPS Assay, 2018), Brooks et al. reported on the association between the Oncotype DX Genomic Prostate Score (GPS) and long-term (20 year) cancer outcomes following radical prostatectomy in a stratified cohort of 423 patients treated between 1987 and 2004. Death from other causes was a competing risk in the Cox regression of cause-specific hazards used for estimating absolute risk. The authors found that the GPS test appeared to have a low false discovery rate and was independently associated with both 20-year risk of distant metastases (DM) and prostate cancer-specific mortality (PCSM). Multivariable analysis with regression to the mean correction for this cohort estimated hazard ratios of 2.24 (95% CI, 1.49 to 3.53) and 2.30 (95% CI, 1.45 to 4.36) for DM and PCSM respectively, per 20-unit increase in GPS. The researchers concluded that the use of GPS testing can provide risk assessment of long-term outcomes in prostate cancer beyond just clinical factors and suggest that prospective studies should be pursued to validate the results found in this study.

Eggener et al. (2019, included in Hayes, Oncotype DX GPS Assay, 2018) performed a multi-center study seeking to validate the 17 gene Oncotype DX Genomic Prostate Score (GPS) gene expression assay when used on biopsy tissue to predict adverse pathology in a group of 1200 prospectively enrolled individuals with very low-, low-, and favorable intermediate-risk prostate cancer. A prespecified sub analysis of GPS from biopsy and its relationship with adverse pathology found on RP was performed on the group of participants who immediately proceeded to RP. A total of 114 individuals underwent RP and of those, 40 had adverse pathology. In this study, GPS results were shown to be a significant predictor of adverse pathology based on results of univariable analysis (odds ratio per 20 GPS units [OR/20 units]: 2.2; 95% CI 1.2-4.1; p = .008). Significance persisted after adjustments were made for biopsy Gleason score, clinical T-stage and logPSA (OR/20 units: 1.9; 95% CI 1.0-3.8; p = .04), or NCCN risk group (OR/20 units: 2.0; 95% CI 1.1-3.7; p = .02). The researchers also evaluated the impact of GPS scores on physician and patient attitudes about decision-making related to their management; Decisional Conflict Scores improved significantly (from 27 to 14) after GPS testing was performed. Based on the overall results, the authors concluded that the GPS assay was confirmed to be an independent predictor of adverse pathology at surgery and was also related to a reduction of patient conflict in terms of decision-making.

In an effort to evaluate the current utility of gene expression classifiers (GECs) related to management of newly diagnosed prostate cancer, Hu et al. (2018) conducted an observational study including individuals diagnosed with localized prostate cancer. Three GECs results (Decipher Prostate Biopsy, Oncotype Dx Prostate and Prolaris), along with data on how the results were used, were collected to determine practice patterns, predictors of the use of GEC and the effect of GEC results on the management of prostate cancer. Using the Michigan Urological Surgery Improvement Collaborative registry, the researchers determined that 18.8% of 3,966 individuals newly diagnosed with prostate cancer underwent testing with a GEC. The rate of use of GEC varied in individual practice settings from 0% to 93% and individuals that that had GEC testing were more likely to have lower prostate specific antigen level, lower Gleason score, lower clinical T stage and fewer positive cores (all p < .05). For those individuals with clinically favorable cancer risk, rate of active surveillance was significantly different among individuals with GEC results above the threshold (46.2%), those with a GEC results below the threshold (75.9%) and individuals who did not have GEC testing (57.9%). Based on these results, the authors estimate that for every nine individuals with favorable cancer risk that participate in GEC testing, one additional individual may be managed with active surveillance. Individuals with favorable-risk prostate cancer whose GEC results classified them as low risk were more likely to be managed with active surveillance than those who did not undergo testing, per the results of the multivariable analysis (odds ratio, 1.84; p = .006). The researchers concluded that that is currently high levels of variability among practices with regard to the use of GEC testing, but for individuals with clinically favorable risk, GEC can significantly increase the rate of active surveillance. Additional follow up to help determine whether the use of GEC testing should be included in the initial care of individuals with prostate cancer to improve clinical outcomes is encouraged.

A Molecular Test Assessment produced by Hayes evaluated the Oncotype DX GPS for utility in clinical decision-making for individuals with newly diagnosed, localized prostate cancer who met NCCN criteria for very low, low, or favorable intermediate-risk prostate cancer and were eligible for active surveillance. In terms of clinical validity, the body of evidence consistently favors use of the GPS assay to assist with management strategies for such individuals, however more clinical utility studies reporting on primary outcomes are recommended (Hayes, Oncotype DX Genomic Prostate Score [GPS] Assay [Genomic Health Inc.], 2018, updated 2022).

Brand et al. (2016) performed a meta-analysis of two independent clinical validation studies of a 17-gene biopsy-based genomic assay (Oncotype Dx Prostate Cancer Assay) as a predictor of favorable pathology at radical prostatectomy. Patient-specific meta-analysis was performed on data from 2 studies (732 patients) using the Genomic Prostate Score (GPS; scale 0-100) together with Cancer of the Prostate Risk Assessment (CAPRA) score or NCCN risk group as

predictors of the likelihood of favorable pathology (LFP). Risk profile curves associating GPS with LFP by CAPRA score and NCCN risk group were generated. Patient-specific meta-analysis generated risk profiles ensure more precise estimates of LFP with narrower confidence intervals either study alone. GPS added significant predictive value to each clinical classifier. The authors concluded that a model utilizing GPS and CAPRA provided the most risk discrimination, and in a decision curve analysis, greater net benefit was shown when combining GPS with each clinical classifier compared with the classifier alone. Although the clinical characteristics of the 2 patient cohorts were similar, there were nonetheless some key differences in the representation of different racial groups and higher risk patients. The risk estimates were numerically different in the 2 studies, although the confidence levels overlapped.

## *Clinical Practice Guidelines*

### National Comprehensive Cancer Network (NCCN)

NCCN Guidelines for Prostate Cancer (NCCN Prostate Cancer, v4.2024) state that although full assessment of their clinical utility requires prospective randomized clinical trials, which are unlikely to be done, the panel believes that patients with low or favorable intermediate disease and life expectancy greater than or equal to 10 years may consider the use of Oncotype DX Prostate during initial risk stratification. The guidelines recommend Oncotype Dx Prostate 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 received treatment for prostate cancer and are candidates for active surveillance or definitive therapy.

## HBA1/HBA2 (Alpha Globin 1 and Alpha Globin 2) (e.g., Alpha Thalassemia, Hb Bart Hydrops Fetalis Syndrome, HbH Disease)

Genetic testing for Alpha-thalassemia is used to diagnosis Hb Bart syndrome in a fetus with prenatal onset of generalized edema and pleural and pericardial effusions as a result of congestive heart failure induced by severe anemia. Usually, death occurs in the neonatal period. It is also used to diagnose Hemoglobin H disease, which usually develops in the first year of life. It is also used for carrier testing (Tamary and Dgany, 2024).

In a Hayes Molecular Test Assessment on Alpha-Thalassemia, it is noted that the genetic aspects of alpha-thalassemia are complicated in that it is caused by sequence variants affecting the hemoglobin, alpha 1 (*HBA1*) and hemoglobin, alpha 2 (*HBA2*) genes. For confirmation of diagnosis in an individual showing clinical features of HbH disease, Hayes assigns a rating of C (potential but unproven benefit). For postnatal confirmation of diagnosis in an individual with clinical features of Hb Bart hydrops fetalis syndrome, Hayes assigns a rating of D1 (no proven benefit and/or not safe). For identification of a newborn who is affected with alpha-thalassemia, Hayes assigns a rating of C. For carrier testing in an individual with a positive hematological test, from a family with confirmed alpha-thalassemia, from a high-risk ethnic background, or who is the reproductive partner of a known alpha-thalassemia carrier, Hayes assigns a rating of B (some proven benefit). For prenatal diagnosis or preimplantation genetic diagnosis to identify a fetus or embryo affected with alpha-thalassemia in a couple with known variants, Hayes assigns a rating of B (Hayes, Molecular Test Assessment Alpha-Thalassemia, 2015).

Molecular pathology tests for diseases or conditions that manifest severe signs or symptoms in newborns and in early childhood or that result in early death are generally not relevant to a Medicare member. Prenatal diagnostic testing and carrier screening are not a Medicare benefit.

## HBB (Hemoglobin, Subunit Beta) (e.g., Sickle Cell Anemia, Beta Thalassemia, Hemoglobinopathy)

Genetic testing is used to diagnose Beta-thalassemia which presents between 6-24 months of age with pallor due to severe anemia, poor weight gain, stunted growth, hepatosplenomegaly, and jaundice. The diagnosis of β-thalassemia is established in a proband older than age 12 months by identifying microcytic hypochromic anemia, anisopoikilocytosis with nucleated red blood cells on peripheral blood smear, absence of iron deficiency, and reduced or complete absence of hemoglobin A (HbA) and elevated hemoglobin A2 (HbA2) and often hemoglobin F (HbF) on hemoglobin analysis. Identification of biallelic pathogenic variants in *HBB* on molecular genetic testing can diagnosis those younger than 12 months of age who have a positive or suggestive newborn screening result and/or unexplained microcytic hypochromic anemia with anisopoikilocytosis and nucleated red blood cells on peripheral blood smear. It is also used for prenatal testing, preimplantation genetic testing, and carrier testing (Langer, 2024).

Sickle cell disease is caused by mutations in the *HBB* gene. Signs and symptoms typically start in early childhood (MedlinePlus, 2024c). It is diagnosed with a simple blood test. In the U.S., it is most often found at birth during routine newborn screening tests (CDC, 2024).

Methemoglobinemia, beta-globin type affects red blood cell function and is caused by mutations in the *HBB* gene. It can cause cyanosis which typically initially appears around 6 months of age (MedlinePlus, 2015a). The diagnosis is confirmed by arterial or venous blood gas with co-oximetry, which will speciate hemoglobin to determine the methemoglobin concentration and percentage (Ludlow et al. 2023).

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## Hearing Loss Panel (e.g., Non-Syndromic Hearing Loss, Usher Syndrome, Pendred Syndrome)

Nonsyndromic hearing loss (NSHL) is defined as partial or total hearing loss that does not occur with other medical conditions or symptoms. It is estimated that up to 3/1000 children are born with hearing loss in one or both ears. The frequency of hearing loss increases with age; by age 85 hearing loss is experienced by more than half of all people (MedlinePlus, 2016b).
Hayes conducted two reports; one that evaluated the clinical utility of genetic testing in those with or suspected of congenital and/or prelingual nonsyndromic hearing loss (2019, updated 2022) and a second report that evaluated the clinical utility of genetic testing in individuals with or suspected of postlingual nonsyndromic hearing loss (2019, updated 2022). Both reports found there was insufficient evidence to support genetic testing in these patient populations. Per Hayes, there were no studies identified that would inform decision making that resulted in improved patient management or influenced outcomes.

Palmer et al. (2013) performed a study to assess the impact of genetic testing on psychological well-being in adults diagnosed with hearing loss at birth to 6 years of age. The study included 263 eligible patients. A psychological evaluation was performed before and after genetic testing. The authors concluded that there is limited evidence to suggest that knowing genetic test results would impact patient perceived control, depression, and anxiety. (This study is included in the Hayes 2019 Genetic Testing in Patients with or Suspected of Postlingual Nonsyndromic Hearling Loss Clinical Utility Evaluation).

Pendred syndrome is usually associated with hearing loss and goiters. Usually, severe to profound hearing loss is apparent at birth. Less often, hearing loss does not develop until later in infancy or early childhood (MedlinePlus, 2016c).

Usher syndrome is an inherited disease that can cause major hearing loss and retinitis pigmentosa. In type 1, deafness is at birth. Type II accompanies moderate to severe hearing loss. Type III are born with normal hearing but develop problems with vision and then hearing loss. There is no cure for Usher syndrome (MedlinePlus, 2016d).

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## Hereditary Peripheral Neuropathies Panel

Charcot-Marie-Tooth (CMT) disease is a disorder that damages the peripheral nerves, which can cause loss of sensation and atrophy of the muscles in the feet, legs, and hands (MedlinePlus, 2020).

There is no cure for CMT, and maintaining mobility, flexibility, and muscle strength is important. Starting a treatment program early may delay or reduce nerve degeneration and muscle weakness before it progresses to the point of disability. Medications can be prescribed for severe nerve pain. Ongoing research includes efforts to identify more of the mutant genes and proteins that cause the various disease subtypes, discover the mechanisms of nerve degeneration and muscle atrophy with the goal of developing interventions to stop or slow down these debilitating processes, and to develop therapies to reverse nerve degeneration and muscle atrophy (NIH, 2024).

The diagnosis of CMT disease is based on physical symptoms, family history and clinical tests. These include nerve conduction velocity (NCV) electromyogram (EMG). Treatment is symptomatic and supportive, and there is no cure. With recent advances in molecular genetic testing using both deletion duplication analysis and next generation sequencing (NGS) for patients with a clinical diagnosis of CMT, a genetic cause can be found in about 60% of patients (NORD, 2021)

The large number of CMT causing genes is often challenging for clinicians and patients when trying to determine the underlying genetic diagnosis. There is little information available to guide which gene to test and testing a patient for mutations in all commercially available CMT genes is not realistic. Family planning and prognosis may require an accurate

genetic diagnosis and current treatment trials depend on knowing the genetic cause of a patient's CMT even if no cures are presently available (Miller et al. 2011).

Hayes Molecular Test Assessment for Charcot-Marie-Tooth Type 1A (*PMP22*) (2008, updated 2012) describes Charcot-Marie-Tooth disease Type 1A (CMT1A) as a peripheral demyelinating neuropathy caused by a 1.5-megabase (Mb) duplication of chromosome 17 at band p11.2. Product names include complete CMT Evaluation (#400) or *PMP22* Duplication/Deletion DNA Test (#131) (Athena Diagnostics Inc.); Inherited Peripheral Neuropathies (Charcot-Marie-Tooth Type 1A [CMT1A] and Hereditary Neuropathy with Liability to Pressure Palsies [HNPP]) FISH analysis (#8467) (Medical Genetics Laboratories [MGL] at Baylor College of Medicine [BCM]). For confirmation of diagnosis in an individual with suspected CMT based on clinical findings, Hayes assigns a rating of C (potential but unproven benefit). For an asymptomatic individual with a confirmed family history of CMT1A to establish personal risk, Hayes assigns a rating of C. For an oncology patient with unexplained or preexisting familial neuropathy consistent with CMT, Hayes assigns a rating of B (some proven benefit. Published evidence indicates that safety and impact on health outcomes are at least comparable to standard treatment/testing. However, there are outstanding questions regarding long-term safety and impact on health outcomes, clinical indications, contraindications, optimal treatment/testing parameters, and/or effects in different patient subpopulations). For prenatal or preimplantation genetic diagnosis of CMT1A, Hayes assigns a rating of B.

Hereditary neuropathy with liability to pressure palsies (HNPP) is a genetic disorder causes recurrent acute sensory and motor neuropathy in a single nerve or multiple nerves. Symptoms usually start at age 20-30s as non-painful focal sensory and motor neuropathy. There is no cure for HNPP and treatment is only to manage symptoms (Chrestian, 2020).

Hereditary sensory neuropathy type I (HSN1) is a genetic disorder that causes abnormalities impacting the nerves, especially of those of the hands and feet. Symptoms appear on average at 37 years old as hearing loss, and cognitive decline (dementia), sensory neuropathy. There is no cure for HSN and treatment is only to manage symptoms (NORD, 2017a).

Hereditary sensory and autonomic neuropathy type 1E (HSAN1E) is a rare genetic disorder that presents symptomatically usually at age 20-30s as hearing loss, cognitive decline, and sensory neuropathy. There is no cure for HSAN and treatment is only to manage symptoms (NORD, 2017b).

Hereditary spastic paraplegia (HSP) is an inherited neurologic disorder that manifests as difficulty walking due to muscle weakness and muscle tightness (spasticity) in the legs. There is no cure for HSP and treatment is only to manage symptoms (NORD, 2017c).

Screening services such as pre-symptomatic genetic tests and services used to detect an undiagnosed disease or disease predisposition and prenatal diagnostic testing are not a Medicare benefit.

## Hereditary Retinal Disorders Panel (e.g., Retinitis Pigmentosa, Leber Congenital Amaurosis, Cone-Rod Dystrophy)

Genetic testing is used to diagnose hereditary retinal disorders such as retinal pigmentosa, Leber congenital amaurosis, and cone-rod dystrophy. Retinal pigmentora first manifests as loss of night vision in children. Signs and symptoms are usually limited to vision loss (MedlinePlus, 2010). Leber congenital amaurosis presents as severe visual impairment starting at birth or shortly after birth (MedlinePlus, 2022b). Cone-rod dystrophy usually first manifest in children as decreased visual acuity and increased sensitivity to light (MedlinePlus, 2018a). It is also used for prenatal testing, preimplantation genetic testing, and carrier testing (Kumaran et al. 2023).

Molecular pathology tests for diseases or conditions that manifest severe signs or symptoms in newborns and in early childhood or that result in early death are generally not relevant to a Medicare member. Prenatal diagnostic testing and carrier screening are not a Medicare benefit.

## HTT (Huntingtin) (e.g., Huntington Disease)

The *HTT* gene provides instruction for making a protein called huntingtin, a protein that appears to play a role in neurons in the brain and is essential for normal development before birth. One region of the *HTT* gene contains a DNA segment known as CAG (cytosine, adenine, and guanine) trinucleotide repeat. Normally, the CAG segment is repeated 10 to 35 times within a gene. While individuals with a range of CAG repeats between 27 to 35 time are not at risk of developing symptoms of HD, they may be at risk of having a child with an allele in the Huntington Disease (HD)-causing range. HD diagnosis is made based on family history, clinical symptoms, and the detection of an expansion of 36 or more CAG trinucleotide segments in the *HTT* gene by molecular genetic testing, although individuals with 36 to 39 CAG repeats do not always develop symptoms of HD. Of note, is that pathogenic $(CAG)_n$ repeat expansions in *HTT* cannot currently be

detected by clinical sequence-based multigene panels, exome sequencing, or genome sequencing. The mean age of onset for HD is 35-45 years and the median survival time after onset is 15 to 18 years (Caron et al. 2020).

Pérez-Oliveira et al. (2024) conducted a multi-center study to investigate whether the number of CAG repeats of *HTT* is associated with the risk of developing certain tauopathies and its influence as a modulator of the clinical and neuropathological phenotype, and to evaluate the potential of polyglutamine staining as a neuropathological screening. The study included brain samples of 588 patients with neuropathological diagnoses of tauopathies (34 with corticobasal degeneration (CBD), 98 with progressive supranuclear palsy (PSP) and 456 with Alzheimer's disease (AD)) and a control group of 1070 patients that included 44 neuropathological controls. The authors reported that they identified significant differences in the number of patients with pathological *HTT* expansions in the CBD group (2.7%) and PSP group (3.2%) compared to control subjects (0.2%) and that there was a significant increase in the size of the *HTT* CAG repeats in the AD group compared to the control group. The authors concluded that the results indicated a link between *HTT* CAG repeat expansion with other non-HD pathology, which suggests that they could share common neurodegenerative pathways and that their findings support that genetic or histological screening for *HTT* repeat expansions should be considered in tauopathies. Limitations of the study included the retrospective design, the small number of cases per disease entity for CBD and PSP, and the lack of controls for possible concomitant pathology seen in neurodegenerative diseases.
Scarabino et al. (2022) conducted a follow-up study to validate their hypothesis that leukocyte telomere length (LTL) was strongly correlated with the estimated time to clinical onset in pre-HD subjects. The study included 90 participants, including 45 controls (mean age 41.9 +/- 10.5 years, 32.4% males) and 45 pre-HD patients (42.1% males) at baseline (T0) with a mean age of 41.9 +/- 10.4 years and then again after clinical onset at follow-up (T1) at a median of three years (mean age at follow-up was 45.3 +/- 10.2 years). The mean age at clinical HD onset was 44.4 +/- 10.7 years, which was about 2.5 (2.5 +/- 0.99) years before HD clinical onset. The CAG range in the participants was 39-51 repeats. The authors reported that, in pre-HD subjects at T0, LTL was significantly reduced by 22% compared to the controls and by 14% from T0 at T1, and that no relationship was observed between the LTL and CAG numbers in subjects that carried different CAG repeats at T0 and at T1. The authors also reported that the receiver operating characteristic (ROC) curve analysis showed that LTL measurement was extremely accurate in discriminating pre-HD subjects from the controls and pre-HD from manifest HD which yields a robust prognostic value in pre-HD subjects. The authors concluded that their follow-up study showed a marked reduction in telomere length in the pre-HD patients about 2.5 years before HD onset compared to the controls and independent of CAG size, and that the homogeneity allows a common cut-point of less than 0.70 T/S to be identified between pre-HD and manifest HD. The authors recommended longitudinal studies to complete the real timing of telomere shortening. Limitations of the study include the single-center design and the small sample size.

Fang et al. (2022) conducted a study to analyze potential protospacer adjacent motif (PAM) sites immediately following the genomic DNA/sgRNA complementary region to identify candidate single nucleotide polymorphisms (SNPs) for allele-specific CRISPR-Cas9-mediated targeting. The authors sequenced 1056 individuals with HD, including blood samples from individuals with HD from a French cohort and genomic DNA samples from the CHDI Foundation’s Enroll-HD cohort. The authors reported that they developed computational tools (NanoBinner and NanoRepeat) to de-multiplex the data, detect repeats, and phase the reads on the expanded or the wild-type *HTT* allele, and that one SNP that was common to 30% of individuals with HD of European ancestry emerged from the analysis and that this SNP was confirmed as a strong candidate for allele-specific deletion of the expanded *HTT* allele (m*HTT*) in human HD cell lines. The authors also reported that up to 57% HD individuals may be candidates for allele-specific editing through combinatorial SNP targeting. The authors conclude that they developed an experimental and computational workflow to resolve the SNP haplotypes near exon-1 of the *HTT* gene for allele specific editing in individuals affected with HD and that their workflow could be applied to other repeat expansion diseases to facilitate the design of guided RNAs for allele-specific gene editing. Limitations of the study include the single center design and the study’s focus on the SNP detection.

A 2008 (updated 2012) Hayes Molecular Test Assessment on Huntington Chorea/Disease (HD) for Diagnostic, Predictive, and Prenatal or Preimplantation Genetic Diagnosis Purposes states that that cause of HD is an expansion of a CAG trinucleotide repeat in exon 1 in the huntingtin (*HTT*) gene. For diagnosis of HD in patients with suspected HD in the absence of a family history of HD, Hayes assigns a rating of C (potential but unproven benefit). For diagnosis of HD in patients with suspected HD from families in which there is a history of HD, Hayes assigns a rating of D1 (no proven benefit and/or not safe). For asymptomatic individuals from families in with a history of HD to define personal risk, Hayes assigns a rating of D2 (insufficient evidence). For asymptomatic individuals from families with a history of HD to define risk of transmission, Hayes assigns a rating of B (some proven benefit). For prenatal testing with family history of HD, Hayes assigns a rating of B. For preimplantation testing from parents with penetrant genetic variation for HD, Hayes assigns a rating of C.

Screening services such as pre-symptomatic genetic tests and services used to detect an undiagnosed disease or disease predisposition, prenatal diagnostic testing, and carrier screening are not a Medicare benefit.

## IKBKAP (Inhibitor of Kappa Light Polypeptide Gene Enhancer in B-Cells, Kinase Complex-Associated Protein) (e.g., Familial Dysautonomia)

Genetic testing is used to diagnosis familial dysautonomia, a debilitating disorder present from birth. Symptoms include gastrointestinal dysfunction, recurrent pneumonia, vomiting crises, altered sensitivity to pain and temperature, blood pressure instability, hypotonia, decreased or absent deep tendon reflexes, decreased taste and absence of fungiform papillae of the tongue, alacrima determined either by history in infants older than 3 months of age or the Schirmer test, progressive vision loss, developmental delay, and intellectual disability. There is decreased life expectancy. It is also used for prenatal testing, preimplantation genetic testing, and carrier testing (Bar-Aluma, 2021).

Molecular pathology tests for diseases or conditions that manifest severe signs or symptoms in newborns and in early childhood or that result in early death are generally not relevant to a Medicare member. Prenatal diagnostic testing and carrier screening are not a Medicare benefit.

## JAK2 (Janus Kinase 2) and MPL (MPL Proto-Oncogene, Thrombopoietin Receptor) (Myeloproliferative Disorders)

### *Myeloproliferative Disorders*

Myeloproliferative disorders are a group of conditions that cause abnormal growth of blood cells in the bone marrow. They include PV, ET, PMF, and chronic myelogenous leukemia (CML). The WHO further classifies PV, ET, and PMF as Philadelphia chromosome negative myeloproliferative neoplasms (MPNs). The diagnosis of a MPN is suspected based upon clinical, laboratory, and pathological findings (i.e., bone marrow morphology). MPNs are related, but distinct from, myelodysplastic syndromes (MDS). In general, MDS are characterized by ineffective or dysfunctional blood cells, while MPN are characterized by an increase in the number of blood cells.

### *Polycythemia Vera (PV)*

PV is a chronic MPD characterized by increased hemoglobin, hematocrit, and red blood cell mass. There is an associated increased risk for thrombosis and transformation to acute myelogenous leukemia (AML) or PMF; however, patients are often asymptomatic. Criteria for a diagnosis of PV are based upon complete blood count (CBC) and clinical features. The *JAK2 V617F* mutation is present in the vast majority of PV, accounting for approximately 90% of cases. Functionally similar mutations in *JAK2* exon 12 account for most remaining cases of *JAK2 V617F* mutation-negative PV. Together, they are identified in 98% of PV cases and lead to high diagnostic certainty.

Among the proposed revised WHO criteria for diagnosis is presence of the somatic *JAK2 V617F* mutation or functionally similar exon 12 mutation. Absence of a *JAK2* mutation, combined with normal or increased serum erythropoietin level, greatly decreases the likelihood of a PV diagnosis. WHO proposed revision criteria for PV do not address additional molecular markers, including *CALR* mutation status.

### *Essential Thrombocythemia (ET)*

ET is a disorder of sustained increased platelet count. The majority of ET patients (60%) carry a somatic *JAK2 V617F* mutation, while a smaller percentage (5-10%) have activating *MPL* mutations. Revision to the WHO criteria for diagnosis of ET has been proposed and includes exclusion of PV, PMF, CML, MDS, or other myeloid neoplasm. Also included in the proposed major criteria for diagnosis is demonstration of somatic *JAK2 V617F* mutation or *MPL* exon 10 mutation (Tefferi et al. 2014). Proposed criteria additionally state that 70% of patients without a *JAK2* or *MPL* mutation carry a somatic mutation of the *CALR* gene. Among confirmed ET cases, mutations in *CALR* are more common than *MPL*. Positive *CALR* mutation status is suggested as indicating a more indolent course (Klampfl et al. 2013).

### *Primary Myelofibrosis (PMF)*

PMF is a rare disorder in which the bone marrow is replaced with fibrous tissue, leading to bone marrow failure. Clinical features are similar to ET. The approximate incidence is 1 in 100,000 individuals. Persons can be asymptomatic in the early stages of the disease. For such patients, treatment may not initially be necessary. Progression of the disease can include transformation to AML. Treatment is generally symptomatic and aimed at preventing complications.

Demonstration of a clonal marker is important for diagnosis. Somatic molecular markers in PMF patients are similar to those in patients with ET, and include *JAK2 V617F*, *MPL,* and *CALR*. Somatic mutations in *JAK2* are identified in 50-60% of PMF cases, and *MPL* mutations in 10%. Mutations in *CALR* are less common than *JAK2*, but more common than *MPL*.

Rumi et al. (2014) performed a study to evaluate *JAK2* or *CALR* mutation status in defining subtypes of essential thrombocythemia (ET) and the impact on clinical course and outcomes. 1,235 ET or PV patients were included in the study. The mutant allele burden was lower in *JAK2*-mutated than in *CALR*-mutated essential thrombocythemia. Patients

with *JAK2* (*V617F*) mutations were more advanced in age, had a more elevated white blood cell count and hemoglobin level, and lower serum erythropoietin and platelet count compared to those with *CALR* mutation. Hematologic parameters of patients with *JAK2*-mutated ET or polycythemia vera (PV) were correlated to the mutant allele burden. Although no polycythemic transformation was seen in *CALR*-mutated patients, the cumulative risk was 29% at 15 years in those with *JAK2*-mutated ET. No substantial difference in myelofibrotic transformation was found between the 2 subtypes of ET. Patients with *JAK2*-mutated ET and those with PV had a comparable thrombosis risk, which was two times more than that of patients with the *CALR* mutation. The authors concluded the findings support the notion that *JAK2*-mutated ET and PV represent different phenotypes of a single myeloproliferative neoplasm. *CALR*-mutated ET is a distinct disease entity.

Pardanani et al. (2006) performed a cohort study on *MPL515* mutations in myeloproliferative and other myeloid disorders. DNA from 1,182 patients with myeloproliferative and other myeloid disorders and 64 healthy controls were tested for *MPL515* mutations, regardless of *JAK2V617F* mutational status: 290 with myelofibrosis with myeloid metaplasia (MMM), 318 with essential thrombocythemia (ET), 242 with polycythemia vera (PV), 118 with chronic myelomonocytic leukemia, 88 with myelodysplastic syndrome, and 126 with acute myeloid leukemia (AML). *MPL515* mutations, either *MPLW515L* (n = 17) or a previously undescribed *MPLW515K* (n = 5), were noted in 20 patients. The diagnosis of patients with mutant *MPL* alleles at the time of testing was de novo MMM in 12 patients, post-ET MMM in 1, MMM in blast crisis in 3, and ET in 4. 6 patients harbored both the *MPLW515L* and *JAK2V617F* alleles. The authors concluded that there were *MPLW515K* or *MPLW515L* mutations in patients with ET OR MMM at an approximate frequency of 5% and 1%, respectively, but are not seen in patients with PV or other myeloid disorders. *MPL* mutations may occur simultaneously with the *JAK2V617F* mutation, which suggests that these alleles may have functional complementation in myeloproliferative disease.

## *Clinical Practice Guidelines*

### British Committee for Standards in Haematology

The thrombopoietin receptor *MPL* is one of several *JAK2* cognate receptors and is considered essential for myelopoiesis. The mutation frequency of *MPL* mutations associated with myeloproliferative disorders is substantially less (< 10%) than *JAK2* mutations. The guideline group for the British Committee for Standards in Haematology (Harrison et al. 2010) recommended a modification to the 2008 WHO criteria for ET to include the presence of an acquired pathogenetic mutation (e.g., in the *JAK2* or *MPL* genes). Therefore, *MPL* gene testing may be indicated for individuals who would meet WHO's diagnostic criteria for MPD if a clonal marker were identified.

## KIT (V-Kit Hardy-Zuckerman 4 Feline Sarcoma Viral Oncogene Homolog)

The NCCN Biomarkers Compendium assigns a 2A Rating for *KIT* and Myeloproliferative Neoplasms (MPN).

Cattaneo et al. (2021) performed a single-center cohort study to evaluate the bone marrow morphology and the clinical-laboratory parameters of triple-negative (lack of demonstrable mutations affecting *JAK2*, *CALR*, or *MPL,* which is found in about 10% of patients with essential thrombocythemia (ET) and 5–10% of those with primary myelofibrosis (PMF)) ET patients, as well as to determine their molecular profile using next-generation sequencing (NGS) to identify any potential clonal biomarkers. 40 triple-negative ET patients were included in the study. Nucleotide variants were identified in 35 out of 40 patients. 29 subjects harbored 1 or 2 variants and 6 cases displayed 3 or more concomitant nucleotide changes. The most frequent sequence variants involved the *TET2* gene (55.0%), followed by *KIT* (27.5%). Histologically, most of the cases showed a classical ET morphology. Prevalent megakaryocytes morphology was more often polymorphic with a mixture of giant megakaryocytes with hyperlobulated nuclei, normal and small sized maturing elements, and naked nuclei. In 5 cases a mild degree of reticulin fibrosis (MF-1) was evident together with an increase in the micro-vessel density. By means of NGS enabled identification of nucleotide variants in most cases, thus the findings suggest that a significant proportion of triple-negative ET patients do have a clonal disease. In analogy with driver genes-mutated MPNs, these findings may prevent issues arising concerning triple-negative ET treatment, especially when a cytoreductive therapy may be warranted.

Chen et al. (2016) performed a systematic review and meta-analysis to evaluate the prognostic significance of *KIT* mutations in core-binding factor acute myeloid leukemia (CBF-AML). Negative effect was indicated on relapse risk of CBF-AML (RR [relative risk], 1.43; 95%CI [confidence interval], 1.20–1.70) and t(8;21) AML (RR, 1.70; 95% CI, 1.31–2.21), not on OS of CBF-AML (RR, 1.09; 95% CI, 0.97–1.23), CR (OR [odds ratio], 0.95; 95% CI, 0.52–1.74), relapse risk (RR, 1.12; 95% CI, 0.90–1.41) or OS (RR, 1.03; 95% CI, 0.90–1.18) of inv(16) AML. Subgroup analysis of t(8,21) AML showed negative effect of *KIT* mutations on CR (OR, 2.03; 95%CI: 1.02–4.05), relapse risk (RR, 1.89; 95%CI: 1.51–2.37) and OS (RR, 2.26; 95%CI: 1.35–3,78) of non-Caucasians, not on CR (OR, 0.61; 95%CI: 0.19–1.95) or OS (RR, 1.12; 95%CI: 0.90–1.40) of Caucasians. The authors concluded that *KIT* mutations in CBF-AML to be included in the initial routine diagnostic workup and stratification system of t(8,21) AML. Limitations of the study were that the data was abstracted from published reports at a study-level analysis, thus one must consider the substantial effect of heterogeneity.

Another limitation was that observational prospective studies for rare diseases are hard to conduct and the observational nature of the studies pose residual confounders.

Mikami et al. (2013) analyzed c-kit and α-smooth muscle actin expression to identify precursors for clinical gastrointestinal stromal tumor (GIST). Pathology files from Kitasato University East Hospital were reviewed and 74 lesions of small gastric stromal tumors were included in the study. 68 of 74 lesions were classified into 4 representative groups according to the expression of c-kit and α-smooth muscle actin (αSMA): group A, c-kit diffusely positive and αSMA negative (18 cases); group B, c-kit diffusely positive and αSMA focally positive (13); group C, c-kit focally positive and αSMA diffusely positive (27); and group D, c-kit negative and αSMA diffusely positive (10). Groups A and B of c-kit diffuse expression revealed increased cellularity and labeling indices of p27(Kip1) and Ki-67 than did groups C and D of diffuse αSMA expression. Incidence of *KIT* exon 11 mutation in groups A and B was 86% (25/29) and groups C and D was 0% (0/20). The authors concluded that small gastric stromal tumors with c-kit diffuse expression were considered precursors for clinical GIST because they were substantially different from c-kit focally positive or negative tumors. The mutation of *KIT* is considered an early event in tumorigenesis of GIST.
Carvajal et al. (2011) performed a single-group, open-label, phase 2 trial at 1 community and 5 academic oncology centers in the U.S. to assess the clinical effects of imatinib mesylate in melanoma harboring *KIT* alterations patients. 295 patients with melanoma screened for *KIT* mutations and amplification were included in the study. 51 cases with such alterations were identified and 28 of these patients were treated who had advanced unresectable melanoma stemming from mucosal, acral, and chronically sun-damaged sites. 2 complete responses lasting 94 (ongoing) and 95 weeks, 2 durable partial responses lasting 53 and 89 (ongoing) weeks, and 2 transient partial responses lasting 12 and 18 weeks among the 25 evaluable patients were studied. The overall durable response rate was 16% (95% confidence interval [CI]), 2%-30%), with a median time to progression of 12 weeks (interquartile range [IQR], 6-18 weeks; 95% CI, 11-18 weeks), and a median overall survival of 46.3 weeks (IQR, 28 weeks-not achieved; 95% CI, 28 weeks-not achieved). Response rate was greater in cases with mutations affecting recurrent hotspots or with a mutant to wild-type allelic ratio of more than 1 (40% vs 0%, P = .05). This indicated a positive selection for the mutated allele. The authors concluded that within patients with advanced melanoma harboring *KIT* alterations, treatment with imatinib mesylate results in substantial clinical responses in a subset of patients. Responses may be limited to tumors harboring *KIT* alterations of proven functional relevance. Trial Registration clinicaltrials.gov Identifier: NCT00470470.

Handolias et al. (2010) performed an observational study to assess the clinical responses with imatinib or sorafenib in melanoma patients expressing mutations in *KIT*. 32 metastatic acral or mucosal melanoma patients were screened for *KIT* exons 11, 13 and 17 mutations. *KIT* mutations were identified in and in 6% of acral melanomas and 38% of mucosal melanomas. 1 patient was treated with sorafenib and 3 patients were treated with imatinib. All 4 patients responded to treatment, but 3 have since advanced within the brain.

Fontalba et al. (2006) performed a comparative study to identify *c-Kit* gene mutations in polycythemia vera patients. Imatinib mesylate has been reported to have clinical activity in the treatment of polycythemia vera (PV), suggesting the involvement of one of the kinases targeted by this inhibitor, including *PDGFR* and *c-Kit*. Activating *c-Kit* mutations have been noted in patients with mastocytosis and other myeloid disorders such as acute myeloid leukemia. The study evaluated the presence of mutations of *c-Kit* in polycythemia vera patients. 7 out of 20 patients carried missense mutations in the *c-Kit* gene and no sequence variation was detected in 15 healthy controls.

Curtin et al. (2006) evaluated array comparative genomic hybridization data from 102 primary melanomas (28 from acral skin, 38 from mucosa, and 18 from skin with and 18 from skin without chronic sun-induced damage) for DNA copy number aberrations specific to melanoma subtypes where mutations in *BRAF* and *NRAS* are uncommon. A narrow amplification on 4q12 was found, and candidate genes within it were studied. Oncogenic mutations in *KIT* were found in 3 of 7 tumors with amplifications. Examination of all 102 primary melanomas found mutations and/or copy number increases of *KIT* in 36% of acral, 39% of mucosal, and 28% of melanomas on chronically sun-damaged skin, but in no (0%) melanomas on skin without chronic sun damage. 79% of tumors with mutations and 53% of tumors with multiple copies of *KIT* showed elevated *KIT* protein levels. The authors concluded that *KIT* is a valuable oncogene in melanoma. Since the majority of the *KIT* mutations that were found in melanoma also occur in imatinib-responsive cancers of other types, imatinib may offer an immediate therapeutic benefit for a substantial proportion of the global melanoma burden.

## MCOLN1 (Mucolipin 1) (e.g., Mucolipidosis, Type IV)

Genetic testing is used to diagnosis Mucolipidosis IV, a very rare lysosomal storage disorder characterized by severe psychomotor delay, progressive visual impairment, and achlorhydria. Symptoms manifest usually by the end of the first year of life as delayed developmental milestones and impaired vision. It is also used for prenatal testing, preimplantation genetic testing, and carrier testing (Misko et al. 2021).

Molecular pathology tests for diseases or conditions that manifest severe signs or symptoms in newborns and in early childhood or that result in early death are generally not relevant to a Medicare member. Prenatal diagnostic testing and carrier screening are not a Medicare benefit.

## MECP2 (Methyl CpG Binding Protein 2) (e.g., Rett Syndrome)

Genetic testing is used to diagnose Rett Syndrome, a progressive neurodevelopmental disorder mostly affecting girls. Rett Syndrome is characterized by apparently normal psychomotor development at 6-18 months of age, followed by a short period of developmental stagnation, then with rapid regression in motor and language skills and then long-term stability. In boys, it is used to diagnose severe neonatal-onset encephalopathy, which is characterized by a relentless clinical course that follows a metabolic-degenerative type of pattern, involuntary movements, abnormal tone, severe seizures, and abnormal breathing. Often, death occurs before 2 years of age. It is also used for prenatal testing, preimplantation genetic testing, and carrier testing (Kaur, 2019).

In a Hayes Molecular Test Assessment on *MECP2* Testing for Rett Syndrome and Other Disorders, for testing for *MECP2* sequence variants in patients who meet established clinical diagnostic criteria for classic or variant RS, Hayes assigns a rating of C (potential but unproven benefit). For testing for *MECP2* sequence variants in patients who have some symptoms of RS but do not meet established clinical diagnostic criteria, Hayes assigns a rating of B (some proven benefit. Published evidence indicates that safety and impact on health outcomes are at least comparable to standard treatment/testing. However, there are outstanding questions regarding long-term safety and impact on health outcomes, clinical indications, contraindications, optimal treatment/testing parameters, and/or effects in different patient subpopulations). For prenatal testing for *MECP2* sequence variants in the parents of children with RS who meet the established clinical diagnostic criteria and for whom there is evidence that RS was inherited rather than having occurred sporadically, Hayes assigns a rating of B. For testing for *MECP2* sequence variants in disorders other than RS, including autism, Angelman syndrome, X-linked intellectual disability, intellectual disability, *MECP2* duplication syndrome, schizophrenia, and other psychiatric disorders, Hayes assigns a rating of D2 (insufficient evidence) (Hayes, Molecular Test Assessment *MECP2* Testing for Rett Syndrome and Other Disorders, 2010, updated 2014).

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## MTHFR (5,10-Methylenetetrahydrofolate Reductase)

Valeriani et al. (2023) performed a comprehensive systematic review and meta-analysis of Factor V Leiden, prothrombin, *MTHFR*, and *PAI-1* gene polymorphisms in patients with arterial disease. The study included 377 studies for 98,186 patients (32,791 CVD, 62,266 CHD, 3129 PAD) and 108,569 controls. Overall, 37,249 patients had *G1691A*, 32,254 *G20210A*, 42,546 *MTHFR C677T*, 8889 *MTHFR A1298C*, and 19,861 *PAI-1 4G/5G* gene polymorphisms. In CVD patients, PPs were 6.5 % for *G1691A*, 3.9 % for *G20210A*, 56.4 % for *MTHFR C677T*, 51.9 % for *MTHFR A1298C*, and 77.6 % for *PAI-1*. In CHD, corresponding PPs were 7.2 %, 3.8 %, 52.3 %, 53.9 %, and 76.4 %. In PAD, PPs were 6.9 %, 4.7 %, 55.1 %, 52.1 %, and 75.0 %, respectively. Strongest ORs in CVD were for homozygous *G1691A* (2.76; 95 %CI, 1.83-4.18) and for homozygous *G20210A* (3.96; 95 %CI, 2.05-7.64). Strongest ORs in CHD were for homozygous *G1691A* (OR 1.68; 95%CI, 1.02-2.77) and *G20210A* (heterozygous 1.49 95%CI, 1.22-1.82; homozygous 1.54 95%CI, 0.79-2.99). The OR for *PAI-1* 4G/4G in PAD was 5.44 (95%CI, 1.80-16.43). Data for *MTHFR C677T* and *A1298C*, and *PAI-1* gene polymorphisms are weaker than for other forms of inherited thrombophilia. No significant differences between cases and controls were found, except for *MTHFR C677T* in cerebrovascular disease and of *PAI-1 4G* in peripheral arterial disease. Specific subgroups with higher PPs and ORs were identified according to age and region.

Bezemer et al. (2007) performed a large multicenter study to assess the effect of the *MTHFR* genotype and the risk of venous thrombosis. DNA from patients in the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA Study), was collected which included 4,375 patients with first deep vein thrombosis or pulmonary embolism and from 4,856 control subjects. *MTHFR 677C- >T* was not associated with venous thrombosis risk (odds ratio [95% confidence interval], 0.99 [0.91-1.08] for the *CT* genotype and 0.94 [0.81-1.08] for the *TT* genotype). Stratification by known risk factors for venous thrombosis showed no association in specific groups. The authors concluded that *MTHFR 677C-- > T* was not associated with venous thrombosis risk. The narrow confidence interval excludes even a minor effect. Slight elevation of homocysteine levels as a result of *MTHFR 677TT* do not appear to cause venous thrombosis. For clinical purposes, there is no rationale for measuring the *MTHFR 677C-- > T* variant.

## *Clinical Practice Guidelines*

### American College of Medical Genetics and Genomics (ACMG)

ACMG (Hickey et al. 2013) published a practice guideline on the lack of evidence for *MTHFR* polymorphism testing. Among a number of recommendations, ACMG experts concluded that *MTHFR* polymorphism genotyping should not be ordered as part of the clinical evaluation for thrombophilia or recurrent pregnancy loss and that *MTHFR* polymorphism genotyping should not be ordered for at-risk family members. However, in an addendum in 2020, it is noted that the document no longer meets the criteria for an evidence-based practice guideline by the College and has been reclassified as a Clinical Practice Resource.

### British Society of Haematology

In an updated guideline for thrombophilia testing by Arachchillage et al. (2022), it is noted that a large number of variants in other genes with a wide range of prevalence have been reported to spark discussions regarding increased risk of thrombosis. Included are variants of *SERPINE1* (encoding plasminogen activator inhibitor type 1) (*PAI-1*), methylenetetrahydrofolate reductase (*MTHFR*), and factor XIII as well as variants linked to the quantitative changes in procoagulant factors. However, either their association with thrombosis is not compellingly consistent or their impact is too minor to change management. Therefore, they should not be included in thrombophilia panels at present.
In summary, despite many earlier publications suggesting a link between *MTHFR* polymorphisms and a risk for a wide spectrum of obstetric and cardiovascular complications, it is now accepted that *MTHFR* genotype alone is not associated with venous thromboembolism (VTE). There is no clinical indication for *MTHFR* genotyping in any population. There is broad consensus in the medical literature that *MTHFR* genotyping has no clinical utility in any clinical scenario. This testing is considered investigational and is not a Medicare benefit.

## Nuclear Encoded Mitochondrial Genes Panel (e.g., Neurologic or Myopathic Phenotypes) and Whole Mitochondrial Genome [e.g., 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), Kearns-Sayre Syndrome, Chronic Progressive External Ophthalmoplegia]

Genetic testing is used to diagnose mitochondrial diseases such as Kearns-Sayre syndrome (KSS), Leber hereditary optic neuropathy (LHON), Leigh syndrome, and mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS) syndrome. Kearns-Sayre syndrome affects many parts of the body but especially the eyes (progressive external ophthalmoplegia) and symptoms usually appear before the age of 20 (MedlinePlus, 2011b). Leber hereditary optic neuropathy is an inherited vision loss that usually begins in teens or twenties (MedlinePlus, 2013). Leigh syndrome manifests as progressive loss of mental and movement abilities and is first seen in the first year of life. Death usually follow 2-3 years later due to respiratory failure (MedlinePlus, 2023c). MELAS symptoms of muscle weakness and pain, loss of appetite, vomiting, recurrent headaches, and seizures usually appear in childhood (MedlinePlus, 2013).

In a primary mitochondrial disorders overview (Chinnery, 2021), the author notes that comprehensive genomic testing does not require a clinician to determine the likely involved gene. Comprehensive testing includes genome sequencing, exome sequencing, and mitochondrial sequencing which can analyze nuclear DNA and mtDNA simultaneously. However, some mtDNA pathogenic variants may not be identified in leukocyte DNA. Genomic testing may not be as accurate as targeted single-gene testing or multigene molecular genetic testing panels due to false negative rates that vary by genomic region. Therefore, most laboratories validate positive results by using a second, well-established method. Some DNA variants, such as large deletions or duplications (> 8-10 bp in length), epigenetic alterations, and triplet repeat expansions, may not be detected through genomic testing.

In a Hayes Precision Medicine Insight on Whole Mitochondrial Genome Sequencing, there appeared to be minimal support in individuals with suspected mitochondrial disorders, based on a review of abstracts. This review included no reported changes in patient management or decision making, one that reported diagnostic yield but changes in clinical management or patient outcomes were not reported, and no comparison of its utility against other testing methods. Review of professional guidelines and position statements appeared to show weak support for individuals with suspected mitochondrial disorders. 4 guidelines were identified including ACMG, National Health Service Rare Mitochondrial Disorders Working Group, Association for Clinical Genomic Science, and Mitochondrial Medicine Society. All 4 were expert consensus based, and 2 had overlapping authorship. They generally recommended mitochondrial DNA testing for patients with suspected mitochondrial disorders. The guidelines varied in respect to whether focused testing versus more comprehensive testing methods should be used as a first step in genetic diagnosis. (Hayes Precision Medicine Insight Whole Mitochondrial Genome Sequencing, 2024).

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## myPath® Melanoma

Melanoma is an aggressive cancer with an estimated 100,640 cases and 8,290 deaths in 2024 (ACS, 2024a). The lifetime risk of developing melanoma in the United States is approximately 2.6% (1 in 38) for Caucasians, 0.1% (1 in 1,000) for African Americans, and 0.6% (1 in 167) for Latinos. Melanoma is more common in men overall, but before age 50 the rates are higher in women than in men. The average age of people diagnosed with melanoma is 65. Many melanomas are curable if detected early and diagnosed accurately. The five-year survival for localized melanoma is > 99%, compared with only 35% among patients with distant metastases (ACS, 2024b).

Melanoma can be difficult to diagnose, particularly in its earliest stages, yet accurate diagnosis of melanocytic neoplasms is vital to optimal patient outcomes. Histopathologic examination has long been the gold standard for melanoma diagnosis, and while it is adequate for most cases, evidence suggests that approximately 15-20% of all biopsied melanocytic neoplasms are difficult to diagnose by histopathology alone (Shoo et al. 2010, Veenhuizen et al 1997, Ronen et al. 2021). Subspecialty training and experience in dermatopathology is associated with improved diagnostic accuracy and subsequent clinical management of patients with challenging melanocytic lesions (Ronen et al. 2021, Piepkorn et al. 2019, Tosteson et al. 2021, Elder et al. 2018). However, even experienced dermatopathologists disagree in some cases, and, depending on the type of lesions evaluated, diagnostic discordance may be substantial (Piepkorn et al. 2019, Tosteson et al. 2021, Farmer et al. 1996, Cerroni et al. 2010). In equivocal cases, patients may receive diagnoses that are indeterminate or inaccurate, leading to inappropriate treatment. Unnecessary re-excisions, sentinel lymph node biopsies, and protracted clinical follow-up may result when a diagnostically challenging benign lesion is reported as indeterminate (Hawryluk et al. 2012, McGinnis et al. 2002). Conversely, a diagnostically challenging melanoma mistakenly classified as a benign nevus may result in undertreatment and subsequent progression to late-stage melanoma (Hawryluk et al. 2012, McGinnis et al. 2002). Consequently, adjuncts to histopathology have been sought in efforts to improve diagnostic accuracy in equivocal cases.

Gene expression profiles (GEP) can serve as beneficial adjuncts to histopathology in the evaluation of equivocal melanocytic lesions. The myPath® Melanoma assay (Castle Biosciences, Phoenix, AZ) is a 23-gene expression profile (23-GEP) developed to provide an objective, reproducible, and accurate adjunctive method for differentiating malignant melanoma from benign nevi (Warf et al. 2015, Clarke et al. 2015, Clarke et al. 2017, Ko et al. 2017). The test is intended for use by dermatopathologists confronting primary cutaneous melanocytic neoplasms for which the diagnosis of malignant melanoma versus benign nevus is equivocal/uncertain (i.e., a clear distinction between benign or malignant cannot be achieved using clinical and/or histopathological features alone). Use of the test in these cases increases definitive diagnoses, and evidence suggests it may reduce unnecessary procedures in benign lesions (Cockerell et al. 2016, Cockerell et al. 2017).

The myPath® Melanoma test quantifies the expression of 23 genes by quantitative reverse transcription-polymerase chain reaction (RT-PCR). Fourteen of the 23 genes are known to be over-expressed by malignant melanomas relative to benign nevi. The remaining 9 are stably expressed reference genes which allow correction for sample-to-sample variations in RT-PCR efficiency and errors in sample quantification (normalization). The signature genes represent 3 distinct pathways that contribute to melanoma pathogenesis, including aspects of melanocyte differentiation as well as characteristics of the tumor microenvironment such as cell-cell signaling and tumor-induced host immune responses (Warf et al. 2015, Clarke et al. 2015). The test uses 5 to 7 standard-thickness (4-5 µm) sections taken from the routinely processed formalin-fixed paraffin-embedded (FFPE) tissue of the existing biopsy specimen, allowing its integration into routine clinical practice and its use even in small, early-stage lesions. The quantified expression of all 23 genes is combined algorithmically and reported as a single numerical score. That number, (the myPath® Melanoma 'score'), is plotted on a scale that depicts the entire range of scores observed in clinical validation studies (Clarke et al. 2015). Physicians receive a report showing this single numerical score and the corresponding classification: 'likely malignant', 'likely benign', or 'indeterminate'.

Histopathology can accurately classify many melanocytic neoplasms and currently serves as the 'gold' standard for the diagnosis of melanoma. In line with standard practice, therefore, adjunctive molecular tests for melanoma diagnosis have largely been developed and initially evaluated using histopathology as the reference standard. Clarke et al. (2015) and Clarke et al. (2017) performed test validation studies of the myPath® Melanoma test which demonstrated greater than 90% diagnostic accuracy by comparison to concordant histopathologic diagnoses (diagnoses arrived at independently by multiple expert dermatopathologists).

To further assess accuracy using a reference standard independent of histopathologic diagnosis and confirm genuine clinical utility, a third clinical validation study was performed (Ko et al. 2017) in which the test result was compared to the eventual clinical outcomes of tested patients. In a cohort of 182 melanocytic neoplasms collected from patients with

documented outcomes (distant metastases for malignant melanomas and median 6 + year uneventful follow-up for benign nevi), the myPath® Melanoma score differentiated malignant melanoma from benign nevi with a sensitivity of 93.8% and a specificity of 96.2%.

Clarke et al. (2020) also performed a test validation study to assess the accuracy of myPath® Melanoma in differentiating benign nevi from melanoma by comparing test results with clinical outcomes. 125 cases fulfilled criteria for diagnostic uncertainty (69.1%; 95% CI: 61.8–75.7%). Test sensitivity and percent negative agreement in these cases were9 0.4% (95% CI: 79.0–96.8%) and 95.5% (95% CI: 87.3–99.1%), respectively. The authors concluded myPath® Melanoma has high diagnostic accuracy in diagnostically uncertain cases when evaluated against clinical outcomes. Overall, clinical studies have shown sensitivity and specificity ranges of 90-94% and 91-96%, respectively, for the 23-GEP (Clarke et al. 2015, Clarke et al. 2017, Ko et al. 2017, Clarke et al. 2020).

Cockerell et al. (2016) performed a clinical utility study which quantified the influence of the myPath® Melanoma score on both the final diagnoses and the treatment recommendations made by board-certified dermatopathologists for 218 prospectively submitted diagnostically challenging (equivocal or uncertain) melanocytic neoplasms encountered during routine clinical practice. Comparison of pre-test and post-test diagnoses demonstrated a 56% increase in definitive diagnoses with use of the myPath® score (a 30% increase in definitive diagnoses of benign nevus and a 12.4% increase in definitive diagnoses of malignant melanoma). In addition, treatment recommendations provided by dermatopathologists changed for 49% of patients after receiving the myPath® result, with 76.6% of those changes aligned to the test result.

Cockerell et al. (2017) performed a second clinical utility study which assessed the relationship between test result and change in treatment as measured by pre-test dermatopathologist recommendation and post-test actual treatment delivered to a patient by the dermatologist. A cohort of 77 patients with pre-test diagnoses of “indeterminate” (equivocal, uncertain) were followed throughout their clinical course. The myPath® test produced definitive scores for all 77 neoplasms, and after a median 12-month follow-up period, the tested patients’ dermatologists disclosed the actual treatment carried out in each case. The treatment differed from the pre-test recommendation in 55 of 77 (71.4%) cases, 44 of which produced a benign myPath® test result. Re-excision was the pre-test treatment recommendation for 41 of these 44 cases, yet re-excision was ultimately performed in just 7, indicating that a benign myPath® test result enabled dermatologists to forego further intervention in 33 of the 41 cases, yielding an 80.5% reduction in re-excisions.

## Oncotype DX® Breast Cancer Assay

There are many laboratory tests developed to detect genetic variation in breast tumor tissue, particularly gene expression tests. These results may be used to predict distant recurrence risk for women with early-stage breast cancer (BC). In turn, this may help with the decision of whether to include adjuvant chemotherapy.

In 2022, Griguolo et al. explored the evidence on the most widely used, commercially available gene-expression signatures (Oncotype DX, MammaPrint, PAM50, EndoPredict, and Breast Cancer Index [BCI]) for individuals receiving neoadjuvant therapy for hormone receptor-positive/human epidermal growth factor receptor 2-negative breast cancer (HR +/HER2- BC). The authors evaluated the data for the association of gene expression signatures and responses to neoadjuvant chemotherapy (NCT) or neoadjuvant endocrine treatment (NET) and the clinical suggestions from the data to guide clinical decision-making in early HR +/HER2- BC. A consistent association was observed between higher risk (as per gene expression signatures) and higher pathological complete response (pCR) rate after NCT across the gene expression assays studied. Association between lower risk based on gene expression signatures and higher pCR after NET was observed. The evidence, however, is limited and based on small retrospective studies. Larger prospective trials are needed to confirm results for the use of gene expression assays in this context. The researchers assert that the potential use of gene expression signatures to assist with selection of neoadjuvant therapy (chemotherapy versus endocrine therapy) in early BC merits further exploration.

Harnan and colleagues (2019) conducted a systematic review and economic analysis to determine the efficacy and cost-effectiveness of the tumor profiling tests Oncotype DX, MammaPrint, Prosigna, EndoPredict, and immunohistochemistry 4 (IHC4). Studies included individuals with estrogen receptor-positive (ER +), HER2-, stage I, or II cancer with zero to three positive lymph nodes (LN + ). The review included 153 articles on all five tests. In all five tests, the proportions of individuals who were lymph node-negative (LN0) getting endocrine monotherapy, 9% to 33%, were categorized as high-risk, according to the literature. For individuals who were LN +, three tests: Prosigna, EPClin, and IHC4 plus clinical factors (IHC4 + C), categorized more (38% to 76%) individuals who were LN + than those who were LN0 as high-risk according to the studies of endocrine monotherapy. Oncotype DX categorized high-risk in the LN0 and LN + subsets as equal. Oncotype DX classified more individuals as low-risk in LN + when compared to other tests (57% in Oncotype DX vs. 4% to 28% in other tests), but worse 10-year distant recurrence/relapse-free survival/distant recurrence/ relapse-free interval outcomes (82% in Oncotype DX vs. 95% to 100% in other tests). An increase of 1% to a decrease of 23% was seen in UK studies and a reduction of 0% to 64% across European studies on the net change of individuals who were

recommended chemotherapy or decision pre/posttest. Limitations included gaps in the literature, the risk of bias, and limited data relating to the ability of Oncotype DX and MammaPrint to predict benefits from chemotherapy. Additional long-term studies can show the impacts and changes in chemotherapy decisions for Oncotype DX and MammaPrint. The authors concluded that the evidence indicates that all the tests deliver prognostic data regarding the risk of relapse, although greater variation was seen in individuals with LN + status than those with LN 0 status.

Oncotype Dx Breast (Genomic Health, Redwood City, CA) is a test that analyzes the expression of a panel of 21 genes within a tumor to determine a Recurrence Score (RS) which may correspond to a likelihood of BC recurrence within 10 years. The test was initially developed for women with early-stage invasive BC with early-stage cancers that are LN0, and subsequently evidence was gathered on individuals with up to 3 ipsilateral nodes positive. These individuals are typically treated with anti-hormonal therapy, such as tamoxifen or aromatase inhibitors, and Oncotype Dx® can help determine if chemotherapy should be added to the treatment regimen (Evaluation of Genomic Applications in Practice and Prevention [EGAPP] Working Group, 2016).

Nash and colleagues (2023) investigated the benefit of chemotherapy based on RS in younger women (aged 40-50) who were eligible for oncotype testing. Participants were selected from the National Cancer Database (NCBD) and grouped by age, RS, nodal status, and receipt of chemotherapy. A total of 15,422 individuals met inclusion criteria for the study. Of these 43.5% received chemotherapy. Log-rank tests were used to assess for differences between groups and Kaplan-Meier curves compared the unadjusted OS between groups. The analysis revealed that individuals who received chemotherapy were more likely to have higher-stage and higher-grade tumors, tumors that were PR-negative, and higher RS ($p < 0.001$ for all). RS was prognostic for OS regardless of nodal status. After adjustment, chemotherapy was associated with a significant improvement in OS only in the pN1 RS 31-50 subgroup ($p = 0.02$). The authors concluded that RS remains prognostic in younger individuals with early-stage HR-positive, HER2- BC. The survival benefit with chemotherapy was only found in those aged 40-50 with pN1 disease and RS of 31-50. As such, chemotherapy decision-making should be especially preference-sensitive in women aged 40-50 with intermediate RS, where survival benefit may not be enhanced for many women.

The 21-gene expression assay (Oncotype DX Breast Recurrence Score) is commonly and increasingly used to assist with decision-making regarding adjuvant chemotherapy in ER +/HER2- BC with one to three positive lymph nodes (N1) disease. To evaluate patterns in practice related to the use of the recurrence score for decision-making regarding chemotherapy and survival outcomes in these individuals, Li et al. (2023) retrospectively evaluated 35,137 individuals with T1-2N1M0 and ER +/HER2- BC from the Surveillance, Epidemiology, and End Results (SEER) Oncotype DX Database. Both breast cancer specific survival (BCSS) and overall survival (OS) were included in the assessment. In this study, older age, lower tumor grade, T1 stage, fewer positive lymph nodes, and progesterone receptor-positive disease (all $p < 0.05$) were all associated with use of the 21-gene test. RS had a significant association with chemotherapy treatment in the group that had the 21-gene test, whereas age was the primary factor significantly associated with chemotherapy treatment in the group that did not receive 21-gene testing. For individuals who underwent 21-gene testing, the probably of chemotherapy was 30.8%; in the group who did not undergo the 21-gene test, probably of chemotherapy was higher at 64.1%. Based on multivariate prognostic analysis, use of the 21-gene test was associated with both improved BCSS ($p < 0.001$) and OS ($p < 0.001$) when compared to individuals who did not receive the test. From this data, the authors concluded that the 21-gene assay is related to lower rates of adjuvant chemotherapy use and improved survival outcomes. They indicate their support for the use of the 21-gene assay in individuals with ER +/HER2- BC with N1 disease.

In a 2022 systematic review and network meta-analysis, Davey et al. evaluated the Oncotype DX 21-gene RS for its ability to estimate locoregional recurrence (LRR) in ER +/HER2- BC. The review uncovered 16 articles together with 21,037 individuals. The average RS was 17.1, and the average follow-up was 66.4 months. Employing standard RS cut-offs, 49.7% of individuals had RS < 18 (3944/7935), 33.8% had RS 18–30 (2680/7935), and 16.5% had RS > 30 (1311/7935). Those with RS 18–30 and RS > 30 were significantly more likely to experience LRR than those with RS < 18. Using the TAILORx cut-off, 16.2% of individuals had RS < 11 (1974/12,208), 65.8% had RS 11–25 (8036/12,208), and 18.0% with RS > 30 (2198/12,208). LRR rates were comparable for individuals with RS 11–25; however, those with RS > 25 had a considerable risk of LRR versus those with RS < 11. The authors concluded that RS testing correctly estimates the risk of LRR for individuals being treated with the intent to cure early-stage ER +/HER2− BC. RS testing is a valid method to measure the risk of distant disease recurrence; however, awareness of its ability to predict LRR is significant to create effective locoregional control of the breast and axilla. Future prospective, randomized studies can confirm the predictive value of RS for estimating LRR and the application of RS to create suitable locoregional control in high-risk cases.

In 2021, Kalinsky et al. published the results of a prospective randomized clinical trial (RCT) to find the effect of chemotherapy on invasive disease-free survival in individuals with positive lymph-node disease and determine whether

the RS based on the 21 gene assay (Oncotype Dx) influenced the outcome. A total of 5018 women with hormone-receptor-positive, HER2- BC, 1 to 3 positive axillary lymph nodes, and an RS of 25 or lower were randomly grouped into an endocrine therapy alone subset or a chemotherapy with endocrine (chemoendocrine) therapy subset. The intention-to-treat analysis included the participants who declined the assigned treatment, with 402 (16.2%) participants allocated to chemoendocrine therapy and 144 (5.8%) given to endocrine treatment. The trial did not show a clinically applicable or statistically significant rise in invasive disease-free survival with the addition of adjuvant chemotherapy to endocrine therapy in the global population with the same characteristics. For this trial, 67% of post-menopausal participants had no chemotherapy advantage. Dissimilarity, adjuvant chemotherapy led to a relative growth of 40% in invasive disease-free survival and a relative rise of 42% in distant relapse-free survival (RFS) among premenopausal women. Invasive disease-free survival at five years was 91.9% among post-menopausal women in the endocrine-only group and 91.3% in the chemoendocrine group, with no chemotherapy advantage. In the group of premenopausal women, invasive disease-free survival at five years was 89.0% with endocrine-only therapy and 93.9% with chemoendocrine treatment, with a comparable rise in distant relapse-free survival. The trial showed that between premenopausal women with 1 to 3 positive lymph nodes (N1) and an RS of 25 or less, individuals who received chemoendocrine therapy had a lengthier invasive disease-free survival and distant RFS than those who received endocrine-only treatment. In contrast, post-menopausal women with the same characteristics did not profit from adjuvant chemotherapy.
Hayes published a Molecular Test Assessment addressing the use of the Oncotype DX Breast Recurrence Score for individuals with ER +, HER2-, lymph node positive BC to determine the capability of the test to estimate the risk of distant recurrence and the predict the likelihood of chemotherapy benefit in 2020. For individuals with N1 disease, limited but consistent evidence supports the use of the Oncotype DX test for predicting the risk of 9-year distant recurrence, but there is insufficient evidence supporting the test's ability to predict the benefit of chemotherapy. Oncotype DX may improve outcomes for individuals with N1 cancer by lessening the total population of individuals treated with chemotherapy, thereby avoiding detrimental side effects. Insufficient evidence was found to support the use of Oncotype DX testing for estimating the risk of distant recurrence and the potential benefit of chemotherapy for individuals with N2 disease (4 to 9 positive lymph nodes) (Hayes, Oncotype DX Breast Recurrence Score [Genomic Health Inc.] for Lymph Node–Positive Patients, 2020, updated 2023).

In a 2020 Hayes Molecular Test Assessment, the Oncotype DX Breast Recurrence Score was assessed as a prognostic indicator for 9-year distant BC recurrence and predictive indicator for chemotherapy benefit in individuals diagnosed with ER +, HER2-, and node-negative (N0) invasive BC. The evidence presented in the assessment suggests that the Oncotype DX test can estimate the risk of distant recurrence and the likely benefit of chemotherapy for guiding proper treatment decisions for individuals, thus impacting provider management and decisions related to therapy. Additional study addressing the range of scores necessary for predicting the likelihood of chemotherapy benefits in specific subgroups is recommended. Clinical utility studies reporting health outcomes after recurrence score-based treatments are needed as well (Hayes, Oncotype DX Breast Recurrence Score for Lymph Node–Negative Patients [Genomic Health Inc.] 2020, updated 2023).

Poorvu et al. (2020) evaluated women less than 40 years of age with early-stage ER + and HER2- BC to decide if the 21-gene RS could inform chemotherapy recommendations. The prospective TAILORx phase 3 trial enrolled 509 individuals and the RS assay was performed either clinically (189 participants) or on banked specimens (320 individuals). The median follow-up time was 6 years. Of the 509 individuals, 300 (59%) had N0 BC and 195 of them had a RS of 11-25, of which 86 received chemotherapy. The 6-year distant recurrence free survival (DRFS) varied by the RS with < 11 associated with 94.4% N0 and 92.3% N1. For those with RS 11-25, DRFS was 96.9% N0 and 85.2% N1 and for those with RS > 26, the DRFS was 85.1% N0 and 71.3% N1. The researchers concluded that the assay is prognostic for young women with N0 and limited N1.

Wang et al. (2019) examined the value of Oncotype Dx when determining the prognosis in female individuals with BC and tumor stage 1-2 (tumor is 20-55mm), LN + and no evidence of metastasis (T1-2 N1M0). The study reviewed 4059 cases to categorize them to prognostic stages IA and IIB and used data derived from the National Cancer Institute's limited use Surveillance, Epidemiology, and End Results (SEER) 18 registry databases, released in November 2017. Cases in the SEER database was linked to RS results from assays performed by Genomic Health. All cases with RS had negative HER2, and the authors selected female ER + invasive ductal carcinoma (IDC) cases in T1-2N1M0 stage with Oncotype RS results diagnosed between 2004 and 2012. Individuals were categorized into low-risk (RS < 11), intermediate-risk (RS 11–25), and high-risk (RS > 25) groups. The median age of the individuals was 59 years. Of these participants, 2898 (71.4%) had stage T1 cancer, 1854 (45.7%) had stage $N_{1mic}$ cancer, 743 (18.3%) had grade 3 cancer, and 3746 (92.3%) had positive PR status. They were stratified into the RS low-risk group (794, 19.6%), the RS intermediate-risk group (2667, 65.7%), and 598 (14.7%) were in the RS high-risk group. The high-risk group tended to have younger individuals, larger tumors, a higher percentage of grade 3 disease, negative PR, and more advanced cancer staging. They also had more frequent use of chemotherapy. Otherwise, the RS groups did not differ much in race, N stage, surgery, or radiation. In terms of pathological prognostic stages, there were 2781 individuals (68.5%) in stage IA, 829 (20.4%) in stage IB, 360

(8.9%) in IIA, and 89 (2.2%) in IIB. The distributions of clinical and pathological characteristics, including BCSS and OS, were compared between RS and pathological staging groups using a variety of statistical analysis. The median follow-up period was 57 months. The results showed a statistically significant correlation ($p < .001$) between the RS groups and pathological stage results. In the low and high-risk RS groups, the BCSS and OS were similar between RS and pathological staging groups. In the intermediate RS group, however, survival rates differed significantly between RS staging and pathological staging. The survival rates were inversely correlated with the escalation of prognostic stages. Similar trends were seen in the high-risk group but were not statistically significant. In this retrospective study, RS was an independent prognosticator for BCSS, and with pathological stage for OS. The authors concluded that Oncotype Dx could complement the prognostic staging system in N + individuals.

Wolmark et al. (2016) assessed the utility for a 21 gene RS in predicting distance recurrence (> 5 years) in stages I and II BC in high and low expressing ESR1 groups within a cohort of 3,060 individuals from the National Surgical Adjuvant Breast and Bowel project, all of whom had undergone tamoxifen therapy. Overall, the authors found that RS consistently predicted distant recurrence; low RS had a low risk of distant recurrence. In a subgroup analysis, it was noted that individuals with a low RS and N1, the risk of distant recurrence was 7.9%. In those with N2, the risk of distant recurrence was 16.7%.

## *Clinical Practice Guidelines*

### National Comprehensive Cancer Network (NCCN)

NCCN Guidelines for Breast Cancer (NCCN Breast Cancer, v5.2024) states that, "the 21-gene assay (Oncotype Dx) is preferred by the NCCN Breast Cancer Panel for prognosis and prediction of chemotherapy benefit. Several commercially-available gene-based assays are useful in determining prognosis by predicting distant recurrence, local recurrence, or survival. Of these, only one, the 21-gene assay (Oncotype Dx) has been clinically validated for predicting the benefit of adding adjuvant chemotherapy to further reduce the risk of recurrence. 21-gene assay (Oncotype DX) in Node-negative, HR-positive, HER2-negative disease: The 21-gene recurrence score (RS) is one of the most validated multigene assays. In the West German Plan B study, patients (n = 110) with lymph node-positive, HR-positive, HER2-negative tumors, and a RS of ≤ 11, were found to have a 5-year disease-free survival of 94.4% when treated with endocrine therapy alone (Nitz et al. 2017). In a secondary analysis of a prospective registry of patients with HR-positive, HER2-negative, lymph node-positive tumors, the 5-year risk of distant recurrence in patients with a RS of < 18, treated with endocrine therapy alone was 2.7% (Stemmer et al. 2017). These 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. 355,356 There is a clear benefit from adjuvant chemotherapy in patients with node positive, HR-positive, HER2-negative tumors, if the RS is high (≥ 31). In a secondary analysis of the SWOG 8814 trial of patients with HR-positive, lymph node-positive tumors, high RS (≥ 31) was predictive of chemotherapy benefit. This study evaluated breast cancer specimens from node-positive, HR-positive postmenopausal patients (n = 367) randomized to endocrine therapy with tamoxifen alone or chemotherapy with CAF followed by tamoxifen (Albain et al. 2010). Compared with tamoxifen alone, treatment with CAF among patients with a high RS (≥ 31) resulted in improved 10-year DFS (55% vs. 43%; HR 0.59, 95% CI 0.35-1.01) and OS (73% vs. 54%; HR 0.56, 95% CI 0.31-1.02).350 The Southwest Oncology Group (SWOG) S1007 RxPONDER trial (NCT01272037), assigned patients with 1-3 lymph node-positive nodes, HR-positive, HER2-negative breast cancer and a RS ≤ 25 to standard endocrine therapy with or without adjuvant chemotherapy. The results showed that the addition of adjuvant chemotherapy to endocrine therapy improved invasive disease–free survival among premenopausal—but not postmenopausal—women with hormone receptor–positive, HER2-negative, node-positive breast cancer and a 21-gene assay recurrence score up to 25 (Kalinsky et al. 2021).

### National Institute for Health and Care Excellence (NICE)

The 2018 NICE guidelines on tumor profiling tests for guiding adjuvant chemotherapy choices in early BC offer recommendations for EndoPredict (EPclin score), Oncotype DX Breast Recurrence Score, Prosigna, MammaPrint, and IHC4 + C. NICE endorses EndoPredict (EPclin score), Oncotype DX Breast Recurrence Score, and Prosigna as possibilities for guiding adjuvant chemotherapy decisions for individuals with ER +, HER2- and LN0 including micrometastatic disease; early BC if the following indications are met:

- The individual has an intermediate risk of distant recurrence via a validated tool such as PREDICT or the Nottingham Prognostic Index.
- The data provided by the test would aid the individual's choice, with their physician, whether to have adjuvant chemotherapy considering their preference.
- The companies offer the tests to the NHS with the discounts arranged in the access proposals; and
- The physicians and companies make prompt, comprehensive, and linkable record-level test information obtainable to the National Cancer Registration and Analysis Service as designated in the information collection arrangements arranged with NICE.

## Oncotype DX® Breast DCIS Score™ Test (Ductal Carcinoma in Situ)

Ductal carcinoma in situ (DCIS) is a heterogeneous group of neoplastic lesions confined to the breast ducts and lobules. It is one of the most commonly diagnosed breast conditions, accounting for approximately 20% of newly diagnosed breast cancers in the United States (Ward et al. 2015). Women diagnosed with DCIS are at risk for local recurrence, which may be either DCIS or progression to invasive breast carcinoma. The management of patients with DCIS is an area of controversy and historically treatment has included both surgical excision and radiation therapy (Zujewski et al. 2011). Following surgical excision alone local recurrences occur in approximately 25% to 30% of women by 10 years (Correa et al. 2010). The addition of radiation therapy has been reported to reduce local recurrence risk by approximately 50% but has not been demonstrated to prolong overall or disease-free survival (Correa et al. 2010). In an observational study of patients diagnosed with DCIS from 1988 to 2011, prevention of invasive in-breast recurrence with radiation therapy after lumpectomy did not improve 10-year breast cancer-specific mortality compared with lumpectomy alone (Narod et al. 2015). Therefore, treating all women with radiation therapy following surgical excision may represent overtreatment for many, especially given that the majority of cases do not recur following surgery alone. Clinical and pathologic features do not reliably predict the risk of recurrence; therefore, validated biomarkers are needed that identify patients at low risk of local recurrence for whom less treatment is indicated and conversely distinguish patients at high risk of progression to invasive disease for whom more intensive treatment regimens are appropriate.

### *Test Description*

The DCIS Score is a ribonucleic acid (RNA) based assay measuring the expression of 5 proliferation genes, progesterone receptor (PR), GSTM1 and 5 reference genes (Figure 1) with results reported as a numerical score along with accompanying interpretive information. The assay is performed on formalin fixed paraffin-embedded (FFPE) tissue blocks containing DCIS. The DCIS Score was developed based upon analyses of multiple correlative science studies comparing gene expression profiles between invasive and DCIS tumor samples (Baehner et al. 2008). An algorithm was developed using scaling and category cut-points based on the analysis of the DCIS Score result in a separate cohort of DCIS patients (Baehner et al. 2012).

### Figure 1: Genes Comprising the DCIS Score

| Proliferation Group | Hormone Receptor Group | Reference Group |
|---|---|---|
| • Ki67<br>• STK15<br>• Survivin<br>• CCNB1 (cyclin B1)<br>• MYBL2 | • PR<br>• GSTM1 | • ACTB (ß -actin)<br>• GAPDH<br>• RPLPO<br>• GUS<br>• TFRC |

### *Test Performance*

Solin et al. (2013) performed an initial test validation of the DCIS Score result in a prospectively designed study of archived tumor specimens from 327 patients who participated in the previously described E5194 trial (Hughes et al. 2009), a prospective cooperative group trial that evaluated 5- and 10-year ipsilateral breast event (IBE) rates after local excision alone in a selected population of patients with DCIS. The study met its primary objective, as the DCIS Score result was predictive of the 10-year risk of any IBE. The DCIS Score result as a continuous variable was significantly associated with developing an IBE (hazard ratio [HR]/50 units = 2.31, 95% CI = 1.15-4.49; p = 0.02). Using 3 pre-specified risk groups (low < 39, intermediate 39-54, and high = 55), the 10-year risk of any IBE (DCIS or invasive carcinoma) was 10.6% in the low-risk group compared to 26.7% in the intermediate risk group and 25.9% in the high-risk group; the risk stratification between the 3 groups was significant (log rank p = 0.006). The risk for developing ipsilateral invasive carcinoma was only 3.7% in the low-risk group compared to 19.2% in the high-risk group (log rank p = 0.003). Approximately 70% of all patients enrolled in the study were in the low-risk group. In multivariable analyses, the DCIS Score result, tumor size, and menopausal status were identified to be statistically significant predictors of the risk of local recurrence (p = 0.02). The HR for the score remained unchanged after adjusting for tumor size and menopausal status thereby demonstrating that the DCIS Score result provides independent prognostic information beyond these risk factors.

Rakovitch et al. (2015) performed a second prospectively designed clinical validation study of the Oncotype DX® Breast DCIS Score Assay conducted in a population-based cohort of women diagnosed with DCIS and treated with breast conserving therapy alone from 1994-2003 in Ontario, Canada. The final study cohort included 718 patients of whom 571 had negative surgical margins. Median follow-up was 9.6 years. The study found the DCIS Score result to independently predict and quantify local recurrence risk. In the primary analysis, the DCIS Score result was significantly associated with any local recurrence in estrogen receptor positive patients (HR/50 units = 2.26, 95% CI = 1.41-3.59; p < 0.001) as well as all patients regardless of estrogen receptor status (HR = 2.15; 95% CI = 1.43-3.22; p < 0.001). For the same pre-specified risk groups (low < 39, intermediate 39-54, and high = 55), the 10-year risk of a local invasive carcinoma recurrence was 8.0% in the low-risk group compared with 20.9% and 15.5% in the intermediate and high-risk groups, respectively; the risk

stratification between the 3 groups was significant (p = 0.03). The risk of developing a DCIS local recurrence was 5.4% in the low-risk group compared with 14.1% and 13.7% in the intermediate and high-risk groups, respectively (p = 0.002). In multivariable analysis, the DCIS Score result was a significant predictor of local recurrence (HR/50 units = 1.68, 95% CI = 1.08-2.62; p = 0.02) and provided independent recurrence risk information beyond clinical and pathologic measures including age at diagnosis, tumor size, grade, necrosis, multifocality, and subtype. The primary analyses were restricted to patients with clear margins; however, secondary analysis included all patients regardless of surgical margins. The HR in the expanded cohort, adjusting for margin status and other clinical and pathological features, was 2.11 (95% CI = 1.43-3.09; p < 0.001) indicating that the DCIS Score result effectively risk-stratifies patients regardless of margin status.

## *Analytical and Clinical Performance of the Oncotype DX® DCIS Assay*

- **Intended Use**: To assess the average 10 year rate for any ipsilateral breast event (DCIS or invasive carcinoma) in women diagnosed with DCIS who had breast conserving surgery with negative margins and are considering adjuvant radiation therapy.
- **Validated Specimen Type(s)**: FFPE tissue.

### Analytical Performance

<table>
<tr><th>Description</th><th colspan="3">Results</th></tr>
<tr><td rowspan="4">Precision, within RNA extract (2 operator; 2 runs on different days; 2 manufacturing reagent lot; 5 PCR robots; 9 PCR detection systems; 75 paired RNA extracts run all in CLIA lab; expected score range 3-86*)</td><td colspan="3">Within RNA Extracts</td></tr>
<tr><td>DCIS Score Category</td><td>N</td><td>STD</td></tr>
<tr><td>Low</td><td>36</td><td>1.04</td></tr>
<tr><td>Int-High</td><td>39</td><td>1.09</td></tr>
<tr><td rowspan="4">Precision, between tumor block sections (2 operator; 2 runs on different days; 2 manufacturing reagent lot; 5 PCR robots; 9 PCR detection systems; 39 unique tumor blocks run all in CLIA lab; expected score range 3-86*)</td><td colspan="3">Between Consecutive Tumor Block Sections</td></tr>
<tr><td>DCIS Score Category</td><td>DCIS Score Category</td><td>DCIS Score Category</td></tr>
<tr><td>Low</td><td>19</td><td>2.11</td></tr>
<tr><td>Int-High</td><td>20</td><td>3.96</td></tr>
<tr><td>Analytical sensitivity: Minimum input</td><td colspan="3">Total RNA: 110 ng extracted from tumor tissue.</td></tr>
<tr><td rowspan="6">Critical reagent closed/shelf-life stability (GHI conducted shelf-life stability unless stated otherwise)</td><td colspan="3">Reverse Transcription Kit: Stability from date of receipt through the manufacturer's labeled expiration date with 12 months of on-site storage at -20 °C ± 5 °C.</td></tr>
<tr><td colspan="3">GSP pool (gene specific primers for reverse transcription): 9 months at -20 °C ± 5 °C</td></tr>
<tr><td colspan="3">Reverse Transcription Positive control: 2 years at -80 °C ± 10 °C.</td></tr>
<tr><td colspan="3">P3 Plate: 9 months -80 °C ± 10 °C.</td></tr>
<tr><td colspan="3">Human gDNA (quantitative PCR positive control): 6 months at + 5 °C ± 3 °C.</td></tr>
<tr><td colspan="3">Quantitative PCR Master Mix: 18 months from date of manufacturing.</td></tr>
<tr><td rowspan="6">Critical reagent open/in use stability (GHI conducted operational stability unless stated otherwise)</td><td colspan="3">Reverse Transcription Kit: Use within 2 shifts after opening kit and prior to manufacturer's labeled expiration date at -20 °C ± 5 °C.</td></tr>
<tr><td colspan="3">GSP pool (gene specific primers for reverse transcription): Freeze thaw no more 10x.</td></tr>
<tr><td colspan="3">Reverse Transcription Positive control: Single Use Tube.</td></tr>
<tr><td colspan="3">P3 Plate: Freeze thaw no more than 10x. Use within 1 day 5 °C ± 3 °C.</td></tr>
<tr><td colspan="3">Human gDNA (quantitative PCR positive control): 6 months at +5 °C ± 3 °C.</td></tr>
<tr><td colspan="3">Quantitative PCR Master Mix; 3 months after thaw at 5 °C ± 3 °C. Up to 3 hours prior to qPCR plate assembly at room temperature (18 °C to 25 °C).</td></tr>
<tr><td colspan="3">Assembled Quantitative PCR plates: 24 hours at room temperature (18 °C to 25 °C).</td></tr>
<tr><td>Specimen stability, primary</td><td colspan="3">FPET slice in tube 6 months at room temperature (18 °C to 25 °C).</td></tr>
</table>

| Description | Results |
|---|---|
| Specimen stability, intermediate (extracted RNA) | Within 1 day 5 °C ± 3 °C.<br>Within 5 days -20 °C ± 5 °C.<br>Within 365 days at -80 °C ± 10 °C |
| Specimen stability, intermediate (cDNA Sample plate) | Within 3 months at -20 °C ± 5 °C. |

*DCIS Score risk groups were specified prior to first clinical validation study (DCIS Score: Low < 39, Intermediate 39-54, High = 54). Actual range of DCIS scores for samples used for precision studies were DCIS Score Low 3-37 and DCIS Score Int-High 40-86.

## Clinical Performance

The Oncotype DX® DCIS Score is a continuous measure that provides predicted risks of an ipsilateral breast event for individual patients over a continuum of gene expression, reflecting the continuous nature of tumor biology. Statistics. such as sensitivity and specificity, were designed to evaluate the general predictive ability of binary (dichotomous) predictors of the presence or absence of a disease or condition, rather than prediction of the risk of a future event, and have limitations in the assessment of continuous predictors of risk (Cook et al. 2007, Moons et al. 2003, Pepe et al. 2008). A more appropriate statistical assessment of the predictive accuracy of the DCIS Score for risk groups is demonstrated by the width of the 95% confidence intervals for estimates of 10-year risk of an IBE within each risk group, shown in the table below.

The Oncotype DX® DCIS Score was validated in 2 clinical studies encompassing the indicated patient population. Both clinical validation studies were conducted under IRB-approved protocols with pre-specified analytical and quality acceptance criteria, statistical analysis plans, and endpoints. All clinical studies were conducted on the platform (device) after assay performance requirements (above) were specified and independently validated.

| Description | Results | |
|---|---|---|
| | **Solin et al. (2013) (n = 327 patients)** | **Rakovitch et al. (2015) (n = 571 patients)** |
| Hazard ratio/50 units | 2.31[a]<br>(95% CI = 1.15 - 4.49)<br>p = 0.02 | 2.15[b]<br>(95% CI = 1.43 - 3.22)<br>p < 0.001 |
| **Number (%) of Patients** | | |
| Low DCIS Score | 230 (70%) | 355 (62%) |
| Intermediate/High DCIS Score | 97 (30%) | 216 (38%) |
| **10-year Risk of Local Recurrence (95% CI)** | | |
| Low DCIS Score | 10.6% (6.9-16.2%) | 12.7% (9.5-16.9%) |
| Intermediate/High DCIS Score | 26.2% (18.1-37.0%) | 30.1% (23.9-37.5%) |
| Overall Proportion with IBE[c] | 46/327 (14.1%) | 100/571 (17.5%) |

[a]Adjusted for tamoxifen use (pre-specified primary analysis)
[b]No covariate adjustment; all patients (irrespective of ER status) with negative resection margins
[c]Ipsilateral breast event (DCIS or invasive carcinoma)

## *Decision Impact and Health Economic Studies*

Alvarado et al. (2015) performed a prospective multicenter clinical utility study evaluating the impact of the DCIS Score result upon treatment recommendations for radiation therapy (XRT). Eligible women had newly diagnosed histologically documented DCIS and were candidates for breast conserving therapy. Physicians completed standardized questionnaires that captured their estimates of local recurrence risk and treatment recommendations for XRT, prior to and after receiving the DCIS Score results. A total of 115 evaluable patients from 10 US centers were included in final analyses. Study results found a significant change in the proportion of patients receiving recommendations for XRT pre- vs post-DCIS Score result (P = 0.008; McNemar's test). Pre-assay, 73.0% of patients were recommended to receive XRT; this was reduced to 59.1% post-assay. Overall integration of the DCIS Score result into clinical management decisions resulted in a 31.3% change in XRT recommendations. Changes in treatment were bidirectional, indicating that the information was useful both for identifying patients at lower risk of recurrence for whom XRT may be omitted, as well as those at higher risk who may be appropriate candidates for more intensive modalities.

Manders et al. (2017) performed a second prospective multicenter clinical utility study, 27 surgeons and 27 radiation oncologists at 13 US centers provided estimates of local recurrence risk and XRT recommendations for 127 patients, before and after DCIS Score results were known. Baseline characteristics of this patient cohort were similar to those of the first clinical utility study. Post-assay, 26.4% of recommendations changed overall , representing 22.0% of recommendations by radiation oncologists and 30.7% of recommendations by surgeons. The DCIS Score result was the most frequently cited reason for post-assay treatment recommendations.

Young et al. (2014) reported a retrospective health economic study from a single center involving 38 patients for whom the DCIS Score assay had been ordered. In this cohort, 26 patients (68%) had DCIS Score results and local recurrence risk considered low enough to omit radiation from their course of therapy. The authors concluded that the assay has the potential to be cost-saving to the healthcare system and spare many patients from the adverse effects associated with radiation therapy. A cost-effectiveness modeling study comparing the Oncotype DX® Breast DCIS Score Assay to standard clinical assessment to determine treatment recommendation for radiation therapy has been reported by Alvarado et al. (2012). The study found that on average, the assay was more cost-effective than the clinical assessment strategy by approximately $1000/patient, with similar life expectancies (17.15 vs 17.11, respectively) and quality-adjusted life-years (QALYs) (16.777 vs 16.789).

## *Criteria for Coverage*

The Oncotype DX® DCIS assay is covered only when the following clinical conditions are met:

- Pathology (excisional or core biopsy) reveals ductal carcinoma in situ of the breast (no pathological evidence of invasive disease), and
- FFPE specimen with at least 0.5 mm of DCIS length, and
- Patient is a candidate for and is considering breast conserving surgery alone as well as breast conserving surgery combined with adjuvant radiation therapy, and
- Test result will be used to determine treatment choice between surgery alone vs. surgery with radiation therapy, and
- Patient has not received and is not planning on receiving a mastectomy.

## OVERA® and ROMA™

Coleman et al. (2016) performed a clinical validity evaluation in a non-concurrent prospective study of 493 preoperatively collected serum specimens from premenopausal and postmenopausal women presenting with an adnexal mass requiring surgical intervention. Overa® test scores were determined based on the analysis of archived serum specimens, and the patients were stratified into low or high-risk groups for finding malignancy on surgery. The analysis examined whether patient referral to a gynecologic oncologist was supported when dual assessment was determined to be positive (either Overa® or clinical assessment was positive, or both were positive). A dual assessment was considered negative when both Overa® and clinical assessment were negative. Among the 493 study participants, 92 (19%) had a final pathology diagnosis of malignancy. The clinical performance of the Overa® assay, when combined with presurgical physician assessment demonstrated sensitivity of 93.5%, specificity of 64.8%, PPV of 37.9%, and NPV of 97.7%. Overall, the addition of Overa® testing to presurgical physician assessment correctly identified 75% ($P < 0.001$) of the malignancies missed by physician assessment alone. This study had several limitations, including a small patient population and a retrospective study design. Additionally, preoperative physician assessment was not uniform across the patient population and this may have introduced bias into the study. Also the method used for combining clinical assessment and Overa® test results was to consider the test positive if either clinical assessment or Overa® test was positive. Thus, in practice, Overa® testing would not be necessary if clinical assessment alone indicated cancer. Using Overa® testing in this manner guarantees that Overa® testing will be more sensitive and less specific than clinical assessment alone, even if it has no better than chance capability of detecting ovarian cancer. Sensitivity improved from 74% to 94% and specificity decreased from 93% to 65%. Finally, this study was funded by the manufacturer. This single study provides very-low-quality evidence for the clinical validity of the Overa® test.

Wang et al. (2014) published a meta-analysis of studies evaluating the diagnostic accuracy of the ROMA™ algorithm and comparing it to the performance of single markers HE4 and CA 125. To be included in the meta-analysis, studies had to investigate both HE4 and CA 125 or calculate ROMA™, include women with ovarian cancer and benign gynecologic disease, use pathology diagnosis as the reference standard, and collect blood samples before treatment was initiated. A total of 32 studies met these inclusion criteria; six of these were conducted in the United States. Findings of the overall pooled analysis of diagnostic accuracy are presented in Table 1. Findings were similar when diagnostic performance in premenopausal women and postmenopausal women were evaluated separately. ROMA™ had similar or higher sensitivity than HE4 and CA 125, and HE4 had the highest specificity.

**Table 1 - Diagnostic Performance of ROMA™ Compared with HE4 and CA 125 From Wang et al. (2014): Meta-Analysis Findings**

| Test | No. Studies | Sensitivity | Specificity |
|---|---|---|---|
| ROMA™ | 14 | 85.3 (81.2-88.6) | 82.4 (77.4-86.5) |
| HE4 | 28` | 76.3 (72.0-80.1) | 93.6 (90.0-95.9) |
| CA 125 | 28 | 79.2 (74.0-83.6) | 82.1 (76.6- 86.5) |

Dayyani et al. (2016) conducted a meta-analysis comparing ROMA™ with HE4 and CA 125 in patients with suspected ovarian cancer. Six studies met the inclusion criteria, four of which were included in the Wang et al. (2014) meta-analysis. Two studies were published in 2014 or later. ROMA™ had statistically higher area under the curve (AUC) values than either HE4 or CA 125 alone (0.921, vs 0.899, and 0.883 for HE4 and CA 125, respectively). Findings of the pooled analysis of diagnostic accuracy are shown in Table 2.

**Table 2 - Diagnostic Performance of ROMA™ Compared With HE4 and CA 125 From Dayyani et al. (2016): Meta-Analysis Findings ROMA™**

| Test | No. Studies | Sensitivity % (95% CI) | Specificity %, 95% CI |
|---|---|---|---|
| ROMA™ | 6 | 87.3 (75.2-94.0) | 85.5 (71.9-93.2) |
| HE4 | 6 | 68.2 (69.3-90.1) | 85.1 (71.6-92.8) |
| CA 125 | 6 | 79.6 (66.3-88.5) | 82.5 (66.2- 91.9) |

Minar et al. (2018) performed a retrospective comparative study to compare the Risk of Ovarian Malignancy Algorithm (ROMA) and the Copenhagen Index (CPH-I) to differentially diagnose ovarian tumors. 267 women who attended University Hospital Brno, Czech Republic were included in study. 110 women had benign tumors, 42 had borderline ovarian tumors, and 115 had malignant tumors. The 2 indices showed comparable discriminatory performance with no significant variances. In the differentiation of benign tumors from all stages of borderline tumor and ovarian cancer, ROMA had a sensitivity of 71% and a specificity of 88%. CPH-I had a sensitivity of 69% and a specificity of 85%. The authors concluded that CPH-I has the potential to be a tumor index that is independent of menopausal status. It may be an easy alternative to ROMA in basic medical care settings.

Al Musalhi et al. (2016) performed a prospective, cross-sectional study to evaluate the validity and compare the performance of cancer antigen-125 (CA-125), human epididymis protein 4 (HE4), the risk of malignancy index (RMI), and the risk of ovarian malignancy algorithm (ROMA) in the diagnosis of ovarian cancer in patients with ovarian lesions found during their preoperative work-up. The study included 213 cases of various types of malignant (23%) and benign (77%) ovarian tumors. CA-125 demonstrated the greatest sensitivity (79%) when looking at the total patient population. The sensitivity was 67% in premenopausal women when divided by age. CA-125 had lower sensitivity (89%) compared to RMI, HE4, and ROMA (93% each) in postmenopausal women. A high specificity of 90% was found for HE4 in the total patient population, 75% in postmenopausal women and 93% in premenopausal women. CA-125 had the greatest specificity (79%) in postmenopausal women. CA-125 and RMI were often elevated in benign gynecological conditions particularly in endometriosis in comparison to HE4 and ROMA. Modifications of the optimal cut-offs for the four parameters were also studied. CA-125 and RMI showed a substantial increase in their specificity if the cut-off was increased to ≥ 60 U/mL for CA-125 and to ≥ 250 for RMI. For HE4, improvement in its specificity in postmenopausal women when its cut-off was increased to140 pmol/L was observed. The authors concluded that HE4 and ROMA displayed an extremely high specificity but were less sensitive than CA-125 and RMI in premenopausal women. In postmenopausal women, they were of comparable sensitivity and were very useful in distinguishing benign endometriosis or ovarian tumors from ovarian cancer. Modifying the cut-off values of the different markers resulted in a greater accuracy compared to the standard cut-offs, but at the expense of decreased sensitivity.

Terlikowska et al. (2016) performed a test validation study to assess HE4, CA125 and ROMA in the differential diagnosis of benign and malignant adnexal masses. 224 women were evaluable which included 120 premenopausal women with an average age of 36 and 104 postmenopausal women with an average age of 63. Using the ROC analysis, although no statistical variances were found among their AUCs, the ROMA algorithm appears to be impactful in gathering the diverse performance of HE4 and CA125. The AUC for HE4, CA125 and ROMA for all patients were: 0.895; 0.879 and 0.918, respectively. At established new ideal cutoff values for HE4, CA125 and ROMA the authors discovered greater specificity in postmenopausal in comparison to premenopausal women (96.9 vs 89.8 % and 97.7 vs 84.1 % and 95.9 vs 89.1 %, respectively). The sensitivity of HE4 in premenopausal and postmenopausal women was comparable (83.5 vs 83.8 %). CA125 was the greatest in premenopausal women (87.0 vs 84.1 %). HE4, CA125 and ROMA had high negative predictive value (97.6, 93.9 and 94.4 %, respectively). The authors concluded that the ROMA algorithm demonstrated the greatest diagnostic performance to differentiate epithelial ovarian cancer from benign ovarian disease. High specificity of

HE4 and CA125 was revealed while differentiating ovarian benign diseases from epithelial ovarian cancer in postmenopausal women and the high sensitivity of CA125 in identifying epithelial ovarian cancer in premenopausal patients.

Cho et al. (2015) performed a multi-center comparative clinical study aiming to compare CA125, HE4, and risk of ovarian malignancy algorithm (ROMA) in predicting epithelial ovarian cancer in Korean women with a pelvic mass. Prospectively, serum from 90 Korean women with ovarian mass was collected before surgery. Serum from 79 normal populations without ovarian mass was collected for the control group. The HE4 and CA125 data was registered and assessed separately and ROMA was calculated for each sample. 23 ovarian cancers and 67 benign tumors and were assessed. Median serum levels of HE4 and CA125, and ROMA score were substantially greater in patients with ovarian cancer than those with benign ovarian tumor and normal population. In ROC curve analysis for women with a pelvic mass, area under the curve (AUC) for HE4 and ROMA was greater than CA125. Statistical differences in each study compared to CA125 were marginal. Sub-analysis showed that AUC for HE4 and ROMA was greater than AUC for CA125 in post-menopausal women with a pelvic mass, but no statistically significant differences were found. The authors concluded that the data suggested that both HE4 and ROMA score showed greater performance than CA125 for the detection of ovarian cancer in women with a pelvic mass. HE4 and ROMA may be a helpful independent diagnostic marker for epithelial ovarian cancer in Korean women. Limitations of the study included small sample size.

Diagnostic performance of the ROMA™ test was evaluated for FDA approval in a prospective, blinded clinical trial using thirteen demographically mixed subject enrollment sites with company sponsorship (K103358). Patients all presented with an adnexal mass and were scheduled to undergo surgery. An Initial Cancer Risk Assessment (ICRA) was performed to determine the detection of benign versus malignant lesions before testing. The prevalence of cancer was 15%. Using pathologic diagnosis as the gold standard, test performance, when combined with presurgical assessment for benign disease, was as follows in the hands of a mixed population of generalist and specialist physicians:

| Measure | ICRA alone | ICRA with ROMA testing |
|---|---|---|
| Sensitivity | 77% | 91% |
| Specificity | 84% | 67% |
| PPV | 46% | 33% |
| NPV | 96% | 98% |

Both tests, when added to pre-testing clinical assessment, produced a fall in the positive predictive value of diagnosis with a small increase in the negative predictive value. The changes observed in the negative predictive value were of uncertain statistical and clinical significance.

Moore et al. (2014) performed a prospective multicenter study to evaluate ROMA™ in conjunction with clinical assessment, using either positive clinical assessment or positive ROMA™ as a positive test (similar to the recommended usage for OVA1®). Using this method of combining tests guarantees a higher sensitivity and lower specificity for the combined test than for either test alone. Used in this way, ROMA™ would only need to be evaluated in patients with a negative clinical assessment. In this study, 461 women were enrolled, of whom a total of 86 (19%) had a malignancy. Combined assessment improved sensitivity from 77.9% to 89.7%, but worsened specificity from 84.3% to 67.2%.

It is important to note that all of the above literature assessed ROMA™ as a stand-alone test and did not evaluate diagnostic performance in conjunction with clinical assessment, as the test was intended to be used. Therefore, the ability to draw conclusions regarding the test's diagnostic performance is limited.

## *Clinical Practice Guidelines*

### The American Congress of Obstetricians and Gynecologists (ACOG)

ACOG recommend at level C scientific evidence (based primarily on consensus and expert opinion) that physicians can use serum biomarker panels as an alternative to CA125 level alone when evaluating women with adnexal masses to determine the need for referral to or consultation with a gynecologic oncologist when an adnexal mass requires surgery. Consultation with or referral to a gynecologic oncologist is recommended at a level B scientific evidence (based on limited or inconsistent scientific evidence) for patients with an elevated score on a formal risk assessment test (ACOG, 2016).

### The Society for Gynecologic Oncology (SGO)

In May 2013, the Society for Gynecologic Oncology (SGO) issued a position statement on multiplex serum testing for women with pelvic masses stating that, "Blood levels of five proteins in women with a known ovarian mass have been reported to change when ovarian cancer is present. Tests measuring these proteins may be useful in identifying women

who should be referred to a gynecologic oncologist. Recent data have suggested that such tests, along with physician clinical assessment, may improve detection rates of malignancies among women with pelvic masses planning surgery. Results from such tests should not be interpreted independently, nor be used in place of a physician's clinical assessment. Physicians are strongly encouraged to reference the American Congress of Obstetricians and Gynecologists' 2011 Committee Opinion "The Role of the Obstetrician-Gynecologist in the Early Detection of Epithelial Ovarian Cancer" to determine an appropriate care plan for their patients." SGO does not formally endorse or promote any specific products or brands. (SGO, 2013)

### National Comprehensive Cancer Network (NCCN)

The NCCN ovarian cancer guidelines (NCCN Ovarian Cancer/Fallopian Tube Cancer/Primary Peritoneal Cancer, v3.2024) state, "the FDA has approved the use of ROMA, OVA1, or OVERA for estimating the risk for ovarian cancer in those with an adnexal mass for which surgery is planned, and have not yet been referred to an oncologist. Although the American Congress of Obstetricians and Gynecologists (ACOG) has suggested that ROMA and OVA1 may be useful for deciding which patients to refer to a gynecologic oncologist, other professional organizations have been non-committal. Not all studies have found that multi-biomarker assays improve all metrics (ie, sensitivity, specificity, positive predictive value, negative predictive value) for prediction of malignancy compared with other methods (eg, imaging, single-biomarker tests, symptom index/clinical assessment). Currently, the NCCN Panel does not recommend the use of these biomarker tests for determining the status of an undiagnosed adnexal/pelvic mass."

### National Institute for Health and Care Excellence (NICE)

There is currently not enough evidence to recommend the routine adoption of the IOTA ADNEX model, Overa®, RMI I (at thresholds other than 200 or 250), ROMA™ or IOTA Simple Rules in secondary care in the NHS to help decide whether to refer people with suspected ovarian cancer to a specialist multidisciplinary team (MDT). The NICE guideline on ovarian cancer recommends that people with an RMI I of 250 or more are referred to a specialist MDT. Evidence suggests that there is no substantial change in accuracy if the threshold for RMI I is lowered to 200. The IOTA ADNEX model, Overa®, RMI I (at thresholds other than 250), ROMA™ and IOTA Simple Rules show promise. Further research is recommended on test accuracy and the impact of the test results on clinical decision-making (NICE, 2017).

In summary, improving early detection and prevention of ovarian cancer is a priority in women's health. To date, none of the multimarker serum tests addressed in this policy have been shown to reliably screen, improve quality of life, or decrease mortality in women with ovarian cancer. Given that the literature has the limitations as outlined above and in conjunction with the position of the American College of Obstetrics and Gynecology, The Society for Gynecologic Oncology, and the National Cancer Institute, coverage of these tests must await larger, prospective, non-industry funded data on long term outcomes including quality of life, improvement in survival and impact on mortality in women with ovarian cancer.

## PDGFRA (Platelet - Derived Growth Factor Receptor, Alpha Polypeptide)

The *PDGFRA* gene provides instructions for making a protein called platelet-derived growth factor receptor alpha (PDGFRA), which is part of a family of proteins called receptor tyrosine kinases (RTKs). Receptor tyrosine kinases transmit signals from the cell surface into the cell through signal transduction. The PDGFRA protein is found in the cell membrane of certain cell types where a specific protein, called platelet-derived growth factor, attaches (binds) to it. This binding turns on (activates) the PDGFRA protein, which then triggers other proteins inside the cell by adding a cluster of oxygen and phosphorus atoms (a phosphate group) at specific positions (a process called phosphorylation). This process leads to activating a series of proteins in multiple signaling pathways. The PDGFRA protein, through the signaling pathways it stimulates, plays a crucial role in controlling vital cellular processes such as cell growth, division (proliferation), and cell survival. This underscores the importance of PDGFRA protein signaling in the development of various cell types throughout the body (MedlinePlus, 2021a).

Through a randomized trial, Joensuu et al. (2023) explored the influence of *KIT* and *PDGFRA* mutations on overall survival (OS) for those with gastrointestinal stromal tumors (GIST) treated with adjuvant imatinib. The results of this trial showed that during a median follow-up time of 10 years, 164 RFS events and 76 deaths occurred. Most people were re-treated with imatinib when GIST recurred. Those with *KIT* exon 11 deletion or indel mutation treated with three years of adjuvant imatinib survived longer than patients treated for one year [10-year OS 86% versus 64%, respectively; HR, 0.34; 95% confidence interval (CI), 0.15-0.72; P = 0.007], and also had more prolonged RFS (10-year RFS 47% versus 29%; HR, 0.48; 95% CI, 0.31-0.74; P < 0.001). People with the *KIT* exon nine mutation had unfavorable OS regardless of the duration of adjuvant imatinib. The authors concluded that compared with one year of imatinib, three years of adjuvant imatinib led to a 66% reduction in the estimated risk of death and a high 10-year OS rate in the subset of those with a *KIT* exon 11 deletion/indel mutation.

Qu et al. (2016) performed a single center study analyzing the long-term outcomes of imatinib in FIP1L1/PDGFRA associated chronic eosinophilic leukemia (CEL). 33 patients in China were included in the study. At median follow up of 64 months, 94% of patients with F/P mutated CEL had complete hematologic remission (CHR). After a median of 3 (1.5-12) months, 97% had complete molecular remission (CMR). 24 cases were given maintenance therapy, with an average CMR duration of 43 (5-88) months. Imatinib therapy was stopped in 8 cases, including 4 relapse cases, and 4 patients who maintained CHR or CMR after stopping therapy with an average time of 47 (2-74) months. 1 case showed primary resistance with a *PDGFRA T674I* mutation. The authors concluded that F/P mutated CEL has a great long-term prognosis after imatinib therapy. Imatinib 100 mg is satisfactory to induce remission, and a single 100 mg weekly dose sustains a durable remission. A subgroup of patients may sustain a durable remission after stopping therapy with a CMR.

# Pigmented Lesion Assay (PLA) (Oncology Melanoma)

## *Background*

Invasive and in situ cutaneous melanoma is a type of skin cancer that is diagnosed in over 100,000 patients annually in the United States. Over 8,200 people die from cutaneous melanoma in the US per year (ACS, 2024). Detecting melanomas at their earliest stages (melanoma in situ (MIS) / Stage 1) impacts disease outcome and patient survival. The 5-year relative survival rate from diagnosis for localized, early melanoma is over 99%, but 35% for melanoma that has spread to distant sites (ACS, 2024). The generally well accepted approach to assessing pigmented lesions includes visual inspection followed by surgical biopsy and histopathologic analysis of the biopsied tissue (Anderson et al. 2018, Argenziano et al. 2012, Duffy et al. 2012, Friedman et al. 2009, Nault et al. 2015, Reddy et al. 2013, Rigel et al. 2010, Schafer et al. 2006, Strazzula et al. 2014, Wilson et al. 2012). One large study (Anderson et al. 2018) assessing dermatologists' biopsy decisions using existing decision-making tools is approximately 25. In another study (Lott et al. 2018), roughly 24 biopsies were needed to diagnose 1 invasive melanoma, and roughly 12 biopsies were needed to detect either invasive melanomas or MIS. In summary, this approach results in many biopsies that do not lead to a melanoma diagnosis. Guidelines from the American Academy of Dermatology recommend that a prebiopsy photograph be taken to help with clinical / pathologic correlation (Elmore et al. 2017).

Additionally, the diagnostic yield of early-stage melanoma on biopsied tissue is limited. Histopathologic assessment of early-stage biopsied melanoma tissue is challenging and has significant discordance between pathologists (Elder et al. 2018, Malvehy et al. 2014, Ferris et al. 2018). It also appears that under interpretation is more common than over-interpretation of a patient who has had a biopsy (Malvehy et al. 2014, Ferris et al. 2018), which is tantamount to missed diagnoses. Additionally, while fellowship-trained or board-certified dermatopathologists tend to have a higher accuracy than other pathologists, even among this group, under-interpretation is highly prevalent (Malvehy et al. 2014).

In summary, conventional melanoma care may lead to both biopsies of non-malignant lesions, and even in those patients who do have a biopsy the diagnosis of a malignancy may be missed. As such, there is potential clinical utility for a test that can either spare a patient the need for a biopsy.

The Pigmented Lesion Assay (PLA) was developed to address fill the niche of reducing the biopsy rate of non-malignant lesions.

## *PLA Test Description*

It is a gene expression test using samples collected via adhesive patches providing a non-invasive alternative to the surgical biopsy pathway in the assessment of pigmented skin lesions (Ferris et al. 2018, Ferris et al. 2017, Gerami et al. 2017, Jansen et al. 2018, Rivers et al. 2018, Wachsman et al. 2011, Yao et al. 2016, Yao et al. 2017). The test is positive if *LINC00518* and/or *PRAME* (2 genes known to be overexpressed in melanoma) are detected (Horn et al. 2013, Pozzobon et al. 2014). The PLA is based on a platform technology for non-invasive genomic testing of the skin that allows the analysis of samples collected with an adhesive patch (Yao et al. 2017). Four patches are placed on a lesion. For each patch, the margin of the lesion is outlined by the clinician. This outlined tissue is dissected away from the surrounding tissue by the processing laboratory, and RNA is extracted only from the lesional tissue. In contrast to histopathologic sectioning, the adhesive patch method of tissue sampling allows the collection of tissue from the entire the lesion in the plane of the skin surface. Further, genomic information obtained by adhesive patch sampling of the stratum corneum contains information from deeper epidermal cells.

## *Bioplausibility*

The PLA has been validated against hotspot driver mutations in melanoma (e.g., *BRAF* other than *V600E*, *NRAS*, and the *TERT* promoter) that are associated with disease progression and histopathologic findings, such as mitotic counts and ulceration (Cancer Genome Atlas Network 2015, Griewank et al. 2014, Heidenreich et al. 2014, Hodis et al. 2012, Horn et al. 2013, Pozzobon et al. 2014, Shain et al. 2016, Shain et al. 2015).

## *Analytical and Clinical Validation*

The analytical and clinical performance of the PLA is supported by multiple investigational studies as discussed below.

As noted above, trained pathologists may disagree over their assessments of pigmented skin lesions, and comparisons of pathologists' opinions to each other or to a consensus group have been used as reference standard in studies. The clinical validity for the PLA has been assessed both using pathologist opinion and longitudinal patient outcomes.

Following early work identifying that the expression of *LINC* and *PRAME* can accurately classify pigmented lesions using a simple 2-gene detection methodology, a classifier method based on these 2 genes was used in an independent test set (Jansen et al. 2018).

The performance metrics of the PLA were validated by Gerami et al. (2017) against consensus panel histopathologic assessment clearly demonstrating the test's clinical validity in the assessment of early stage pigmented lesions. In this study samples were collected prospectively from multiple dermatology practices and centers, in patients 18 years of age or older, and from pigmented lesions that were suspicious for melanoma, meeting one or more ABCDE criteria. Clinically obvious or frank melanomas were excluded. Lesions were simultaneously sampled using the adhesive patch and surgically biopsied. Biopsy specimens underwent pathologic diagnosis from 3 independent dermatopathologists, and lesions that received a concordant diagnosis from all 3 dermatopathologists were enrolled in the study. Overall, 11% of lesions sampled had a discordant pathological read and were excluded, creating a reference set upon which there was diagnostic agreement among pathologists. A blinded evaluation of these concordant biopsy samples was performed against the PLA result. An initial training set of 157 lesions was tested and demonstrated a 91% sensitivity, 53% specificity. An independent validation set was subsequently studied that included 398 pigmented lesion samples (87 melanomas, 253 atypical pigmented lesions, 53 non-melanocytic lesions). All melanomas enrolled in the study were classified as very early stage and were either MIS or Stage 1 with a median Breslow thickness < 0.5 mm. The PLA demonstrated a sensitivity of 91% and a specificity of 69%.

A separate study assessing the clinical performance of the PLA in patients who received longitudinal follow-up was also done by Ferris et al. (2018). This study included available outcomes and clinical management decisions for PLA- and PLA + cases at 4 US dermatology practices using the PLA commercially. Cases were reviewed with a minimum of 6 months to 9 months follow-up, with 273 samples of this ongoing effort having 12 months follow up. Serial dermatoscopy studies indicate that melanomas have detectable visual changes within 3 months and recommended surveillance guidelines are 3-6 months (Altamura et al. 2008). For the 381 lesions evaluated in this study, the sensitivity was 95% and the specificity was 91%. While the sensitivity in this study is similar to that found in the histopathologic validation (Gerami et al. 2017), the specificity is higher.

## Summary of Analytical and Clinical Validation

## Summary of Performance and Utility (Table 1)

**Table 1: Published Studies and Manuscripts Demonstrating PLA Validation and Utility**

| | |
|---|---|
| **Analytical Validity** | Yao et al. (2016). "Analytical Characteristics of a Noninvasive Gene Expression Assay for Pigmented Skin Lesions." Assay and Drug Development Technologies 14.6 (2016): 355-363. |
| **Clinical Validity** | Yao et al. (2017). "An Adhesive Patch-Based Skin Biopsy Device for Molecular Diagnostics and Skin Microbiome Studies." Journal of Drugs in Dermatology 16.10 (2017): 611-618. |
| **Clinical Validity** | Gerami et al. (2017) "Development and validation of a noninvasive 2-gene molecular assay for cutaneous melanoma." J Am Acad Dermatol 76.1 (2017): 114-120.<br>• 398 validation samples, 157 training samples.<br>• PLA performance accuracy: 91% sensitive and 69% specific, NPV 99%. |
| **Clinical Validity and Utility** | Ferris et al. (2018) Real-World Performance and Utility of a Non-Invasive Gene Expression Assay to Evaluate Melanoma Risk in Pigmented Lesions. Melanoma Research 2018.<br>• Analysis of 381 patients, yielding 51 PLA + and 330 PLA- tests.<br>• PLA sensitivity 95%, specificity 91%.<br>• The test guides clinical management of lesions:<br>  ○ 99% of PLA- tests underwent surveillance pathway<br>  ○ 100% of PLA + tests received biopsy<br>• Zero missed melanomas in the follow up period<br>• Number of biopsies needed per melanoma found 2.7<br>• Number of excisions needed per melanoma found 1.6<br>• Visual assessment/histopathology pathway sensitivity 84% |

| | |
|---|---|
| **Clinical Validity** | Ferris et al. (2017) Utility of a noninvasive 2-gene molecular assay for cutaneous melanoma and effect on the decision to biopsy. JAMA Dermatology 153:675-680.<br>• 45 dermatologists evaluated 60 clinical and dermatoscopic images plus patient and lesion history.<br>• Both sensitivity and specificity improved with PLA results over clinical evaluation alone (specificity 32%→ 57%; sensitivity 95% →99%). |

## *Clinical Utility*

A review of over 20,000 commercial PLA results indicated that 88% of reported PLA tests were negative and 12% were positive (Wachsman et al. 2011). This combined with the finding in a 2017 study (Lott et al. 2018) of 18,715 surgical biopsies of pigmented lesions showing that 83% of the lesions biopsied were either benign or mildly atypical lesions, suggests that if the test has sufficient clinical performance to rule out melanoma (i.e., adequate sensitivity and negative predictive value), and treating clinicians use the test results as intended, it should result in significantly fewer unnecessary biopsies without compromising melanoma outcomes. Clinical performance is reviewed above. Here clinical decision making following the use of the test is reviewed.

In the Ferris et al. (2018) longitudinal follow-up study mentioned above,18 99% of the 330 PLA negative lesions were managed by dermatologists with surveillance. Three of the PLA- lesions that were biopsied in the follow-up period were done so at the patient's insistence. One PLA- lesion was simultaneously surgically biopsied (not the intended use of the test) and adhesive patch sampled and was diagnosed as MIS. There were 0 missed melanomas found in the follow-up period. Of 51 PLA + test results, 100% were managed by dermatologists with a surgical biopsy. Nineteen (37%) of these cases were MIS / Stage 1 invasive melanomas with a thickness of < 0.5 mm and demonstrating a Number needed to biopsy (NNB) of 2.7 (51/19).

In an additional utility study by Ferris et al. (2017), 45 dermatologists who regularly evaluate pigmented lesions, assessed 60 cases containing dermatoscopic and lesional images (8 melanoma and 52 nevi with known pathologic concordance) with full patient and lesion history. The photographic/dermatoscopic analysis design of this study provided information nearly identical to the dermatologist's primary clinical visual assessment used to make biopsy decisions and is therefore more relevant than typical decision impact studies that involve select case information review with and without a test result. Cases/images were initially presented without PLA results, and the dermatologists were asked to make a biopsy decision for suspicion of melanoma. The 60 cases were then shuffled and presented again, including the PLA test data. Again, dermatologists were asked to make a biopsy decision for suspicion of melanoma. Outcomes included changes in biopsy decisions, specificity, and sensitivity. Biopsy decisions increased from 750 to 1331. Assuming correctness of the reference diagnosis, the specificity of the biopsy decision increased by 1.8-fold with the PLA (32%-56%, $p < 0.001$). The sensitivity also improved to approximately 99% ($p = 0.01$) with the PLA, even with significant increases in specificity.

Most recently, Ferris et al. (2019) reviewed 12-month management decisions and outcomes for patients testing using the PLA. The study involved retrospective chart reviews of 734 lesions that were PLA(-) and a registry of 175 pigmented lesions tested using the PLA. Among the 734 PLA(-) lesions, 13 were biopsied within one year. Of these 13 biopsied lesions, 11 were nevi with various degrees of atypia, 1 was a basal cell carcinoma and 1 was a squamous cell carcinoma. None were melanomas. In the registry cohort, 1433 of 1575 total lesions were PLA(-), and in follow-up only 2 had a surgical evaluation within a year. One of these had a scoop excision and was found to be a melanocytic nevus. The other was a squamous cell carcinoma removed by Mohs surgery. Of the 142 PLA( + ) lesions in the registry cohort 96.5% were biopsied.

## PIK3CA (Phosphatidylinositol-4, 5-Biphosphate 3-Kinase, Catalytic Subunit Alpha)

André et al. (2021) reported on the final overall survival results from SOLAR-1 randomized controlled trial in alpelisib plus fulvestrant for *PIK3CA*-mutated, hormone receptor-positive, human epidermal growth factor receptor-2-negative patients with advanced breast cancer. Postmenopausal women and men with HR +, HER2- ABC whose disease advanced on or after aromatase inhibitor (AI) were randomized 1 : 1 to receive alpelisib (300 mg/day) plus fulvestrant (500 mg every 28 days and once on day 15) or placebo plus fulvestrant. Plasma ctDNA was collected at baseline and tested for a *PIK3CA* mutation using the Qiagen therascreen *PIK3CA* RGQ polymerase chain reaction (PCR) kit. Overall survival (OS) in the *PIK3CA*-mutant cohort was evaluated by Kaplan-Meier methodology and a one-sided stratified log-rank test was carried out with an O'Brien-Fleming efficacy boundary of. In the *PIK3CA*-mutated cohort (n = 341), median OS [95% confidence interval (CI)] was 39.3 months (34.1-44.9) for alpelisib-fulvestrant and 31.4 months (26.8-41.3) for placebo-fulvestrant [hazard ratio (HR) = 0.86 (95% CI, 0.64-1.15; P = 0.15)]. OS results did not cross the prespecified efficacy boundary. Median OS (95% CI) in patients with lung and/or liver metastases was 37.2 months (28.7-43.6) and 22.8 months (19.0-26.8) in the alpelisib-fulvestrant and placebo-fulvestrant arms, respectively [HR = 0.68 (0.46-1.00)]. Median times to chemotherapy (95% CI) for the alpelisib-fulvestrant and placebo-fulvestrant arms were 23.3 months (15.2-28.4) and 14.8

months (10.5-22.6), respectively [HR = 0.72 (0.54-0.95)]. There were no new safety signals observed with longer follow-up. The authors concluded that although the analysis did not cross the prespecified boundary for statistical significance, there was a 7.9-month numeric improvement in median OS when alpelisib was added to fulvestrant treatment of patients with *PIK3CA*-mutated, HR +, HER2- ABC. In general, these results further support the statistically significant prolongation of PFS observed with alpelisib plus fulvestrant in this population, which has a poor prognosis due to a *PIK3CA* mutation.

Rugo et al. (2021) performed a non-randomized trial (BYLieve) on alpelisib plus fulvestrant in *PIK3CA*-mutated, hormone receptor-positive advanced breast cancer after a CDK4/6 inhibitor. Cohort A included 127 patients with at least 6 months of follow-up. 121 patients had a centrally confirmed *PIK3CA* mutation, determined by the therascreen test. At data cutoff, average follow-up was 11·7 months (IQR 8·5-15·9). 61 (50·4%; 95% CI 41·2-59·6) of 121 patients were living without disease progression at 6 months. The greatest grade 3 or worse adverse events were hyperglycaemia (36 [28%] of 127 patients), rash maculopapular (12 [9%]), and rash (12 [9%]). Severe adverse events occurred in 33 (26%) of 127 patients. Treatment-related deaths were not reported. The authors concluded that BYLieve demonstrated activity of alpelisib plus fulvestrant with manageable toxicity in patients with *PIK3CA*-mutated, hormone receptor-positive, HER2-negative advanced breast cancer, after progression on a CDK4/6 inhibitor plus an aromatase inhibitor. Study limitations included the fact that there was no comparison group. This trial is registered with ClinicalTrials.gov, NCT03056755 funded by Novartis Pharmaceuticals.

Turner et al. (2021) reported on the BYLieve non-randomized trial and the effectiveness of Alpelisib + Fulvestrant Compared with RealWorld Standard Treatment Among Patients with HR +, HER2–, *PIK3CA*-Mutated Breast Cancer. The BYLieve trial (NCT03056755) confirmed safety and efficacy of alpelisib with fulvestrant for hormone receptor-positive (HR +), human epidermal growth factor receptor-2-negative (HER2-), *PIK3CA*-mutated advanced breast cancer (ABC), after cyclin-dependent kinase 4/6 inhibitor (CDK4/6i) with an aromatase inhibitor (AI) as immediate prior therapy. 855 patients with *PIK3CA*-mutant disease with prior CDK4/6i plus hormone therapy were included in the study; further matching to 120 patients from BYLieve selected 95 patients with no exposure to HER2-targeting agents, clinical study drug, or alpelisib. In postmatching and unadjusted results, primary and secondary endpoints were in favor of treatment with alpelisib with fulvestrant in BYLieve over standard treatments in the real-world cohort. Postadjustment, median PFS for patients treated with alpelisib in BYLieve was 7.3 vs. 3.7 months in the real-world cohort, and 6-month PFS was 54.6% versus 40.1%, respectively. The authors concluded that weighted/matched analysis comparing BYLieve with the real-world setting further corroborates the clinical benefit of alpelisib with fulvestrant for treatment of HR +, HER2-, *PIK3CA*-mutant ABC after CDK4/6i treatment.

André et al. (2019) performed a randomized controlled trial (SOLAR-1) on Alpelisib for *PIK3CA*-Mutated, Hormone Receptor-Positive Advanced Breast Cancer. 572 patients underwent randomization which included 341 patients with confirmed tumor-tissue *PIK3CA* mutations. In the *PIK3CA*-mutated cancer cohort, progression-free survival at a median follow-up of 20 months was 11.0 months (95% confidence interval [CI], 7.5 to 14.5) in the alpelisib–fulvestrant group, in comparison to 5.7 months (95% CI, 3.7 to 7.4) in the placebo–fulvestrant group (hazard ratio for progression or death, 0.65; 95% CI, 0.50 to 0.85). In the cohort without *PIK3CA*-mutated cancer, the hazard ratio was 0.85 (95% CI, 0.58 to 1.25; posterior probability of hazard ratio < 1.00, 79.4%). Overall response in all cohort patients with *PIK3CA*-mutated cancer was greater with alpelisib–fulvestrant than with placebo–fulvestrant (26.6% vs. 12.8%). Among patients with measurable disease in this cohort, the percentages were 35.7% and 16.2%, respectively. In the overall population, the most frequent adverse events of grade 3 or 4 were hyperglycemia (36.6% in the alpelisib–fulvestrant group vs. 0.7% in the placebo–fulvestrant group) and rash (9.9% vs. 0.3%). Grade 3 diarrhea was seen in 6.7% of patients in the alpelisib–fulvestrant group, in comparison to 0.3% of those in the placebo–fulvestrant group. Grade 4 diarrhea was not reported. The percentages of patients who stopped alpelisib and placebo owing to adverse events were 25.0% and 4.2%, respectively. The authors concluded that treatment with alpelisib-fulvestrant prolonged progression-free survival among patients with *PIK3CA*-mutated, HR-positive, HER2-negative advanced breast cancer who previously received endocrine therapy. (Funded by Novartis Pharmaceuticals; SOLAR-1 ClinicalTrials.gov number, NCT02437318).

Francini et al. (2024) conducted a systematic review and meta-analysis to evaluate the intensification approaches and treatment sequencing in metastatic castration-resistance prostate cancer. *PIK3CA/AKT1/PTEN* alterations and immunohistochemistry-assessed *PTEN* loss were found in 40–50% of prostate cancer cases, as indicated in the IPATential150 trial. They are promising predictive biomarkers for AKT inhibitors. The authors concluded that AKT inhibitor combination of ipatasertib + abiraterone extends radiographical progression-free survival (rPFS) in individuals with *PTEN* loss or *PIK3CA/AKT1/PTEN* alterations. However, survival data is immature.

Lau et al. (2024) performed a systematic review to identify genes suggested to have molecular mechanism impacts on the radioresponsiveness of colorectal cancer patients. 24 genes including *PIK3CA* were identified in having roles in pathways including apoptosis, inflammation, DNA damage response/repair, and cancer metabolism, that may impact cancer

radioresponse. This review was limited by the fact that there was a lack of retrospective studies to verify findings, the study was solely on rectal cancer and not colon cancer, and that no meta-analysis was performed.

Lawler et al. (2024) performed a systematic review and meta-analysis regarding prognostic and predictive tumor markers for early (diagnosed before the age of 50) versus late onset colorectal cancer. No significant difference in the prevalence of *PIK3CA* mutations was found for early-onset colorectal cancer. Specifically, a study with over 1,000 distal and rectal tumors displayed no significant age difference in *APC, BRAF*, *KRAS*, *NRAS*, *PIK3CA*, or *TP53*, mutations. The authors concluded that more research is needed to clarify the relationships with novel tumor characteristics including immune markers and to identify molecular subtypes specific to early-onset colorectal cancer that can impact prognosis and treatment. The review was limited by studies published within the last 10 years, significant estimate heterogeneity, and that residual confounding by differences in tumor location or hereditary conditions may have caused bias in the results.

Roncato et al. (2024) performed a systematic review and meta-analysis to evaluate the impact of the most relevant molecular alterations in cancer-related genes of colorectal cancer (for example, *RAS*, *BRAF*, *SMAD4*, *PIK3CA*) as prognostic markers of survival and disease recurrence in patients with metastatic colorectal cancer who were treated by liver metastases resection. Only 3 studies for *PIK3CA* were eligible and no significant association with either recurrence free survival or overall survival could be highlighted. The authors concluded that no conclusion could be drawn for *PIK3CA* due to limited availability of literature.

Sadlecki et al. (2024) performed a systematic review on molecular changes found in borderline ovarian tumors (BOTs) and their significance on overall therapeutic approach. Proto-oncogenes: *BRAF*, *KRAS*, *NRAS*, *ERBB2*, and *PIK3CA* were included in the review. In 1 patient, *PIK3CA*, *BRAF*, and *ERBB2* mutations were found solely in lower grade serous ovarian cancer (LGSC) but not in synchronous serous borderline tumor (SBOT). In another patient, *PIK3CA* mutations were found in both LGSC and SBOT. The implication is that *PIK3CA* and *ERBB2* mutations are significant events occurring during the transformation of serous cystadenoma to SBOT and further to LGSC. Of note, the frequency of *PIK3CA* mutations in LGSCs/SBOTs in the Japanese population seems to be considerably higher than in the Western population. *PIK3CA* mutation may play a primary role in developing LGSCs in Japanese patients. Targeting the *PIK3CA*/*AKT* pathway through molecular therapies seems to be a potentially promising treatment for LGSC in the Japanese population. The authors concluded that molecular studies substantially added to the understanding of borderline ovarian tumor pathogenesis. However, substantial research still needs to be performed to clarify the relationship between ovarian neoplasms and extraneous disease, pinpoint accurate prognostic indicators, and form targeted therapeutic approaches.

## PMP22 (Peripheral Myelin Protein 22) (e.g., Charcot-Marie-Tooth, Hereditary Neuropathy With Liability to Pressure Palsies)

The *PMP22* gene provides instructions for making a protein called peripheral myelin protein 22 (PMP22). This protein is found in the peripheral nervous system, which connects the brain and spinal cord to muscles and to sensory cells that detect sensations such as touch, pain, heat, and sound. Mutations in gene cause several forms of Charcot-Marie-Tooth (CMT) disease, a disorder that damages the peripheral nerves, which can cause loss of sensation and atrophy of the muscles in the feet, legs, and hands (MedlinePlus, 2020c).

There is no cure for CMT, and maintaining mobility, flexibility, and muscle strength is important. Starting a treatment program early may delay or reduce nerve degeneration and muscle weakness before it progresses to the point of disability. Medications can be prescribed for severe nerve pain. Ongoing research includes efforts to identify more of the mutant genes and proteins that cause the various disease subtypes, discover the mechanisms of nerve degeneration and muscle atrophy with the goal of developing interventions to stop or slow down these debilitating processes, and to develop therapies to reverse nerve degeneration and muscle atrophy (NIH, 2024).

The diagnosis of CMT disease is based on physical symptoms, family history and clinical tests. These include nerve conduction velocity (NCV) electromyogram (EMG). Treatment is symptomatic and supportive, and there is no cure. With recent advances in molecular genetic testing using both deletion duplication analysis and next generation sequencing (NGS) for patients with a clinical diagnosis of CMT, a genetic cause can be found in about 60% of patients (NORD, 2021)

More than 80 different genes are associated with CMT. *PMP22* duplication accounts for approximately 50% of all CMT and *PMP22* deletion/duplication analysis is recommended as the first test for all probands with CMT. Because the methodology to detect *PMP22* duplication differs from that used in many multigene panels, this test needs to be ordered separately unless a laboratory explicitly states that *PMP22* deletion/duplication analysis is included in its multigene panel. Symptoms usually begins in the first to third decade and results in weakness and atrophy of the muscles in the hands and/or feet. Testing is also used for prenatal testing, preimplantation genetic testing, and carrier testing (Bird et al. 2024a).

The large number of CMT causing genes is often challenging for clinicians and patients when trying to determine the underlying genetic diagnosis. There is little information available to guide which gene to test and testing a patient for mutations in all commercially available CMT genes is not realistic. Family planning and prognosis may require an accurate genetic diagnosis and current treatment trials depend on knowing the genetic cause of a patient's CMT even if no cures are presently available (Miller et al. 2011).

Hayes Molecular Test Assessment for Charcot-Marie-Tooth Type 1A (*PMP22*) (2008, updated 2012) describes Charcot-Marie-Tooth disease Type 1A (CMT1A) as a peripheral demyelinating neuropathy caused by a 1.5-megabase (Mb) duplication of chromosome 17 at band p11.2, which includes the peripheral myelin protein 22 (*PMP22*) gene. Product names include complete CMT Evaluation (#400) or *PMP22* Duplication/Deletion DNA Test (#131) (Athena Diagnostics Inc.); Inherited Peripheral Neuropathies (Charcot-Marie-Tooth Type 1A [CMT1A] and Hereditary Neuropathy with Liability to Pressure Palsies [HNPP]) FISH analysis (#8467) (Medical Genetics Laboratories [MGL] at Baylor College of Medicine [BCM]). For confirmation of diagnosis in an individual with suspected CMT based on clinical findings, Hayes assigns a rating of C (potential but unproven benefit). For an asymptomatic individual with a confirmed family history of CMT1A to establish personal risk, Hayes assigns a rating of C. For an oncology patient with unexplained or preexisting familial neuropathy consistent with CMT, Hayes assigns a rating of B (some proven benefit). For prenatal or preimplantation genetic diagnosis of CMT1A, Hayes assigns a rating of B.

Screening services such as pre-symptomatic genetic tests and services used to detect an undiagnosed disease or disease predisposition, prenatal diagnostic testing, and carrier screening are not a Medicare benefit.

## Resolution ctDX Lung

In a recent retrospective study conducted by Liu et al. (2024), patients with metastatic *HER2*-mutant NSCLC who underwent prospective clinical ctDNA sequencing and received systemic therapy were analyzed. *HER2* mutations were identified by next-generation sequencing through MSK-IMPACT, MSK-ACCESS or Resolution ctDx Lung™ assay. Primary endpoints were time to the next treatment (TTNT) and overall survival (OS). 63 patients were included in the study. Chemoimmunotherapy was the primary first-line treatment with a median TTNT of 5.1 months, whereas 55.0% of patients who received second-line T-DXd had a median TTNT of 9.2 months. Plasma ctDNA was tested before first-line therapy in 40 patients with a median OS of 28.0 months. 31 of those patients (78.0 %) had identifiable ctDNA. *HER2* mutations were found on ctDNA with an average turnaround time of 13 days, occasionally occurred simultaneously with *MET* and *EGFR* alterations and were monitored longitudinally correlating with treatment response. Those who had detectable baseline ctDNA had substantially shorter OS. 31 patients had at least one detectable ctDNA alteration by ctDx Lung or MSK-ACCESS. The authors concluded that chemoimmunotherapy continues to be main treatment option for metastatic *HER2*-mutant NSCLC. ctDNA can rapidly detect *HER2* and co-mutations. It can potentially guide and monitor ideal first-line therapy. As a negative prognostic biomarker, detectable ctDNA at baseline should be taken into account for patient selection in future studies. Study limitations included the retrospective nature of the study and small sample size. This prevented further multivariable analysis investigating the independent prognostic value of ctDNA. These include the trend that *HER2* mutations occurred more often in younger patients and subgroup analyses comparing the clinical outcomes associated with the different *HER2*-targeted agents. Also, radiologic tumor measurements were not available. Although TTNT reflects duration of clinical benefit as determined by the clinician, it can be impacted by toxicity and treatment interruptions.

In a Hayes Precision Medicine Research Brief of Resolution ctDx Lung (Resolution Bioscience Inc.), it was concluded that based on a review of abstracts, there is insufficient evidence to perform a full assessment for the Resolution ctDX Lung test. Only one study was found that specifically stated Resolution ctDX Lung assay was used (Hayes Precision Medicine Research Brief Resolution ctDx Lung (Resolution Bioscience Inc.), 2020)

Supplee et al. (2019) tested the sensitivity of Guardant360™ (G360, Guardant Health) and ctDx-Lung (Resolution Bioscience) in detecting oncogenic fusions in plasma cell-free DNA. Out of 16 patients assayed known to harbor an *ALK*, *ROS1*, or *RET* in tumor, G360 identified fusions in 7 cases, ctDx-Lung identified fusions in 13 cases, and 3 cases were not identified by G360 or ctDx-Lung. Out of the 7 fusions identified by both assays, G360 reported lower mutant allelic fractions (AF). In cases missed by G360, tumor derived *TP53* mutations were frequently identified confirming the presence of tumor DNA. Raw sequencing data displayed that inverted or out-of-frame variants were overrepresented in cases identified using ctDx-Lung but not by G360. The authors concluded that focusing on complex, clinically actionable mutations using tumor as a reference standard enables evaluation of technical differences in plasma NGS assays that may impact clinical performance. Noting the heterogeneity of fusion sequences observed in non-small cell lung cancer (NSCLC), the authors hypothesize that differences in hybrid capture techniques and bioinformatic calling may be sources of variations in sensitivity among these assays. There needs to be continued efforts to better existing assays to fully leverage their potential to impact patient care. The study was limited by its retrospective design and small sample size. While a good model for evaluating complex variants, actionable fusions are uncommon in NSCLC. Routine clinical plasma

NGS is fairly new, thus precluding a larger sample size. Also, although samples were batched together for sequencing with ctDx-Lung, G360 testing has been in use variably for 2 years. At this time, the G360 assay has evolved through four commercial versions (v14.0- v17.0). Future comparative studies should strive to use latest version of all assays. Another limitation includes that the two plasma specimens were not regularly obtained at the same time. (This study is included in the Hayes 2020 Precision Medicine Research Brief).

## SMN1 (Survival of Motor Neuron 1, Telomeric) (e.g., Spinal Muscular Atrophy)

Genetic testing is used to diagnosis spinal muscular atrophy, which is characterized by muscle weakness and atrophy. The onset of weakness ranges from before birth to adulthood. It is also used for prenatal testing and carrier testing (Prior et al. 2020).

The *SMN1* gene provides instructions for making the survival motor neuron (SMN) protein. SMN is found throughout the body, especially in the spinal cord. This protein is in the group of proteins called the SMN complex, which is essential for the maintenance of motor neurons. These cells are located in the brainstem which connects the spinal cord to the brain. Motor neurons tell skeletal muscles to contract which allows the body to move. Several known mutations in the *SMN1* gene have been found to cause spinal muscular atrophy (SMA). This condition leads to weakness and wasting in the skeletal muscles and progresses with age. *SMN1* has also been associated with Amyotrophic lateral sclerosis (ALS) in a small number of cases (MedlinePlus, 2018b). SMA has been the leading genetic cause of infant mortality. At this time, there is no cure for SMA although there are new disease modifying therapies that are available that are shown to extend life expectancy and allow for further motor milestones more so than without the modifying treatment. Ongoing studies are needed for these replacement therapies for SMA therapy management and preservation of quality of life. (Chaytow et al. 2021).

Molecular pathology tests for diseases or conditions that manifest severe signs or symptoms in newborns and in early childhood or that result in early death are generally not relevant to a Medicare member. Prenatal diagnostic testing and carrier screening are not a Medicare benefit.

## SMPD1 (Sphingomyelin Phosphodiesterase 1, Acid Lysosomal) (e.g., Niemann-Pick Disease, Type A)

Genetic testing is used to diagnose acid sphingomyelinase deficiency (ASMD)/Niemann-Pick disease, which most commonly presents as hepatosplenomegaly detectable by 3 months of age. Usually, failure to grow is apparent by the second year of life. Psychomotor development progresses no further than the 12-month level, followed by relentless neurologic deterioration. It is also used for prenatal testing, preimplantation genetic testing, and carrier testing (Wasserstein and Schuchman, 2023).

Molecular pathology tests for diseases or conditions that manifest severe signs or symptoms in newborns and in early childhood or that result in early death are generally not relevant to a Medicare member. Prenatal diagnostic testing and carrier screening are not a Medicare benefit.

## SNRPN/UBE3A (Small Nuclear Ribonucleoprotein Polypeptide N and Ubiquitin Protein Ligase E3A) (e.g., Prader-Willi Syndrome and/or Angelman Syndrome)

Genetic testing is used to diagnose Angelman syndrome and Prader-Willi Syndrome. Angelman syndrome is characterized by severe developmental delay or intellectual disability, severe speech impairment, unique behavior including frequent smiling, laughing, and excitability, gait ataxia and/or limb tremors, seizures and microcephaly. Delays are first detected at 6 months of age but the unique clinical symptoms do not become apparent until after age one (Dagli et al. 2021). Prader-Willi syndrome is characterized by poor appetite, severe hypotonia, and feeding difficulties in early infancy, followed in early childhood by excessive eating and gradual development of morbid obesity and developmental delays. It is also used for prenatal testing, preimplantation genetic testing, and carrier testing (Driscoll et al. 2023).

In a Hayes Molecular Test Assessment on Angelman Syndrome (AS), it is noted that genetic testing for AS involves the *SNRPN/UBE3A* genes. For symptomatic children for diagnosis of Angelman Syndrome (AS), Hayes assigns a rating of B (some proven benefit). For symptomatic adults for diagnosis of AS, Hayes assigns a rating of C (potential but unproven benefit). For prenatal testing for *UBE3A* variants in familial cases of AS, Hayes assigns a rating of D2 (insufficient evidence) (Hayes, Molecular Test Assessment Angelman Syndrome (AS), 2008, updated 2012).

In a Hayes Molecular Test Assessment on Prader-Willi Syndrome (PWS), it is noted that genetic testing for PWS is usually focused on the *SNRPN* gene. For the diagnosis of PWS in symptomatic neonates, infants, children, or adults, Hayes assigns a rating of B (some proven benefit. Published evidence indicates that safety and impact on health outcomes are at least comparable to standard treatment/testing. However, there are outstanding questions regarding

long-term safety and impact on health outcomes, clinical indications, contraindications, optimal treatment/testing parameters, and/or effects in different patient subpopulations). For prenatal diagnosis of PWS in the presence of risk factors, Hayes assigns a rating of D2 (insufficient evidence) (Hayes, Molecular Test Assessment Prader-Willi Syndrome (PWS), 2008, updated 2012).

Molecular pathology tests for diseases or conditions that manifest severe signs or symptoms in newborns and in early childhood or that result in early death are generally not relevant to a Medicare member. Prenatal diagnostic testing and carrier screening are not a Medicare benefit.

## TERT (Telomerase Reverse Transcriptase)

The *TERT* gene provides instructions for making one component of an enzyme called telomerase. Telomerase maintains structures called telomeres, which are composed of repeated segments of DNA found at the ends of chromosomes. Telomeres protect chromosomes from abnormally sticking together or breaking down. Telomerase is highly active in cells that divide rapidly, such as cells that line the lungs and gastrointestinal tract, bone marrow, and cells of the developing fetus, and most cancer cells (MedlinePlus, 2020d).

In a 2021 systematic review, Olympios et al. provided an overview of the recent advances related to physiopathological mechanisms, diagnosis, and clinical implications of alterations in the promoter region of the telomerase reverse transcriptase (*TERTp*) gene which occurs in 70% to 80% of all glioblastomas. Ninety-two studies were included. The results showed that *TERTp* mutations are the most represented alterations in glioblastoma, suggesting a pivotal role in oncogenesis. The identification of *TERTp* mutations is essential and is currently integrated into glioblastoma diagnostic procedures. However, the prognostic impact remains controversial and a better understanding of the molecular mechanisms are needed for the development of *TERT* targeted therapies, and to date, there are no efficient *TERT* mutation related glioblastoma treatments.

In a 2019 review, Colebatch et al. summarized the function and structure of *TERT* and the implications in cancer. The *TERT* gene plays important roles in normal biology, and perturbations of its regulation play a critical role in a variety of pathological states, especially neoplasia. Germline mutations in *TERT* have been associated with idiopathic pulmonary fibrosis, and more rarely in families with dyskeratosis congenita. *TERT* mutations have also been found in patients with severe emphysema. *TERT* plays a central role in modulating telomerase activity in tumors, resulting in the hallmark of no cell death in neoplasms. A deeper understanding of this gene is pertinent given its potential usage as a biomarker and the future development of possible therapeutic avenues.

## *Clinical Practice Guidelines*

### National Comprehensive Cancer Network (NCCN)

In the NCCN Clinical Practice Guidelines for Central Nervous System Cancers (NCCN v3.2024), *TERT* (Promoter Mutations) recommendations are as follows:

- Recommendation: *TERT* promoter mutation testing is recommended for the workup of gliomas.
- Description: *TERT* encodes telomerase, which is the enzyme responsible for maintaining telomere length in dividing cells. *TERT* mutations found in gliomas are located in its noncoding promoter region, and cause increased expression of the TERT protein.
- Detection: *TERT* mutation can be detected by sequencing the promoter region.
- Diagnostic value: *TERT* promoter mutations are almost 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.
- Prognostic value: In the absence of an *IDH* mutation, *TERT* promoter mutation in diffusely infiltrative gliomas is associated with decreased overall survival compared to similar gliomas lacking *TERT* promoter mutation. Combined *TERT* promoter mutation and *IDH* mutations in the absence of 1p/19q codeletion is an uncommon event. However, such tumors have a prognosis as favorable as gliomas with all three molecular alterations.

## Thyroid Cancer

### *Thyroseq®*

Thyroid cancer is the most common endocrine malignancy, with an estimated 44,020 new United States diagnoses in 2024 (NCI, 2024). Thyroid nodules are exceedingly common with prevalence rates of up to 68%, with higher frequencies in the elderly (Haugen et al. 2016). While the majority (85-93%) of thyroid nodules are benign, diagnostic testing (history and physical, laryngoscopy, hormone and chemistry analysis, ultrasound, CT, FNA, and surgical excision) is required to confirm. Over 600,000 thyroid FNAs are performed every year in the United States, and the number has been increasing

annually by 16% (Steward et al. 2019). Cancer rates vary widely by institution, ranging from 6-48% for Bethesda III and 14-34% for Bethesda IV. Repeat FNA of Bethesda III nodules should be strongly considered as it leads to a more definitive reclassification in 60-65% (Patel et al. 2020). Rates of thyroid surgery-specific postoperative complications (recurrent laryngeal nerve injury, permanent hypoparathyroidism, and postoperative hematoma) in high-volume institution studies range between 0.4-7.4%, but a population-based study found it as high as 12.3% (Papaleontiou et al. 2017).

In 2012, molecular marker testing (MT) became widely available as a potential method to augment risk stratification of indeterminate FNA results, ideally reducing the need for diagnostic thyroid surgery or completion thyroidectomy, with their attendant risks and costs. A patient with a low MT malignancy risk is potentially recommended for surveillance with serial ultrasounds to ensure nodule stability. Conversely, a high malignancy MT result could strengthen a recommendation to move forward with surgical removal (lobectomy or total thyroidectomy). Molecular profiling includes genomic alterations (such as point mutations, insertions, and deletions), gene fusions resulting in rearrangements or translocations, copy number variations, RNA-based gene expression, and/or micro-RNA (miRNA) expression (Patel et al. 2020).

In 2016, the nomenclature of encapsulated follicular variant of papillary thyroid cancer was changed to noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) in recognition of its highly indolent nature. Thus, the value of MT may arise both from the avoidance of surgery and the fact that surveillance is now a safer and more informed option (Zhu et al. 2020). Conversely, given clinical guideline recognition that more limited cancer operation can lead to equivalent outcomes, the impact of molecular testing in directing the extent of surgical resection is diminished (Khan et al. 2020).

Thyroseqv3 is a targeted next-generation sequencing test that interrogates selected regions of 112 thyroid cancer-related genes for point mutations, insertions/deletions, gene fusions, copy number alterations, or gene expression alterations (Steward et al. 2019). A “genomic classifier” assigns a value to each detected genetic alteration based on the strength of association with malignancy: 0 (no association with cancer), 1 (low cancer probability), or 2 (high cancer probability). A score calculated for each sample is a sum of individual values of all detected alterations, with scores 0 and 1 accepted as test negative (score 1 commercially reported as currently negative) and scores 2 and above as positive.

Steward et al. (2019) performed a multicenter prospective, blinded, clinical validation study of ThyroSeqv3, including 247 Bethesda III and Bethesda IV nodules in which both pathologist and clinicians were blinded to MT results, sensitivity was 94% (95% CI, 86%-98%) and specificity 82% (95% CI, 75%-87%). With a cancer/NIFTP prevalence of 28%, the negative predictive value (NPV) was 97% (95% CI, 93%-99%), the positive predictive value (PPV) was 66% (95% CI, 56%-75%), with a benign call rate (BCR) of 61%. The observed 3% false-negative rate was similar to that of benign cytology, and the missed cancers were all low-risk tumors. Results of 10 Bethesda V nodules are not separately reported.

Nikiforova et al. (2018) performed another Thyroseqv3 validation study, 238 surgically removed tissue samples were used as a training set and 175 indeterminate (Bethesda III, n = 84; Bethesda IV, n=74; Bethesda V, n = 17) FNAs were used as a validation set (12). The training set sensitivity was 93.9% (95% CI, 88.4%-96.9%), the specificity 89.4% (95% CI, 81.1%-94.3%), with an accuracy of 92.1% (95% CI, 87.8%-95.0%). The validation set sensitivity was 98.0% (95% CI, 92.9%-99.4%), the specificity 81.8% (95% CI, 71.8%-88.9%), with an accuracy of 90.9% (95% CI, 85.7%-94.3%). The sensitivity and specificity for Hürthle cell lesions in the training set was 92.9% (95% CI, 80.52%-98.50%) and 69.3% (95% CI, 48.21%-85.67%), respectively. A separate case study also showed a benefit in indeterminate Hürthle cell cytopathology (Pearlstein et al. 2018).

Chen et al. (2020) performed an independent, single-center, non-blinded observational study where a total of 50 Bethesda III/IV cytologically indeterminate nodules underwent ThyroSeqv3 testing. Molecular analysis yielded 20 (40%) "positive" results and 24 (48%) "negative" results. Six (12%) results were classified as "currently negative" or "negative but limited." All 20 “positive” patients underwent surgery, as well as both “currently negative” patients (n = 2) and one patient with a “negative but limited” result (n = 1). All 26 “negative” patients and one patient with a “negative but limited” result (n = 1) continued with surveillance. In total, 23 (46%) patients underwent surgery and 27 (54%) patients were followed with conservative management. BCR was calculated as ("negative" and "currently negative")/total, resulting in a BCR of 58%. Ninety-one percent (20 of 22) of the resected target nodules were malignant on final pathology. Since surgery was not performed on test-negative patients, test specificity, sensitivity, and NPV were not available.

One retrospective 2019 review (Ohori et al. 2019) of 224 thyroid nodules with available ThyroSeqv3 Bethesda III or IV cytology had a BCR rate of 75%. In a hypothetical cost-effectiveness analysis (Nicholson et al. 2019), ThyroSeqv3 was superior to diagnostic lobectomy for indeterminate (Bethesda III/IV) nodules.

## Clinical Practice Guidelines

### *American Thyroid Association (ATA)*

Guidelines emphasize reserving MT for thyroid nodules with equivocal clinical, cytopathologic, and radiographic factors. The 2015 American Thyroid Association (ATA) (Haugen et al. 2016) guideline for management of thyroid nodules has several cautious recommendations specific to MT: 1/ 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); 2/ 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 (Weak recommendation, Moderate-quality evidence); 3/ 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 (Weak recommendation, Moderate-quality evidence); and 4/ When surgery is considered for patients with a solitary, cytologically indeterminate nodule, thyroid lobectomy is the recommended initial surgical approach. This approach may be modified based on clinical or sonographic characteristics, patient preference, and/or molecular testing when performed. (Strong recommendation, Moderate-quality evidence).

### *American Association of Endocrine Surgeons (AAES)*

More recent guidelines are even more circumspect due to a combination of interim factors. The American Association of Endocrine Surgeons (AAES) 2020 guidelines (Khan et al. 2020) echo recent heightened concerns with MT (1). They cite the following qualifiers: 1/ “follow-up independent studies have often reported diminished utility;” 2/ “providers and patients may also find it challenging to interpret MT results…potentially leading to over- or under-treatment;” 3/ “patient willingness to continue surveillance,” must be considered before obtaining MT; 4/ use of MT results to make clinical decisions relies on PPV and NPV which are contingent on regional and institutional cancer prevalence for each cytology category; and 5/ “because NIFTP decreases the risk of true malignancy for the indeterminate Bethesda categories the PPV of all MT will be impacted.” In other words as to the last point, given that molecular tests were developed and validated prior to this re-designation (and thus designed to classify this potential benign pathology as malignant), their performance measures have been shown to deteriorate significantly when the NIFTP designation is incorporated in the classification of indeterminate nodules. The impact of NIFTP reclassification is not trivial, as its average prevalence within indeterminate thyroid nodules is estimated to be 61% (range, 33-88%) (17,18). AAES guidelines cite three specific MT recommendations: 1/ If thyroidectomy is preferred for clinical reasons, then MT is unnecessary. (Strong recommendation, moderate-quality evidence); 2/ When the need for thyroidectomy is unclear after consideration of clinical, imaging, and cytologic features, MT may be considered as a diagnostic adjunct for cytologically indeterminate nodules. (Strong recommendation, moderate-quality evidence); and 3/ Accuracy of MT relies on institutional malignancy rates and should be locally examined for optimal extrapolation of results to thyroid cancer risk. (Strong recommendation, moderate-quality evidence). Use of MT for Bethesda V nodules is not endorsed as they cite validation and utility studies as lacking. Specifically with respect for MT to guide extent of surgery, they note: “Further study will determine if genotype provides information that has not already been obtained clinically, by US imaging, and/or by cytologic classification, as well as determine if altering the initial extent of surgery based on MT results will affect outcomes.”

### *National Comprehensive Cancer Network (NCCN)*

National Comprehensive Cancer Network guidelines for Thyroid Carcinoma (NCCN Thyroid Carcinoma, v4.2024) have similar criteria. They make a point of adding that: “Molecular diagnostics may be useful to allow reclassification of follicular lesions (i.e., follicular neoplasm, AUS) as either more or less likely to be benign or malignant based on the genetic profile,” but “should be interpreted with caution and in the context of clinical, radiographic, and cytologic features of each individual patient.”

## ***ThyGeNEXT®, ThyraMIR™***

Xing et al. (2014), performed a retrospective multicenter study that reviewed all known fusion and their prevalence in papillary, poorly differentiated anaplastic, follicular, and medullary carcinomas. The study was a review and no new data was presented. The study conclusion demonstrates the prognostic value and perspectives of the utilization of gene fusions as therapeutic targets. The study conclusion is limited due to clinical utility not being achieved in reporting statistical findings, no available conflict of interest and no patient inhomogeneity. The quality of evidence for this study is moderate due to lack of peer review and the strength was conditional for the same reason.

Xing et al. (2015), performed a retrospective study to investigate the prognostic value of *BRAF V600E* mutation for the recurrence of papillary thyroid cancer in 2099 patients. The study conclusion demonstrates the overall *BRAF V600E* mutation prevalence was 48.5%. *BRAF* mutation was associated with poorer recurrence-free probability in Kaplan-Meier

survival analyses in various clinicopathologic categories. The quality of evidence is high and the strength of recommendation is conditional for the population tested.

Labourier et al. (2015), performed a cross-sectional cohort study conducted at 12 endocrinology centers across the United States. The study results found that mutations were detected with malignant outcome. Among mutation negative specimens, miRNA testing correctly identified 64% of malignant cases and 98% of benign cases. The diagnostic sensitivity and specificity of the combined algorithm was 89% (95% confidence intervals (CI): 73 – 97%) and 85% (95% CI: 75 – 92%), respectively. At 32% cancer prevalence, 61% of the molecular results were benign with a negative predictive value of 94% (95% CI: 85 – 98%). Independently of variations in cancer prevalence, the test increased the yield of true benign results by 65% relative to mRNA-based gene expression classification and decreased the rate of avoidable diagnostic surgeries by 69%. This was purely supposition. The authors concluded: multi-platform testing for DNA, mRNA and miRNA can accurately classify benign and malignant thyroid nodules, increase diagnostic yield of molecular cytology, and further improve the preoperative risk-based management of benign nodules with AUS/FLUS or FN/SFN cytology. The quality of evidence is moderate as this was not peer reviewed, a conflict of interest was present in that one of the authors was employed by the company, and the clinical utility is implied but not proven.

Giordano et al. (2014), performed a case-control study conducted in 413 surgical cases comprising 17 distinct histopathologic categories. The study results found that, in the authors opinion, “standardized and validated multianalyte molecular panels can complement the preoperative and postoperative assessment of thyroid nodules and support a growing number of clinical and translational applications with potential diagnostic, prognostic, or theranostic utility.” The quality of evidence is moderate as this was a validation study only and the clinical utility is not addressed. There is an obvious conflict of interest in that the laboratory represented in authors of this study and the correspondence is through the laboratory.

Landa et al. (2013), performed a retrospective study. The objectives of the study were: 1) to determine the prevalence of *TERT* promoter mutations *C228T* and *C250T* in different thyroid cancer histological types and cell lines; and 2) to establish the possible association of *TERT* mutations with mutations of *BRAF*, *RAS*, or *RET/PTC*. The study results found that *TERT* promoter mutations were found in 98 of 225 (44%) of specimens. *TERT* promoters *C228T* and *C250T* were mutually exclusive. The study conclusion demonstrates potential diagnostic, prognostic and therapeutic are suggested. *TERT* promoter mutations are highly prevalent in advanced thyroid cancers, particularly those harboring *BRAF* or *RAS* mutations which are most often *TERT* promoter wild type. Acquisition of a *TERT* promoter mutation could extend survival of *BRAF*- or *RAS*- driven clones and enable accumulation of additional genetic defects leading to disease progression. The quality of evidence is moderate as this is retrospective of variable tumor types and the clinical utility is only inferred.

MicroRNAs (miRNA) are small noncoding RNAs that regulate gene expression. Research has demonstrated that a number of miRNAs are differentially expressed between benign and malignant thyroid nodules which have led to the development of miRNA based diagnostic lab tests, and in some cases, labs may offer miRNA testing in conjunction with gene variant and expression analysis. Wylie et al. (2016) conducted a study examining genetic variant and miRNA analysis on archived pathology samples from the University of Michigan. The samples consisted of an initial set of 235 aspirates representing 118 nodules with benign cytology, including 13 with surgical outcome (12 benign, 1 malignant), 73 with malignant cytology, including 51 with surgical outcome (1 benign, 50 malignant), and 44 with indeterminate cytology, all with available surgical outcome. The second set of aspirates consisted of 42 distinct nodules with indeterminate cytology and surgical outcome. Thirty-one miRNAs were analyzed as well as 17 genetic alterations in the *BRAF*, *RAS*, *RET* and *PAX8* genes, considered standard mutation testing. Furthermore, 54 samples that were negative by the 17-mutation panel were interrogated using a miRNA classification algorithm, commercially available as the ThyraMIR Thyroid miRNA Classifier, which analyzes in parallel 20 genes through next generation sequencing and 46 mRNA transcripts. The authors found that standard mutation testing alone had a sensitivity of 61%, consistent with the literature. Machine learning was utilized to group miRNA analysis into two groups of miRNAs, classifier A and classifier B. When miRNA classifier A was included in the analysis, the sensitivity rose to 78%, and 94% with classifier B. The authors calculated that this leads to a low residual risk of cancer (8%) among specimens negative by mutation and miRNA testing and corresponds to a calculated improvement from 78–90% NPV to 94–98% NPV at 20–40% cancer prevalence. These results contributed to the development of ThyraMIR. In the small cohort that underwent evaluation by ThyraMIR, the authors report a diagnostic sensitivity of 85% and specificity of 95%.

## *Afirma®*

### First Generation Tests

The Afirma gene expression classifier (AGEC) is an early GEP test that was developed as a rule-out. The landmark publication by Alexander et al. (2012) described the test validated against the gold standard histopathology of known benign or malignant thyroid tissue and classified indeterminate thyroid nodules into benign or suspicious using a

proprietary algorithm based on gene expression signatures. The algorithm assesses the expression of 142 primary genes plus 25 additional genes that filter out rare neoplasms such as medullary carcinoma and renal carcinoma as the sample is processed through a series of "cassettes." This prospective, multicenter test validation study examined 265 of 577 indeterminate nodules from 4812 FNAs (5.5%) collected from 3,789 patients at 43 clinical sites over a 19-month period. The AGEC correctly called 78 of 85 malignant samples suspicious for a sensitivity of 92% and 93 of 180 benign samples were called correctly for a specificity of 52%. These percentages were consistent regardless of the sample category. The prevalence of malignancy (POM) was 24% and 25% for Bethesda category III and IV nodules respectively, yielding a negative predictive value (NPV) of 95% and 94% respectively. Because the POM for category V was much higher at 62%, the respective NPV was 85%. These data suggested that the AGEC could rule out malignancy in over 90% of indeterminate category III and IV nodules. Since then, the test has garnered wide acceptance in clinical practice and the approach has been recommended by professional associations (Haugen et al. 2016, NCCN Thyroid Carcinoma v4.2024).

Silaghi et al. (2021) summarized 25 studies involving 4,538 indeterminate nodules of 4,424 patients who had been evaluated using the AGEC test from May of 2009 to June of 2018.30 The overall sensitivity and specificity across all studies was 97% and 19% respectively with an NPV of 91% and positive predictive value (PPV) of 39%. However, most of the reports are retrospective from single centers and demonstrate variable test performance among institutions.

Some reports indicate variability amongst institutions that differ in POM of indeterminate nodules (Al-Qurayshi et al. 2017). For example, Marti et al. (2015) performed a retrospective study comparing the AGEC-benign call rate between Memorial Sloan Kettering Cancer Center (MSK), a tertiary referral cancer center with a POM of 30-38%, and Mount Sinai Beth Israel (MSBI) a comprehensive health system with a POM of 10-19%, Marti et al found that the NPV at MSK was 86-92% yet 95-98% at MSBI. Conversely the PPVs of GEC-suspicious results were 57.1% and 13.3% respectively with 86% (18/21) of resected GEC-suspicious nodules at MSBI being benign on final pathology. This data matched closely to the predicted PPVs and NPVs and highlights the importance of knowing the POM at each institution.

Valderrabano et al. (2019) performed a systematic review and meta-analysis which included 19 titles totaling 2,568 thyroid nodules. The authors reported that the low resection rate of GEC-benign nodules makes the false-negative and NPV impossible to calculate and the only reliable metrics of benign call rate (BCR, the proportion of nodules tested with a GEC-benign result) and PPV suggested that the initial cohort study is not representative of the populations to which the AGEC was subsequently applied.

In 2011, Nikiforov et al. reported on the efficacy of a gene hot spot panel in 967 FNA samples from indeterminate nodules for variants that commonly occur in thyroid cancer such as *BRAF p.V600E*, *KRAS* codons 12/13, *NRAS* and *HRAS* codon 61 and *RET* and *PAX8* fusions establishing that molecular profiling using FNAs of thyroid nodules can aid in malignancy identification as a rule-in test. This report was followed by further clinical validation studies on Bethesda III and IV nodules using ThyroSeq v2 (TSv2), a panel consisting of additional variant hotspots in genes known to be drivers in thyroid carcinogenesis as well those that develop late with expression analysis of an additional eight genes to determine cell type composition. The larger number of variants examined resulted in a higher sensitivity than the original seven gene panel as well as a higher NPV. In a study by Nikiforov et al. (2014) of 143 FNA samples from patients with Bethesda category IV nodules with known surgical outcomes, the TSv2 test demonstrated a sensitivity and specificity of 90% and 93% respectively with a PPV of 83% and an NPV of 96%. Similar results were obtained for category III nodules (Nikiforov et al. 2015).

However, like the AGEC, variability across multiple institutions has been reported.38-40 For example, in a retrospective analysis of 273 category III and IV nodules from four different institutions, Marcadis et al. (2019) reported variation in test performance and diagnoses. Although sensitivity was similar to what was originally reported by Nikiforov, the specificity was lower (52% vs. 93%). This led to a range of PPVs from 22%-43% across the institutions which is lower than what was originally reported at 83%. A PPV of 22% was reported by Taye et al. (2018) with a PPV of 9% (2/22) and 7% (1/15) across all RAS and NRAS mutations, respectively. The authors noted that many genetic alterations, such as those in the RAS family, appeared to be nonspecific for malignancy and positive reports should be interpreted with care.

Vargas-Salas et al. (2018) performed a systematic review and meta-analysis to evaluate 4 available molecular tests: Afirma-GEC, ThyGenX/ThyraMIR, ThyroSeq v2, and RosettaGX Reveal. 26 articles were included in the systematic review. For Afirma-GEC 12 studies were of high quality, 6 moderate quality, and 1 was poor quality of evidence. For ThyroSeq v2 all studies showed good quality in diagnostic accuracy. For ThyGenX/ThyraMIR and RosettaGX Reveal, the overall quality of evidence was low in comparison to ThyroSeq v2 and Afirma-GEC. Afirma-GEC and ThyroSeq v2 had overall sensitivity close to 90% with similar 95% confidence interval. ThyroSeq v2 specificity was 92% and Afirma-GEC was 52%. ThyGenX/ThyraMIR and RosettaGX Reveal had a sensitivity of 74% when considering the whole cohort and 100% when excluding non-agreement gold standard cases. ThyGenX/ThyraMIR specificity was 85% and RosettaGX Reveal was 74%. The authors concluded that post-validation evidence available for Afirma-GEC and ThyroSeq v2 show

intermediate-to-good quality evidence. A potential limitation of the meta-analysis was that for post-validation studies, only surgical pathology cases were considered. Therefore, cases coined benign by molecular testing were not included.

Jug et al. (2018) conducted a test validation study for ThyroSeq and Afirma GEC testing. The performance of these tests was assessed within the context of ultrasonographic features and with the incorporation of the noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) nomenclature. 304 cases were identified, 119 of which were resected. All cases that met the criteria for NIFTP on excision showed either high-risk mutations on ThyroSeq or a "suspicious" result on Afirma GEC. When NIFTP cases were moved from the malignant to nonmalignant category, the PPV of "positive" tests for both ThyroSeq and Afirma GEC decreased from 42.9% to 14.3% (an absolute decrease of 28.6%) and 30.1% to 25.3% (an absolute decrease of 4.8%), respectively. Zero cases of malignancy were found in the American Thyroid Association (ATA) 2015 "very low suspicion" group, even with a "suspicious" Afirma GEC result. ThyroSeq and Afirma GEC tests both demonstrated decreases in the PPV when NIFTP was considered nonmalignant. In the era of NIFTP, a "positive" test result for either the ThyroSeq or Afirma GEC should be interpreted in light of clinical factors and should not exclude conservative (ie, lobectomy) surgical management. ATA 2015 "very low suspicion" nodules, even with "suspicious" Afirma GEC results, were not found to demonstrate malignancy in this series.

Alexander et al. (2014) conducted a multi-center study where they analyzed all patients who received Afirma GEC testing at 5 academic medical centers between 2010 and 2013. Patient and nodule characteristics, fine needle aspiration cytology, Afirma GEC results, and subsequent clinical or surgical follow-up were obtained from 339 patients. 339 patients received Afirma GEC testing of cytologically indeterminate nodules (165 AUS/FLUS; 161 FN; 13 suspicious for malignancy) and 174 of 339 (51%) indeterminate nodules were GEC benign and 148 GEC were suspicious (44%). GEC results substantially changed care recommendations, as 4 of 175 GEC benign were recommended for surgery in comparison to 141 of 149 GEC suspicious. Out of 121 Cyto Indeterminate/GEC Suspicious nodules surgically removed, 53 (44%) were malignant. Variability in site-to-site GEC performance was confirmed, as the proportion of GEC benign varied up to 29% (P = .58), whereas the malignancy rate in nodules cytologically indeterminate/GEC suspicious varied up to 47% (P = .11). 71 out of 174 GEC benign nodules had documented clinical follow-up for an average of 8.5 months, in which 1 out of 71 nodules proved to be cancerous. The authors concluded that the data confirm originally published Afirma GEC test performance and show its substantial impact on treatment recommendations. Although insignificant site-to-site variation exists, such differences should be anticipated by the practicing clinician. Follow-up of GEC benign nodules thus far verify the clinical utility of this diagnostic test.

## Second Generation Tests

Updated versions of both rule-in and rule-out test types have been developed. The AGEC was replaced by the Afirma Genomic Sequencing Classifier (AGSC) which tests for *BRAF* p.V600E and *RET/PTC* fusion variants, as well as characteristic MTC and parathyroid tissue profiles in addition to a more robust classifier that provides a benign or suspicious result for indeterminate nodules. Patel et al. (2018) performed a blinded, multi-center test validation study to evaluate the performance of AGEC for cytologically indeterminate thyroid nodules. 183 patients were included in study and 142 (77.6%) were women. The average age was 51.7 (22.0-85.0) years. The AGEC had a sensitivity of 91% (95% CI, 79-98) and a specificity of 68% (95% CI, 60-76). At 24% cancer prevalence, the positive predictive value was 47% (95% CI, 36-58) and the negative predictive value was 96% (95% CI, 90-99). If positive, genomic profiling may be used to further inform on risk of malignancy and tumor prognosis. The authors concluded that The AGSC shows high sensitivity and accuracy for detecting benign nodules. Its 36% increase in specificity than that of AGEC may increase the number of patients with benign nodules who can safely not undergo unnecessary diagnostic surgery. Study limitations included the lack of performance data in children, data on when the nodule was biopsied, when sample collection methods other than 1 or 2 dedicated FNA passes were used, and that the cancer prevalence was toward the higher end of the expected range amount Bethesda III and IV nodules.

Nikiforova et al. (2018) performed an analytical performance study of Thyroseq v3. Thyroseq v3 (TSv3), an expanded version of TSv2 containing variant targets in 112 genes as well as copy number alterations (CNAs) in multiple genomic regions and expression analysis of 19 genes. Results are reported as positive (high probability of cancer/NIFTP) or negative (low probability of cancer/NIFTP). Using the training tissue set, ThyroSeq GC identified > 100 genetic alterations, including *BRAF*, *TERT*, *RAS*, *DICER1* mutations, *NTRK1/3*, *BRAF* and *RET* fusions, 22q loss, and gene expression alterations. GC cutoffs were established to differentiate cancer from benign nodules with 93.9% sensitivity, 89.4% specificity, and 92.1% accuracy. This accurately classified most follicular, papillary, and Hurthle cell lesions, medullary thyroid carcinomas, and parathyroid lesions. In the FNA validation set, the GC sensitivity was 98.0%, specificity 81.8%, and accuracy 90.9%. Analytical accuracy studies showed a minimal required nucleic acid input of 2.5 ng, a 12% minimal acceptable tumor content, and test results under variable stress conditions were reproducible. The authors concluded that the ThyroSeq v3 GC analyzes 5 different classes of molecular alterations and provides great precision for identifying all common types of thyroid cancer and parathyroid lesions. The analytical sensitivity, specificity, and robustness of the test have been adequately validated and suggest its appropriateness for clinical use.

Multiple reviews have been performed on these second-generation tests and describe increased performance over their predecessors.

Lee et al. (2022), performed a systematic review and meta-analysis on the diagnostic performance of the second-generation molecular tests in the assessment of indeterminate thyroid nodules. Preliminary pooled studies demonstrated that both assays, AGSC and TSv3, have a high sensitivity (96% and 95% respectively) and high NPV (96% and 92% respectively) demonstrating that either test type can be used to rule out malignancy.43 The AGSC and TSv3 were reported to have a specificity of 53% and 50% with a PPV of 63% and 70% respectively. Although this represents an increase in specificity for the AGSC (12% to 53%) the specificity for TSv3 compared to TSv2 went down (78% to 49.6%). However, the specificity of the tests ranged across multiple studies particularly from single centers suggesting inter-institution variation similar to what was seen in the first-generation tests.

Silaghi et al. (2021) reported similar results in their systematic review and meta-analysis on Thyroseq v3, Afirma GSC, and microRNA panels vs. previous molecular tests in the preoperative diagnosis of indeterminate thyroid nodules. 40 eligible studies were included in the study with 7,831 intermediate thyroid nodules (ITNs) from 7,565 patients. Thyroseq v3 showed the greatest overall performance (AUC 0.95; 95% confidence interval: 0.93–0.97), followed by Afirma GSC (AUC 0.90; 0.87–0.92) and Thyroseq v2 (AUC 0.88; 0.85–0.90). In terms of “rule-out” abilities Thyroseq v3 (NLR 0.02; 95%CI: 0.0–2.69) topped Afirma GEC (NLR 0.18; 95%CI: 0.10–0.33). Thyroseq v2 (PLR 3.5; 95%CI: 2.2–5.5) and Thyroseq v3 (PLR 2.8; 95%CI: 1.2–6.3) had the greatest “rule-in” properties compared to Afirma GSC (PLR 1.9; 95%CI: 1.3–2.8). Evidence for Thyroseq v3 appears to have better quality, notwithstanding the paucity of studies. Both Afirma GEC and Thyroseq v2 performance were impacted by NIFTP reclassification. RosettaGX and ThyGenNEXT/ThyraMIR show prominent preliminary results. The authors concluded that the newly emerged tests, Afirma GSC and Thyroseq v3, designed for a “rule-in” purpose, demonstrated to outperform in capabilities to rule out malignancy, thus surpassing previous tests that are no longer available, Afirma GEC and Thyroseq 2. However, Thyroseq v2 still ranks as the best rule-in molecular test. Limitations of this review and meta-analysis included that the evaluated diagnostic tests could not be compared and ranked due to the limited number of studies that had direct head-to-head comparisons and that only patients with surgical pathology were considered which excluded many benign nodules by molecular testing managed conservatively.

Livhits et al. (2021) performed a randomized clinical trial across nine sites by using both the AGSC and the TSv3 in practice on a rotating monthly basis. Of the 346 samples ultimately tested, 189 and 157 were randomized to the AGSC and TSv3 respectively. For the AGSC test, 19 nodule samples were insufficient for testing, 107 (53.2%) were classified as benign and 73 (36.3%) as suspicious. Twelve of the benign samples were surgically resected and histopathologically classified as benign. Fifty-eight of the suspicious samples were resected and revealed NIFTP in 10 (17.2%) and malignancy in 21 (36.2%). The TSv3 test identified 103 (60.2%) negative nodules, 60 (35.1%) positive, and seven insufficient for testing. Eleven negative nodules were resected, and one was found to be a minimally invasive Hurthle cell carcinoma with capsular invasion only that was resected due to growth during the surveillance period. Of the positive nodules, 49 (81.7%) were resected and histopathological results revealed NIFTP in 11 (22.4%) and malignancy in 20 (40.8%). These data demonstrated high sensitivity (97-100%) and reasonably high specificity (80-85%) for both tests and diagnostic surgery was avoided in approximately half of the patients in the study. However, consistent with similar studies, nodules with benign/negative results were assumed to be benign in the absence of histopathological confirmation.

Therefore, to further assess the false negative rates of the AGSC and TSv3, Kim et al. (2023) performed a prospective study of a single center in patients surveilled over a median of 34 months (range 12-60). They reported that of the 217 indeterminate nodules initially reported with negative or benign results 14 (8%) underwent immediate resection and were all confirmed to be benign. Of the 147 that remained on continued surveillance, 15 were resected during the surveillance period. The minimally invasive Hurthle cell carcinoma initially found to be negative by TSv3, remained the only false positive. Of the 133 test positive nodules, 97 underwent immediate resection and 59 were determined to be cancerous and of those that were initially surveilled, 16 underwent delayed surgery with an additional nine found to be malignant. These data reaffirm the high sensitivity rate previously reported for both assays.

## Molecular Profiles

The variants identified in a nodule can also predict the risk and/or class of malignancy. For example, nodules with “driver” mutations such as *BRAF p.V600E* or pathogenic variants in *RET* have a higher probability of malignancy than those carrying *RAS* or *RAS*-like variants (Haugen et al. 2016). Tumors harboring *BRAF p.V600E* are generally classic PTC that frequently involve regional lymph nodes with a higher rate of metastasis, and *RET* mutations are present in all inherited MTCs and 6-10% of apparent sporadic disease (Haugen et al. 2017, Kim et al. 2021, Tao et al. 2021, Wells et al. 2015, Yip 2015). In contrast, *RAS* alterations (*KRAS*/*NRAS*/*HRAS*) are the most frequently identified in indeterminate thyroid nodules. However, unlike *BRAF p.V600E*, the utility of detecting *RAS* alterations remains uncertain. In a systematic review of 35 studies examining *RAS* mutations published between 2000 and 2015, Najafian et al. (2017) reported a

prevalence of *RAS* mutations in 0-48% of benign nodules and 10-93% of malignant nodules across the studies. 64 articles which included 8,162 patients, of whom 42.5% had benign lesions, met all the study criteria. Out of 35 studies examining *RAS* mutations, the prevalence of *RAS* mutation in benign lesions ranged from 0%-48%. Among 38 studies examining RET/PTC rearrangements, the prevalence in benign lesions ranged from 0%-68%. PAX8/PPAR-gamma rearrangements were studied in 27 studies, with the prevalence in benign lesions ranging from 0%-55%. The authors concluded that the presence of these biomarkers and the vast variation in reports of their prevalence in benign lesions suggests there needs to be caution when including these markers in diagnostic decisions. Further understanding of the significance of these markers, as well as newly discovered markers of thyroid malignancy, may be needed to avoid overtreatment of patients with benign thyroid tumors. In a study of over 1,500 patients, Yip (2015) reported an indolent clinical course and nearly 100% disease free survival at five years for patients with *RAS*-positive nodules. In a single-center prospective cohort study, Guan et al. (2020) also reported that although *RAS* variants were the most frequent alterations detected in more than 500 fine needle biopsies, they provided poor value for prediction of TC since most *RAS* alterations presented in benign nodules and NIFTPs (59% and 13% respectively). However, the presence of a "second-hit" in another gene such as *TERT* or *TP53* significantly increased the risk of malignancy.

### Clinical Practice Guidelines

#### *American Thyroid Association (ATA)*

#### *National Comprehensive Cancer Network (NCCN) Thyroid*

Current clinical guidelines, including the ATA Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer (Haugen et al. 2016), and The National Comprehensive Cancer Network (NCCN) Thyroid Carcinoma guidelines (NCCN Thyroid Carcinoma, v4.2024) endorse the use of molecular tests to further risk stratify patients with indeterminate (Bethesda III and IV) thyroid nodule cytology results, as well as their use in identifying cancer types with challenging cytology such as MTC. Molecular tests can be broadly grouped into "rule-out" tests designed to identify benign nodules thereby placing the patient on surveillance and avoiding surgery, "rule-in" tests that aim to predict the aggressiveness of malignancy and aid in surgical decision making and "general" tests that can act as both rule-in and rule-out. Most currently offered tests utilize Next Generation Sequencing (NGS) methodologies to either assess characteristic gene expression profiles (GEP) or genomic sequence variant profiles that are known to be associated with malignancy.

In the ATA Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer (Haugen et al. 2016), they recommend a complete history and physical focusing on the thyroid gland and adjacent cervical lymph nodes be performed when a thyroid nodule is discovered. Malignancy risk factors include a history of radiation therapy, exposure to ionizing radiation, family history of thyroid cancer, rapid nodule growth, hoarseness, cervical lymphadenopathy, vocal cord paralysis, progressive dysphagia, respiratory symptoms, and fixation of the nodule to surrounding tissue.

## UroVysion™ Bladder Cancer Kit

Zheng et al. (2022) performed a systematic review and meta-analysis on Fluorescence in situ hybridization (FISH) and its role in the surveillance of non-muscle invasive bladder cancer (NMIBC). 15 studies were included in the meta-analysis, which totaled 2,941 FISH evaluations from 2,385 NMIBC patients. The pooled sensitivity of FISH was 68% (95% CI: 0.58–0.76), and the pooled specificity was 64% (95% CI: 0.53–0.74). Subgroup analyses were done in 7 studies without Bacillus Calmette–Guerin (BCG) treatment, the pooled sensitivity was 82% (95% CI: 0.68–0.90), and the pooled specificity was 63% (95% CI: 0.37–0.82). 9 out of the 15 studies used "UroVysion standard" to define positive FISH results, demonstrating a pooled sensitivity of 60% (95% CI: 0.50–0.70) and specificity of 70% (95% CI: 0.61–0.78). The authors concluded that FISH has a satisfactory sensitivity and specificity and could be a potential biomarker when it comes to surveilling NMIBC.

Kavcic et al. (2022) performed a prospective test validation study of UroVysion™ Bladder Cancer Kit (FISH) and the Xpert® Bladder Cancer Detection (Xpert) test. Both tests were done on voided urine samples after negative cystoscopy and negative abdominal ultrasound (US) and/or negative computed tomography urography (CTU). 156 patients with hematuria suspected of having urothelial carcinoma (UC) and 48 patients following up after treatment of UC were included in the study. Out of the 204 patients, 20 had UC. 11 were located in the bladder, 6 in the ureter, and 3 in the renal pelvis. FISH had an overall sensitivity (SN) of 78%, a specificity (SP) of 93%, and a negative predictive value (NPV) of 96%. Xpert had an overall SN of 90%, an SP of 85%, and an NPV of 98%. Both tests had high SN, SP, and NPV with the SP of FISH being substantially higher. The authors concluded that by using FISH and Xpert in addition to cystoscopy, renal and bladder US, and/or CTU in the diagnostic workup of patients with hematuria and follow-up after transurethral resection of the bladder (TURB), a significant number of patients (10%) otherwise missed were found to have UC.

Liem et al. (2017) performed a multicenter, prospective clinical trial to assess whether UroVysion fluorescence in situ hybridization (FISH) can be useful in the early identification of bladder cancer recurrence during treatment with Bacillus Calmette-Guérin (BCG). 3 bladder washouts at different time points during treatment (t 0 = week 0, pre-BCG, t 1 = 6 weeks following TURB, t 2 = 3 months following TURB) were gathered for FISH from patients with bladder cancer treated with BCG. Data on bladder cancer recurrence and duration of BCG maintenance therapy were documented. 36 (31.6%) out of 114 patients had a recurrence after an average of 6 months (range 2-32). There was no significant association found between a positive FISH test at t 0 or t 1 and risk of recurrence. A positive t 2 FISH test was correlated with a higher risk of recurrence. Patients with a positive FISH test 3 months following TURB had a 4.0-4.6 times increased risk of developing a recurrence compared to patients with a negative FISH. Patients with a positive FISH test 3 months following TURB and induction BCG therapy have a greater risk of developing tumor recurrence. The authors concluded that FISH can be a useful additional tool for physicians when deciding a treatment plan. Study limitations included the number of bladder washouts samples that were not available or apt for analysis and the limited number of patients with available FISH results at t 2.

García-Peláez et al. (2013) performed a controlled clinical trial to evaluate fluorescent in situ hybridization (FISH) as a predictor of relapse in urothelial carcinoma. 338 samples from 98 patients with 84 episodes of urothelial carcinoma were included in the study. Testing was performed using the UroVysion kit. FISH showed higher sensitivity regardless of grade, negative predictive value, and accuracy, while specificity and positive predictive value were superior with conventional cytology. In the recurrence, series 19/29 episodes were coherent, 11/19 were positive coherent with urothelial carcinoma all high grade and 8/19 negative coherent, mostly low grade. The authors concluded that FISH demonstrated good sensitivity during a follow up of 24 months and is able to predict recurrence, especially in high grade cases. Findings support a multidisciplinary follow up combining FISH, cytology, and cystoscopy.

## X-linked Intellectual Disability (XLID) Panel (e.g., Syndromic and Non - Syndromic XLID)

Genetic testing is used to diagnose X-linked intellectual disabilities such as alpha-thalassemia X-linked intellectual disability (ATR-X) syndrome, fragile X syndrome, and X-linked intellectual disability Siderius type. ATR-X is characterized by distinctive craniofacial features, hypotonia, genital anomalies, growth impairment including microcephaly and short statue (usually present at birth) and mild-to-profound developmental delay/intellectual disability (Stevenson, 2020). Genetic testing for Fragile X Syndrome (FXS) is used to diagnosis individuals with intellectual disability and/or autism spectrum disorder of unknown cause, and/or other features of FXS. It is also used for carrier screening in women to aid in the decision of having a pregnancy/child with FXS, prenatal diagnosis, and newborn screening (Hayes Clinical Utility Evaluation Genetic Testing For Fragile X Syndrome, 2017, updated 2021). The average age of FXS diagnosis is 42 months for girls and 35-37 months for boys (CDC, 2024). X-linked intellectual disability, Siderius type is characterized by mild to moderate intellectual disability in males. Affected boys usually have developmental delays (MedlinePlus, 2015b).

Hayes Precision Medicine Research Brief for X-Linked Intellectual Disability (XLID) Multigene Panels describes intellectual disability (ID) as a decreased ability to learn and understand complex or new information. About 6.5 million Americans are reported to be impacted by ID. X-linked ID (XLID) accounts for about 5% to 10% of ID in boys and men. XLID is caused by variants in genes on the X chromosome. Girls or women who carry X-linked variants related to XLID are on average either unaffected or less severely impacted than boys or men with the same variant. According to a recent review, over 150 syndromes have been described to be related to XLID, 95 of which have been mapped to specific regions of the X chromosome. Variants have been described in 102 genes in families with syndromic or nonsyndromic XLID. Fragile X syndrome is the most common form of XLID, which is caused by variants in the fragile X mental retardation 1 (*FMR1*) gene. The clinical and genetic heterogeneity of XLID make diagnosis on a molecular level challenging once fragile X syndrome has been ruled out. This heterogeneity, along with advancements in sequencing technology, has led laboratories to develop XLID multigene sequencing panels. However, there have been no published studies that have evaluated the use of the clinically available panels in diagnosing XLID. Therefore, it is currently not possible to evaluate the clinical impact of these panels in regard to the care of individuals with XLID. It was concluded that there is insufficient published evidence to perform a Genetic Test Evaluation (GTE) health technology assessment of the use of multigene panels for diagnosing XLID. Therefore, its adoption or use cannot be recommended at this time. The primary evidence deficiencies for multigene XLID panels are insufficient data on analytical validity, clinical validity, and clinical utility (Hayes Precision Medicine Research Brief X-Linked Intellectual Disability (XLID) Multigene Panels, 2014).

Molecular pathology tests for diseases or conditions that manifest severe signs or symptoms in newborns and in early childhood or that result in early death are generally not relevant to a Medicare member. Screening services such as pre-symptomatic genetic tests and services used to detect an undiagnosed disease or disease predisposition, prenatal diagnostic testing, and carrier screening are not a Medicare benefit.

# U.S. Food and Drug Administration (FDA)

This section is to be used for informational purposes only. FDA approval alone is not a basis for coverage.

## PIK3CA (Phosphatidylinositol-4, 5-Biphosphate 3-Kinase, Catalytic Subunit Alpha)

The U.S. FDA approved Piqray (alpelisib) tablets, to be used in combination with the FDA-approved endocrine therapy fulvestrant, to treat postmenopausal women, and men, with hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, *PIK3CA*-mutated, advanced or metastatic breast cancer (as detected by an FDA-approved test) following progression on or after an endocrine-based regimen.

The FDA also approved the companion diagnostic test, therascreen PIK3CA RGQ PCR Kit, to detect the *PIK3CA* mutation in a tissue and/or a liquid biopsy. Patients who are negative by the therascreen test using the liquid biopsy should undergo tumor biopsy for *PIK3CA* mutation testing.

The FDA granted this application Priority Review designation. The FDA granted approval of Piqray to Novartis. The FDA granted approval of the therascreen PIK3CA RGQ PCR Kit to QIAGEN Manchester, Ltd. Refer to the following website for more information: https://www.fda.gov/news-events/press-announcements/fda-approves-first-pi3k-inhibitor-breast-cancer (Accessed October 29, 2024).

## UroVysion™ Bladder Cancer Kit

The FDA approved UroVysion™ Bladder Cancer Kit in detecting aneuploidy for chromosomes 3, 7, 17, and loss of the 9p21 locus via fluorescence in situ hybridizations (FISH) to aid in diagnosing bladder cancer. Refer to the following website for more information: https://www.accessdata.fda.gov/cdrh_docs/pdf3/P030052b.pdf (Accessed October 29, 2024).

# References


Al Musalhi K, Al Kindi M, Al Aisary F, et al. Evaluation of HE4, CA-125, Risk of Ovarian Malignancy Algorithm (ROMA) and Risk of Malignancy Index (RMI) in the preoperative assessment of patients with adnexal mass. Oman Med J. 2016;31(5):336-344.

Albain KS, Barlow WE, Shak S, et al. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, estrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomized trial. Lancet Oncol 2010;11:55-65.

Alexander EK, Kennedy GC, Baloch ZW, et al. Preoperative diagnosis of benign thyroid nodules with indeterminate cytology. N Engl J Med. 2012;367(8):705-715.

Alexander EK, Schorr M, Klopper J, et al. Multicenter clinical experience with the Afirma gene expression classifier. J Clin Endocrinol Metab. 2014 Jan;99(1):119-25.

Al-Qurayshi Z, Deniwar A, Thethi T, et al. Association of malignancy prevalence with test properties and performance of the gene expression classifier in indeterminate thyroid nodules. JAMA Otolaryngol Head Neck Surg. 2017;143(4):403-408.

Altamura D, Avramidis M, Menzies SW. Assessment of the optimal interval for and sensitivity of short-term sequential digital dermoscopy monitoring for the diagnosis of melanoma. Arch Dermatol. 2008;144(4):502-506.

Alvarado M, Carter DL, Guenther JM, et al. The impact of genomic testing on the recommendation for radiation therapy in patients with ductal carcinoma in situ: A prospective clinical utility assessment of the 12-gene DCIS Score result. J Surg Oncol. 2015;111(8):935-940.

Alvarado MD, Harrison L, Solin LJ, et al. Cost-effectiveness of gene expression profiling for DCIS. Poster presented: San Antonio Breast Cancer Symposium; December 2012; San Antonio, TX.

American Cancer Society. Cancer facts & figures 2024. Available at: https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/2024-cancer-facts-figures.html. Accessed August 13, 2024.

American Cancer Society. Key statistics for melanoma skin cancer. Available at: https://www.cancer.org/cancer/types/melanoma-skin-cancer/about/key-statistics.html. Accessed August 13, 2024a.

American Cancer Society. Surgery for Prostate Cancer. Available at: https://www.cancer.org/cancer/types/prostate-cancer/treating/surgery.html. Accessed September 30, 2024.

American Cancer Society. Survival rates for melanoma skin cancer. Available at: https://www.cancer.org/cancer/types/melanoma-skin-cancer/detection-diagnosis-staging/survival-rates-for-melanoma-skin-cancer-by-stage.html. Accessed August 13, 2024b.

American College of Obstetricians and Gynecologists. ACOG Committee Opinion. Carrier screening for genetic conditions. Number 691, March 2017. Reaffirmed 2023.

American College of Obstetrics and Gynecology Committee on Practice B-G. Practice Bulletin No. 174: evaluation and management of adnexal masses. Obstet Gynecol. 2016;128(5):e210-e226.

American College of Radiology. Lung cancer screening registry. Available at: https://www.acr.org/Practice-Management-Quality-Informatics/Registries/Lung-Cancer-Screening-Registry. Accessed March 9, 2022.

Anderson AM, Matsumoto M, Saul MI, et al. Accuracy of skin cancer diagnosis by physician assistants compared With dermatologists in a large health care system. JAMA Dermatol. 2018;154(5):569-573.

André F, Ciruelos E, Rubovszky G, et al; SOLAR-1 study group. Alpelisib for PIK3CA-mutated, hormone receptor-positive advanced breast cancer. N Engl J Med. 2019 May 16;380(20):1929-1940.

André F, Ciruelos EM, Juric D, et al. Alpelisib plus fulvestrant for PIK3CA-mutated, hormone receptor-positive, human epidermal growth factor receptor-2-negative advanced breast cancer: final overall survival results from SOLAR-1. Ann Oncol. 2021 Feb;32(2):208-217.

Andréasson B, Pettersson H, Wasslavik C, et al. ASXL1 mutations, previous vascular complications and age at diagnosis predict survival in 85 WHO-defined polycythaemia vera patients. Br J Haematol. 2020 Jun;189(5):913-919.

Antoniadi T, Buxton C, Dennis G, et al. Application of targeted multi-gene panel testing for the diagnosis of inherited peripheral neuropathy provides a high diagnostic yield with unexpected phenotype-genotype variability. BMC Med Genet. 2015 Sep 21;16:84.

Arachchillage DJ, Mackillop L, Chandratheva A, et al. Thrombophilia testing: A British Society for Haematology guideline. Br J Haematol. 2022 Aug;198(3):443-458.

Argenziano G, Cerroni L, Zalaudek I, et al. Accuracy in melanoma detection: a 10-year multicenter survey. J Am Acad Dermatol. 2012;67(1):54-59.

Baehner FL, Sangli C, Millward C, et al. Quantitative gene expression analysis using Oncotype DX in ductal carcinoma in situ that is adjacent to invasive ductal carcinoma. Poster presented: San Antonio Breast Cancer Symposium; December 2008.

Baehner FL, Yoshizawa CN, Butler SM, et al. The development of the DCIS Score: scaling and normalization in the Marin Medical Laboratories cohort. Poster presented: ASCO Breast Cancer Symposium; September 2012; San Francisco, CA.

Bar-Aluma BE. Familial Dysautonomia. 2003 Jan 21 [Updated 2021 Nov 4]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1180/. Accessed August 22, 2024.

Bezemer ID, Doggen CJ, Vos HL, et al. No association between the common MTHFR 677C- > T polymorphism and venous thrombosis: results from the MEGA study. Arch Intern Med. 2007 Mar 12;167(5):497-501.

Bidichandani SI, Delatycki MB. Friedreich Ataxia. 1998 Dec 18 [Updated 2017 Jun 1]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1281/. Accessed August 22, 2024.

Bird TD. Charcot-Marie-Tooth Hereditary Neuropathy Overview. 1998 Sep 28 [Updated 2024a Aug 1]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1358/. Accessed November 27, 2024.

Bird TD. Myotonic Dystrophy Type 1. 1999 Sep 17 [Updated 2024b Mar 21]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1165/. Accessed August 22, 2024.

Brand TC, Zhang N, Crager MR, et al. Patient-specific meta-analysis of 2 clinical validation studies to predict pathologic outcomes in prostate cancer using the 17-gene genomic prostate score. Urology. 2016 Mar;89:69-75.

Branford S, Wang P, Yeung DT, et al. Integrative genomic analysis reveals cancer-associated mutations at diagnosis of CML in patients with high-risk disease. Blood. 2018 Aug 30;132(9):948-961.

Brooks MA, Thomas L, Magi-Galluzzi C, et al. GPS assay association with long-term cancer outcomes: twenty-year risk of distant metastasis and prostate cancer-specific mortality. JCO Precis Oncol. 2021 Feb 24;5:PO.20.00325.

Cancer Genome Atlas Network. Genomic classification of cutaneous melanoma. Cell. 2015;161(7):1681-1696.

Caron NS, Wright GEB, Hayden MR. Huntington Disease. 1998 Oct 23 [Updated 2020 Jun 11]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1305/. Accessed August 7, 2024.

Carvajal RD, Antonescu CR, Wolchok JD, et al. KIT as a therapeutic target in metastatic melanoma. JAMA. 2011; 305(22): 2327-34.

Cattaneo D, Croci GA, Bucelli C, et al. Triple-negative essential thrombocythemia: Clinical-pathological and molecular features. A single-center cohort study. Front Oncol. 2021 Mar 12;11:637116.

Centers for Disease Control and Prevention (CDC). About sickle cell disease (SCD). November 7, 2024. Available at: https://www.cdc.gov/sickle-cell/about/index.html. Accessed November 26, 2024.

Centers for Disease Control and Prevention (CDC). Data and statistics on fragile x syndrome. May 16, 2024. Available at: https://www.cdc.gov/fragile-x-syndrome/data/index.html. Accessed November 20, 2024.

Cerroni L, Barnhill R, Elder D, et al. Melanocytic tumors of uncertain malignant potential: Results of a tutorial held at the XXIX symposium of the International Society of Dermatopathology in Graz, October 2008. Am J Surg Path. 2010;34(3):314-326.

Chaytow H, Faller KME, Huang YT, et al. Spinal muscular atrophy: From approved therapies to future therapeutic targets for personalized medicine. Cell Rep Med. 2021 Jul 21;2(7):100346.

Chen T, Gilfix BM, Rivera J, et al. The role of the ThyroSeq v3 molecular test in the surgical management of thyroid nodules in the Canadian public health care setting. Thyroid. 2020 Sep;30(9):1280-1287.

Chen W, Xie H, Wang H, et al. Prognostic Significance of KIT Mutations in Core-Binding Factor Acute Myeloid Leukemia: A Systematic Review and Meta-Analysis. PLoS One. 2016 Jan 15;11(1):e0146614.

Chiaretti S, Tavolaro S, Chia E M, et al. Characterization of ABL1 expression in adult t-cell acute lymphoblastic leukemia by oligonucleotide array analysis. Haematologica/The Hematology Journal. 2007; 92(5): 619-626.

Chinnery PF. Primary Mitochondrial Disorders Overview. 2000 Jun 8 [Updated 2021 Jul 29]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1224/. Accessed November 21, 2024.

Cho HY, Park SH, Park YH, et al. Comparison of HE4, CA125, and Risk of Ovarian Malignancy Algorithm in the Prediction of Ovarian Cancer in Korean Women. J Korean Med Sci. 2015;30(12):1777-1783.

Chrestian N. Hereditary Neuropathy with Liability to Pressure Palsies. 1998 Sep 28 [Updated 2020 Aug 27]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1392/. Accessed August 22, 2024.

Clark MM, Stark Z, Farnaes L, et al. 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. 2018 Jul 9;3:16.

Clarke LE, Flake DD II, Busam K, et al. An independent validation of a gene expression signature to differentiate malignant melanoma from benign melanocytic nevi. Cancer. 2017;123(4):617-628.

Clarke LE, Mabey B, Flake DD II, et al. Clinical validity of a gene expression signature in diagnostically uncertain neoplasms. Pers Med. 2020;17(5):361-371.

Clarke LE, Warf MB, Flake DD II, et al. Clinical validation of a gene expression signature that differentiates benign nevi from malignant melanoma. J Cutan Pathol. 2015;42(4):244-252.

Cockerell C, Tschen J, Billings SD, et al. The influence of a gene-expression signature on the treatment of diagnostically challenging melanocytic lesions. Per Med. 2017;14(2):123-130.

Cockerell CJ, Tschen J, Evans B, et al. The influence of a gene expression signature on the diagnosis and recommended treatment of melanocytic tumors by dermatopathologists. Medicine. 2016;95(40):e4887.

Colebatch AJ, Dobrovic A, Cooper WA. TERT gene: its function and dysregulation in cancer. J Clin Pathol. 2019 Apr;72(4):281-284.

Coleman RL, Herzog TJ, Chan DW, et al. Validation of a second-generation multivariate index assay for malignancy risk of adnexal masses. Am J Obstet Gynecol. 2016;215(1):82 e81-82 e11.

Committee on the Review of Omics-Based Tests for Predicting Patient Outcomes in Clinical Trials; Board on Health Care Services; Board on Health Sciences Policy; Institute of Medicine. Evolution of translational omics: Lessons learned and the path forward. Micheel CM, Nass SJ, Omenn GS, editors. Washington (DC): National Academies Press (US); 2012 Mar 23.

Cook NR. Use and misuse of the receiver operating characteristic curve in risk prediction. Circulation 2007;115:928-35.

Correa C, McGale P, Taylor C, et al. Overview of the randomized trials of radiotherapy in ductal carcinoma in situ of the breast. J Natl Cancer Inst Monogr. 2010;(41):162-177.

Covas Moschovas M, Chew C, Bhat S, et al. Association between Oncotype DX Genomic Prostate Score and adverse tumor pathology after radical prostatectomy. Eur Urol Focus. 2022 Mar;8(2):418-424.

Curtin J, Busam K, Pinkel D, et al. Somatic activation of KIT in distinct subtypes of melanoma. Journal of Clinical Oncology. 2006; 24(26): 4340-4346.

Dagli AI, Mathews J, Williams CA. Angelman Syndrome. 1998 Sep 15 [Updated 2021 Apr 22]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1144/. Accessed August 22, 2024.

Davey MG, Davey CM, Bouz L, et al. Relevance of the 21-gene expression assay in male breast cancer: a systematic review and meta-analysis. Breast. 2022 Aug;64:41-46.

Dayyani F, Uhlig S, Colson B, et al. Diagnostic performance of risk of ovarian malignancy algorithm against CA125 and HE4 in connection with ovarian cancer: A meta-analysis. Int J Gynecol Cancer. 2016;26(9):1586-1593.

Driscoll DJ, Miller JL, Cassidy SB. Prader-Willi Syndrome. 1998 Oct 6 [Updated 2023 Nov 2]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1330/. Accessed August 22, 2024.

Duffy KL, Mann DJ, Petronic-Rosic V, et al. Clinical decision making based on histopathologic grading and margin status of dysplastic nevi. Arch Dermatol. 2012;148(2):259-260.

Eggener S, Karsh LI, Richardson T, et al. A 17-gene panel for prediction of adverse prostate cancer pathologic features: Prospective clinical validation and utility. Urology. 2019;126:76-82.

Elder DE, Piepkorn MW, Barnhill RL, et al. Pathologist characteristics associated with accuracy and reproducibility of melanocytic skin lesion interpretation. J Am Acad Dermatol. 2018;79(1):52-59.e55.

Elmore JG, Barnhill RL, Elder DE, et al. Pathologists' diagnosis of invasive melanoma and melanocytic proliferations: observer accuracy and reproducibility study. BMJ. 2017;357:j2813.

Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. Recommendations from the EGAPP Working Group: does the use of Oncotype DX tumor gene expression profiling to guide treatment decisions improve outcomes in patients with breast cancer? Genet Med. 2016 Aug;18(8):770-9.

Fang L, Monteys AM, Dürr A, et al. Haplotyping SNPs for allele-specific gene editing of the expanded huntingtin allele using long-read sequencing. HGG Adv. 2022 Sep 26;4(1):100146.

Farmer ER, Gonin R, Hanna MP. Discordance in the histopathologic diagnosis of melanoma and melanocytic nevi between expert pathologists. Hum Path. 1996;27(6):528-531.

Ferris LK, Gerami P, Skelsey MK, et al. Real-world performance and utility of a noninvasive gene expression assay to evaluate melanoma risk in pigmented lesions. Melanoma Res. 2018;28(5):478-482.

Ferris LK, Jansen B, Ho J, et al. Utility of a noninvasive 2-gene molecular assay for cutaneous melanoma and effect on the decision to biopsy. JAMA Dermatol. 2017;153(7):675-680.

Ferris LK, Rigel DS, Siegel DM, et al. 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. 2019 May 15;25(5):13030/qt61w6h7mn. Erratum in: Dermatol Online J. 2019 Jun 06;25(6):13030/qt452297hk.

Fontalba A, Real PJ, Fernandez-Luna JL, et al. Identification of c-Kit gene mutations in patients with polycythemia vera. Leuk Res. 2006;30(10):1325-1326.

Francini E, Agarwal N, Castro E, et al. Intensification approaches and treatment sequencing in metastatic castration-resistant prostate cancer: a systematic review. Eur Urol. 2024 Sep 20:S0302-2838(24)02599-5.

Frank JE. Diagnosis and management of G6PD deficiency. Am Fam Physician. 2005 Oct 1;72(7):1277-82.

Friedman RJ, Farber MJ, Warycha MA, et al. The "dysplastic" nevus. Clin Dermatol. 2009;27(1):103-115.

García-Peláez B, Trias I, Román R, et al. Fluorescent in situ hybridization as a predictor of relapse in urothelial carcinoma. Actas Urol Esp. 2013 Jul-Aug;37(7):395-400. English, Spanish.

Gerami P, Yao Z, Polsky D, et al. Development and validation of a noninvasive 2-gene molecular assay for cutaneous melanoma. J Am Acad Dermatol. 2017;76(1):114-120 e112.

Giordano TJ, Beaudenon-Huibregtse S, Shinde R, et al. Molecular testing for oncogenic gene mutations in thyroid lesions: a case-control validation study in 413 postsurgical specimens. Hum Pathol. 2014;45(7):1339-1347.

Gould MK, Ananth L, Barnett PG. A clinical model to estimate the pretest probability of lung cancer in patients with solitary pulmonary nodules. Chest 2007;131(2):383-388.

Gould MK, Donington J, Lynch WR, et al. Evaluation of individuals with pulmonary nodules: when is it lung cancer? Chest. 2013;143(5):e93S-e120S.

Gould MK, Tang T, Liu IA, et al. Recent trends in the identification of incidental pulmonary nodules. Am J Respir Crit Care Med. 2015;192(10):1208-1214.

Griewank KG, Murali R, Puig-Butille JA, et al. TERT promoter mutation status as an independent prognostic factor in cutaneous melanoma. J Natl Cancer Inst. 2014;106(9).

Griguolo G, Bottosso M, Vernaci G, et al. Gene-expression signatures to inform neoadjuvant treatment decision in hr +/her2- breast cancer: available evidence and clinical implications. Cancer Treat Rev. 2022 Jan;102:102323.

Guan H, Toraldo G, Cerda S, et al. Utilities of RAS mutations in preoperative fine needle biopsies for decision making for thyroid nodule management: results from a single-center prospective cohort. Thyroid. 2020;30(4):536-547.

Guglielmelli, P, Gangat N., Coltro G, et al. Mutations and thrombosis in essential thrombocythemia. Blood Cancer J. 2021 Apr;11(77):1-4.

Handolias D, Hamilton AL, Salemi R, et al. Clinical responses observed with imatinib or sorafenib in melanoma patients expressing mutations in KIT. British Journal of Cancer. 2010; 102: 1219-1223.

Harnan S, Tappenden P, Cooper K, et al. Tumour profiling tests to guide adjuvant chemotherapy decisions in early: a breast cancer systematic review and economic analysis. Health Technol Assess. 2019 Jun;23(30):1-328.

Harrison CN, Bareford D, Butt N, et al. Guideline for investigation and management of adults and children presenting with a thrombocytosis. Br J Haematol. 2010;149(3):352-375.

Hartman J, Patki T, Johnson NE. Diagnosis, and management of myotonic dystrophy type 1. JAMA. 2024 Apr 9;331(14):1227-1228.

Haugen BR, Alexander EK, Bible KC, et al. 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. 2016;26(1):1-133.

Haugen BR, Sawka AM, Alexander EK, et al. American Thyroid Association guidelines on the management of thyroid nodules and differentiated Thyroid Cancer Task Force review and recommendation on the proposed renaming of encapsulated follicular variant papillary thyroid carcinoma without invasion to noninvasive follicular thyroid neoplasm with papillary-like nuclear features. Thyroid. 2017;27(4):481-483.

Hawryluk EB, Sober AJ, Piris A, et al. Histologically challenging melanocytic tumors referred to a tertiary care pigmented lesion clinic. J Am Acad Dermatol. 2012;67(4):727-735.

Hayes, Inc. Clinical Utility Evaluation. Clinical utility of whole genome sequencing (WGS) and whole exome sequencing (WGS) in patients with intellectual disability. Hayes Inc. January 21, 2021b, updated March 31, 2023.

Hayes, Inc. Clinical Utility Evaluation. Genetic screening of newborns for risk of hearing loss. Hayes, Inc.; November 22, 2019. Updated June 07, 2022.

Hayes, Inc. Clinical Utility Evaluation. Genetic testing for fragile X syndrome. Hayes, Inc.; February 16, 2017. Updated March 31, 2021.

Hayes, Inc. Clinical Utility Evaluation. Genetic testing for patients with clinically diagnosed autism spectrum disorder. Hayes, Inc.; September 15, 2023.

Hayes, Inc. Clinical Utility Evaluation. Genetic testing in patients with or suspected of congenital and/or prelingual nonsyndromic hearing loss. Hayes, Inc.; June 21, 2019. Updated June 07, 2022.

Hayes, Inc. Clinical Utility Evaluation. Genetic testing in patients with or suspected of postlingual nonsyndromic hearing loss. Hayes, Inc.; June 21, 2019. Updated June 07, 2022.

Hayes, Inc. Clinical Utility Evaluation. Prenatal and preimplantation genetic testing for risk of hearing loss. Hayes, Inc.; December 11, 2019. Updated June 07, 2022.

Hayes, Inc. Clinical Utility Evaluation. Prenatal whole genome sequencing and prenatal whole exome sequencing. Hayes Inc.; June 15, 2020, updated May 12, 2023.

Hayes, Inc. Clinical Utility Evaluation. Whole exome/genome sequencing for neuromuscular diseases and movement disorders in adults. Hayes Inc.; March 9, 2022, updated April 3, 2023.

Hayes, Inc. Clinical Utility Evaluation. Whole exome/genome sequencing for previously undiagnosed pediatric neurodevelopmental disorders. Hayes Inc.; November 12, 2021a, updated October 25, 2022.

Hayes, Inc. Molecular Test Assessment. Alpha-thalassemia. Hayes Inc.; February 5, 2015.

Hayes, Inc. Molecular Test Assessment. Angelman syndrome (AS). Hayes Inc.; November 7, 2008, updated November 13, 2012.

Hayes, Inc. Molecular Test Assessment. Charcot-Marie-Tooth type 1A (PMP22). Hayes Inc.; August 5, 2008, updated July 31, 2012.

Hayes, Inc. Molecular Test Assessment. Friedreich ataxia (FRDA). Hayes Inc.; October 13, 2011, updated October 10, 2014.

Hayes, Inc. Molecular Test Assessment. Huntington chorea/disease (HD) for diagnostic, predictive, and prenatal or preimplantation genetic diagnosis purposes. Hayes, Inc.; April 29, 2008, updated April 25, 2012.

Hayes, Inc. Molecular Test Assessment. MECP2 testing for Rett syndrome and other disorders. Hayes Inc.; January 20, 2010, updated January 16, 2014.

Hayes, Inc. Molecular Test Assessment. Myotonic dystrophy types 1 And 2. Hayes, Inc.; March 9, 2009, updated May 5, 2013.

Hayes, Inc. Molecular Test Assessment. Oncotype DX Breast Recurrence Score (Genomic Health Inc.) for lymph node–positive patients. Hayes Inc.; May 6, 2020, updated April 7, 2023.

Hayes, Inc. Molecular Test Assessment. Oncotype DX Breast Recurrence Score for lymph node–negative patients (Genomic Health Inc.). Hayes Inc.; April 23, 2020, updated April 10, 2023.

Hayes, Inc. Molecular Test Assessment. Oncotype DX Genomic Prostate Score (GPS) assay (Genomic Health Inc.). Hayes Inc.; Nov 12, 2018, updated June 17, 2022.

Hayes, Inc. Molecular Test Assessment. Prader-Willi syndrome (PWS). Hayes Inc.; October 15, 2008, updated November 12, 2012.

Hayes, Inc. Molecular Test Assessment. Spinal and bulbar muscular atrophy (SBMA; Kennedy Disease). Hayes, Inc.; August 16, 2010, updated August 4, 2014.

Hayes, Inc. Precision Medicine Insight. Whole mitochondrial genome sequencing. Hayes Inc.; March 13, 2024.

Hayes, Inc. Precision Medicine Insight. X-linked intellectual disability (XLID) multigene panels. Hayes Inc.; December 4, 2014.

Heidenreich B, Nagore E, Rachakonda PS, et al. Telomerase reverse transcriptase promoter mutations in primary cutaneous melanoma. Nat Commun. 2014;5:3401.

Helfand BT, Paterakos M, Wang CH, et al. The 17-gene Genomic Prostate Score assay as a predictor of biochemical recurrence in men with intermediate and high-risk prostate cancer. PLoS One. 2022 Sep 1;17(9):e0273782.

Hickey SE, Curry CJ, Toriello HV. ACMG Practice Guideline: lack of evidence for MTHFR. Genet Med. 2013;15(2):153-156. Addendum 2020. Available at: https://www.gimjournal.org/article/S1098-3600(21)00799-1/fulltext.

Hodis E, Watson IR, Kryukov GV, et al. A landscape of driver mutations in melanoma. Cell. 2012;150(2):251-263.

Horn S, Figl A, Rachakonda PS, et al. TERT promoter mutations in familial and sporadic melanoma. Science. 2013;339(6122):959-961.

Hou YC, Yu HC, Martin R, et al. Precision medicine integrating whole-genome sequencing, comprehensive metabolomics, and advanced imaging. Proc Natl Acad Sci U S A. 2020 Feb 11;117(6):3053-3062.

Hu C, Wang X. Predictive and prognostic value of gene mutations in myelodysplastic syndrome treated with hypomethylating agents: a meta-analysis. Leuk Lymphoma. 2022 Oct;63(10):2336-2351.

Hu JC, Tosoian JJ, Qi J, et al. Clinical utility of gene expression classifiers in men with newly diagnosed prostate cancer. JCO Precis Oncol. 2018;2:PO.18.00163.

Hughes LL, Wang M, Page DL, et al. Local excision alone without irradiation for ductal carcinoma in situ of the breast: a trial of the Eastern Cooperative Oncology Group. J Clin Oncol. 2009;27(32):5319-5324.

Idiopathic Pulmonary Fibrosis Clinical Research Network; Raghu G, Anstrom KJ, King TE Jr, et al. Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. N Engl J Med. 2012 May 24;366(21):1968-77.

Iniguez CE, Armstrong KW, Cooper Z, et al. Thirty-day mortality after lobectomy in elderly patients eligible for lung cancer screening. Ann Thorac Surg. 2016;101:541-6.

Janes JL, Boyer MJ, Bennett JP, et al. The 17-gene Genomic Prostate Score test is prognostic for outcomes after primary external beam radiation therapy in men with clinically localized prostate cancer. Int J Radiat Oncol Biol Phys. 2023 Jan 1;115(1):120-131.

Jansen B, Hansen D, Moy R, et al. Gene expression analysis differentiates melanomas from Spitz Nevi. J Drugs Dermatol. 2018;17(5):574-576.

Joensuu H, Wardelmann E, Eriksson M, et al. KIT and PDGFRA mutations and survival of gastrointestinal stromal tumor patients treated with adjuvant imatinib in a randomized trial. Clin Cancer Res. 2023 Sep 1;29(17):3313-3319.

Jug R, Datto M, Jiang X. Molecular testing for indeterminate thyroid nodules: Performance of the afirma gene expression classifier and ThyroSeq panel. Cancer Cytopathol. 2018 Jul;126(7): 471-480.

Kalinsky K, Barlow WE, Gralow JR, et al. 21-Gene Assay to Inform Chemotherapy Benefit in Node-Positive Breast Cancer. N Engl J Med. 2021 Dec 16;385(25):2336-2347.

Kaur S, Christodoulou J. MECP2 Disorders. 2001 Oct 3 [Updated 2019 Sep 19]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1497/. Accessed August 22, 2024.

Kavcic N, Peric I, Zagorac A, et al. Clinical evaluation of two non-invasive genetic tests for detection and monitoring of urothelial carcinoma: Validation of UroVysion and Xpert Bladder Cancer Detection Test. Front Genet. 2022 Jun 6;13:839598.

Kelsey CR, Marks LB, Hollis D, et al. Local recurrence after surgery for early stage lung cancer: an 11-year experience with 975 patients. Cancer. 2009;115(22):5218-5227.

Khan TM, Zeiger MA. Thyroid nodule molecular testing: is it ready for prime time? Front Endocrinol (Lausanne). 2020;11:590128.

Kim M, Kim BH. Current guidelines for management of medullary thyroid carcinoma. Endocrinol Metab (Seoul). 2021;36(3):514-524.

Kim NE, Raghunathan RS, Hughes EG, et al. Bethesda III and IV thyroid nodules managed nonoperatively after molecular testing with Afirma GSC or Thyroseq v3. J Clin Endocrinol Metab. 2023 Aug 18;108(9):e698-e703.

Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med. 2013;369(25):2379-2390.

Ko JS, Matharoo-Ball B, Billings SD, et al. Diagnostic distinction of malignant melanoma and benign nevi by a gene expression signature and correlation to clinical outcomes. Cancer Epidemiol Biomarkers Prev. 2017;26(7):1107-1113.

Konkle BA, Nakaya Fletcher S. Hemophilia B. 2000 Oct 2 [Updated 2024 Jun 6]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1495/. Accessed August 22, 2024.

Krantz ID, Medne L, Weatherly JM, et al. NICUSeq Study Group. Effect of whole-genome sequencing on the clinical management of acutely ill Infants with suspected genetic disease: A randomized clinical trial. JAMA Pediatr. 2021 Sep 27: 175(12):1218-1226.

Kratz JR, Eeden SKVD, He J, et al. A prognostic assay to identify patients at high risk of mortality despite small, node-negative lung tumors. Journal of the American Medical Association. 2012b;308(16):1629-1631.

Kratz JR, He J, Van Den Eeden SK, et al. A practical molecular assay to predict survival in resected non-squamous, non-small-cell lung cancer: development and international validation studies. Lancet. 2012a;379(9818):823-832.

Kumaran N, Pennesi ME, Yang P, et al. Leber Congenital Amaurosis / Early-Onset Severe Retinal Dystrophy Overview. 2018 Oct 4 [Updated 2023 Mar 23]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK531510/. Accessed August 22, 2024.

La Spada A. Spinal and Bulbar Muscular Atrophy. 1999 Feb 26 [Updated 2022 Dec 15]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1333/. Accessed August 22, 2024.

Labourier E, Shifrin A, Busseniers AE, et al. 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. 2015;100(7):2743-2750.

Landa I, Ganly I, Chan TA, et al. Frequent somatic TERT promoter mutations in thyroid cancer: higher prevalence in advanced forms of the disease. J Clin Endocrinol Metab. 2013;98(9):E1562-E1566.

Langer AL. Beta-Thalassemia. 2000 Sep 28 [Updated 2024 Feb 8]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1426/. Accessed August 22, 2024.

Lau MYH, Islam Khan MZ, Law HKW. Molecular mechanism of radioresponsiveness in colorectal cancer: a systematic review. Genes (Basel). 2024 Sep 26;15(10):1257.

Lawler T, Parlato L, Warren Andersen S. The histological and molecular characteristics of early-onset colorectal cancer: a systematic review and meta-analysis. Front Oncol. 2024 Apr 26;14:1349572.

Lee E, Terhaar S, McDaniel L, et al. Diagnostic performance of the second-generation molecular tests in the assessment of indeterminate thyroid nodules: a systematic review and meta-analysis. Am J Otolaryngol. 2022;43(3):103394.

Li GQ, Xie SJ, Wu SG, et al. Impact of the 21-gene expression assay on treatment decisions and clinical outcomes in breast cancer with one to three positive lymph nodes. Front Endocrinol (Lausanne). 2023 Feb 16;14:1103949.

Li X, Hayward C, Fong P, et al. A blood-based proteomic classifier for the molecular characterization of pulmonary nodules. Sci Transl Med. 2013;5(207):1-24.

Li X, Lee LW, Brusniak M, et al. An integrated quantification method to increase the precision, robustness, and resolution of protein measurement in human plasma samples. Clin Proteomics. 2015;12(3):1-17.

Liem EIML, Baard J, Cauberg ECC, et al. Fluorescence in situ hybridization as prognostic predictor of tumor recurrence during treatment with Bacillus Calmette-Guérin therapy for intermediate- and high-risk non-muscle-invasive bladder cancer. Med Oncol. 2017 Sep 2;34(10):172.

Liu Y, Yanbin S, Wenli M, et al. Decreased microRNA-30a levels are associated with enhanced ABL1 and BCR-ABL1 expression in chronic myeloid leukemia. Leukemia Research. 2013; 37(3): 349-356.

Livhits MJ, Zhu CY, Kuo EJ, et al. Effectiveness of molecular testing techniques for diagnosis of indeterminate thyroid nodules: a randomized clinical trial. JAMA Oncol. 2021;7(1):70-77.

Lokhandwala T, Bittoni MA, Dann RA, et al. Costs of diagnostic assessment for lung cancer: a medicare claims analysis. Clin Lung Cancer. 2016;18(1):e27-e34.

Lott JP, Boudreau DM, Barnhill RL, et al. Population-based analysis of histologically confirmed melanocytic proliferations using natural language processing. JAMA Dermatol. 2018;154(1):24-29.

Ludlow JT, Wilkerson RG, Nappe TM. Methemoglobinemia. [Updated 2023 Aug 28]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537317/. Accessed November 26, 2024.

Luzzatto L, Nannelli C, Notaro R. Glucose-6-Phosphate Dehydrogenase Deficiency. Hematol Oncol Clin North Am. 2016 Apr;30(2):373-93.

Malinowski J, Miller DT, Demmer L, et al.; ACMG Professional Practice and Guidelines Committee. Systematic evidence-based review: outcomes from exome and genome sequencing for pediatric patients with congenital anomalies or intellectual disability. Genet Med. 2020 Jun;22(6):986-1004.

Malvehy J, Hauschild A, Curiel-Lewandrowski C, et al. Clinical performance of the Nevisense system in cutaneous melanoma detection: an international, multicentre, prospective, and blinded clinical trial on efficacy and safety. Br J Dermatol. 2014;171(5):1099-1107.

Manders JB, Kuerer HM, Smith BD, et al. Clinical utility of the 12-Gene DCIS score assay: impact on radiotherapy recommendations for patients with ductal carcinoma in situ; Ann Surg Oncol. 2017;24:660-668.

Marcadis AR, Valderrabano P, Ho AS, et al. Interinstitutional variation in predictive value of the ThyroSeq v2 genomic classifier for cytologically indeterminate thyroid nodules. Surgery. 2019;165(1):17-24.

Marti JL, Avadhani V, Donatelli LA, et al. Wide inter-institutional variation in performance of a molecular classifier for indeterminate thyroid Nodules. Ann Surg Oncol. 2015;22(12):3996-4001.

McDonagh EM, Thorn CF, Bautista JM, et al. PharmGKB summary: very important pharmacogene information for G6PD. Pharmacogenet Genomics. 2012 Mar;22(3):219-28.

McGinnis KS, Lessin SR, Elder DE, et al. Pathology review of cases presenting to a multidisciplinary pigmented lesion clinic. Arch Dermatol. 2002;138(5):617-621.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); September 1, 2016a. ABL1 gene; Available at: https://medlineplus.gov/genetics/gene/abl1/. Accessed August 5, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); July 1, 2020a. AR gene; Available at: https://medlineplus.gov/genetics/gene/ar/. Accessed August 7, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); March 1, 2018a. Cone-rod dystrophy; Available at: https://medlineplus.gov/genetics/condition/cone-rod-dystrophy/. Accessed August 22, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); August 1, 2009a. Distal hereditary motor neuropathy, type II; Available at: https://medlineplus.gov/genetics/condition/distal-hereditary-motor-neuropathy-type-ii/. Accessed October 23, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); August 1, 2009b. Distal hereditary motor neuropathy, type V; Available at: https://medlineplus.gov/genetics/condition/distal-hereditary-motor-neuropathy-type-v/. Accessed October 23, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); July 1, 2020b. DMPK gene. (update July 2020); Available at: https://medlineplus.gov/genetics/gene/dmpk/. Accessed August 12, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); July 17, 2024a. Fragile XE syndrome. Available at: https://medlineplus.gov/genetics/condition/fragile-xe-syndrome/. Accessed November 20, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); April 12, 2023a. G6PD gene; Available at: https://medlineplus.gov/genetics/gene/g6pd/. Accessed August 7, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); January 24, 2022a. G6PD test; Available at: https://medlineplus.gov/lab-tests/g6pd-test/. Accessed August 22, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); March 31, 2024b. Hemophilia B; Available at: https://medlineplus.gov/ency/article/000539.htm. Accessed November 22, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); September 1, 2016a. ABL1 gene; Available at: https://medlineplus.gov/genetics/gene/abl1/. Accessed August 5, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); April 1, 2017a. Hereditary sensory and autonomic neuropathy type II; Available at: https://medlineplus.gov/genetics/condition/hereditary-sensory-and-autonomic-neuropathy-type-ii/. Accessed October 23, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); July 1, 2011a. Hereditary sensory and autonomic neuropathy type V; Available at: https://medlineplus.gov/genetics/condition/hereditary-sensory-and-autonomic-neuropathy-type-v/. Accessed October 23, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); June 1, 2017b. Hereditary sensory and autonomic neuropathy type IE; Available at https://medlineplus.gov/genetics/condition/hereditary-sensory-and-autonomic-neuropathy-type-ie/. Accessed October 23, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); December 1, 2011b. Kearns-Sayre syndrome; Available at https://medlineplus.gov/genetics/condition/kearns-sayre-syndrome/. Accessed August 22, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); October 6, 2022b. Leber congenital amaurosis; Available at: https://medlineplus.gov/genetics/condition/leber-congenital-amaurosis/. Accessed August 22, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); April 28, 2023c. Leigh syndrome; Available at https://medlineplus.gov/genetics/condition/leigh-syndrome/. Accessed August 22, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); July 1, 2015a. Methemoglobinemia, beta-globin type; Available at: https://medlineplus.gov/genetics/condition/methemoglobinemia-beta-globin-type/#causes. Accessed November 26, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); December 1, 2013. Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; Available at: https://medlineplus.gov/genetics/condition/mitochondrial-encephalomyopathy-lactic-acidosis-and-stroke-like-episodes/. Accessed August 22, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); February 1, 2016b. Nonsyndromic hearing loss; Available at: https://medlineplus.gov/genetics/condition/nonsyndromic-hearing-loss/. Accessed August 8, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); July 13, 2021a. PDGFRA gene; Available at: https://medlineplus.gov/genetics/gene/pdgfra/. Accessed August 12, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); March 1, 2016c. Pendred syndrome; Available at: https://medlineplus.gov/genetics/condition/pendred-syndrome/. Accessed October 28, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); July 1, 2020c. PMP22 gene; Available at: https://medlineplus.gov/genetics/gene/pmp22/. Accessed August 7, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); October 1, 2010. Retinitis pigmentosa; Available at: https://medlineplus.gov/genetics/condition/retinitis-pigmentosa/. Accessed August 22, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); March 14, 2024c. Sickle cell disease; Available at: https://medlineplus.gov/genetics/condition/sickle-cell-disease/#causes. Accessed November 26, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); October 1, 2018b. SMN1 gene; Available at: https://medlineplus.gov/genetics/gene/smn1/. Accessed August 8, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); July 1, 2020d. TERT gene; Available at: https://medlineplus.gov/genetics/gene/tert/. Accessed August 2, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); April 1, 2016d. Usher syndrome; Available at: https://medlineplus.gov/ushersyndrome.html. Accessed October 28, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); July 28, 2021b. What is noninvasive prenatal testing (NIPT) and what disorders can it screen for?; Available at: https://medlineplus.gov/genetics/understanding/testing/nipt/. Accessed August 22, 2024.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US); June 1, 2015b. X-linked intellectual disability, Siderius type. (update July 2020). Available at: https://medlineplus.gov/genetics/condition/x-linked-intellectual-disability-siderius-type/. Accessed August 22, 2024.

Mehta PA, Ebens C. Fanconi Anemia. 2002 Feb 14 [Updated 2021 Jun 3]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1401/. Accessed August 22, 2024.

Miceikaite I, Fagerberg C, Brasch-Andersen C, et al. Comprehensive prenatal diagnostics: Exome versus genome sequencing. Prenat Diagn. 2023 Aug;43(9):1132-1141.

Mikami T, Nemoto Y, Numata Y, et al. Small gastrointestinal stromal tumor in the stomach: Identification of precursor for clinical gastrointestinal stromal tumor using C-KIT and A-Smooth muscle actin expression. Human Pathology. 2013; 44(12): 2628-2635.

Miller LJ, Saporta AS, Sottile SL, et al. Strategy for genetic testing in Charcot-Marie-disease. Acta Myol. 2011 Oct;30(2):109-16.

Minar L, Felsinger M, Cermakova Z, et al. Comparison of the Copenhagen Index versus ROMA for the preoperative assessment of women with ovarian tumors. Int J Gynaecol Obstet. 2018;140(2):241-246.

Misko A, Grishchuk Y, Goldin E, et al. Mucolipidosis IV. 2005 Jan 28 [Updated 2021 Feb 11]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1214/. Accessed August 22, 2024.

Moons KGM, Harrell FE. Sensitivity and specificity should be de-emphasized in diagnostic accuracy studies. Acad Radiol 2003;10:670-2.

Moore RG, Hawkins DM, Miller MC, et al. Combining clinical assessment and the Risk of Ovarian Malignancy Algorithm for the prediction of ovarian cancer. Gynecol Oncol. 2014;135(3):547-551.

Najafian A, Noureldine S, Azar F, et al. RAS Mutations, and RET/PTC and PAX8/PPAR-gamma chromosomal rearrangements are also prevalent in benign thyroid lesions: implications thereof and a systematic review. Thyroid. 2017;27(1):39-48.

Narod SA, Iqbal J, Giannakeas V, et al. Breast cancer mortality after a diagnosis of ductal carcinoma in situ. JAMA Oncol. 2015;1(7):888-896.

Nash AL, Ren Y, Plichta JK, et al. Survival benefit of chemotherapy according to 21-gene recurrence score in young women with breast cancer. Ann Surg Oncol. 2023;30(4):2130-2139.

National Comprehensive Cancer Network (NCCN). Biomarkers compendium, 2A rating for KIT and myeloproliferative neoplasms (MPN). Available at: https://www.nccn.org/compendia-templates/compendia/biomarkers-compendium.

National Comprehensive Cancer Network (NCCN). Clinical practice guidelines in oncology. Breast cancer. V5.2024.

National Comprehensive Cancer Network (NCCN). Clinical practice guidelines in oncology. Central nervous system cancers. V3.2024.

National Comprehensive Cancer Network (NCCN). Clinical practice guidelines in oncology. Chronic myeloid leukemia. V1.2025.

National Comprehensive Cancer Network (NCCN). Clinical practice guidelines in oncology. Ovarian Cancer/Fallopian Tube Cancer/Primary Peritoneal Cancer. V3.2024.

National Comprehensive Cancer Network (NCCN). Clinical practice guidelines in oncology. Prostate cancer. V4.2024.

National Comprehensive Cancer Network (NCCN). Clinical practice guidelines in oncology. Thyroid carcinoma. V4.2024.

National Institute for Health and Care Excellence (NICE). Tests in secondary care to identify people at high risk of ovarian cancer. Diagnostics guidance [DG31]. November 2017.

National Institute for Health and Care Excellence (NICE). Tumor profiling tests for guiding adjuvant chemotherapy choice in early breast cancer. Diagnostics guidance [DG34]. December 2018.

National Institutes of Health. National Cancer Institute Surveillance, Epidemiology, and End Results Program (SEER) Cancer Stat Facts: Thyroid Cancer. Available at: https://seer.cancer.gov/statfacts/html/thyro.html. Accessed August 14, 2024.

National Institutes of Health. National Center for Biotechnology Information. AFF2 gene mental retardation, X-linked, associated with fragile site FRAXE NGS Genetic DNA Test. April 27, 2023. Available at https://www.ncbi.nlm.nih.gov/gtr/tests/classic/605579/. Accessed November 22, 2024.

National Institutes of Health. National Institute of Neurological Disorders and Stroke. Charcot-Marie-Tooth Disease. July 19, 2024. Available at: https://www.ninds.nih.gov/health-information/disorders/charcot-marie-tooth-disease. Accessed August 7, 2024.

National Lung Screening Trial Research Team, Aberle DR, Adams AM, Berg CD, et al. Reduced lung-cancer mortality with low-dose computed tomographic screening. N Engl J Med. 2011;365(5):395.

National Organization for Rare Disorders (NORD). Rare Diseases. Charcot-Marie-Tooth Disease. 2021. Available at: https://rarediseases.org/rare-diseases/charcot-marie-tooth-disease/. Accessed August 7, 2024.

National Organization for Rare Disorders (NORD). Rare Diseases. Hereditary sensory neuropathy type I. 2017a. Available at: https://rarediseases.org/rare-diseases/hereditary-sensory-neuropathy-type-i/. Accessed October 23, 2024.

National Organization for Rare Disorders (NORD). Rare Diseases. Hereditary sensory and autonomic neuropathy type 1E. 2017b. Available at: https://rarediseases.org/rare-diseases/hereditary-sensory-autonomic-neuropathy-type-1e/. Accessed October 23, 2024.

National Organization for Rare Disorders (NORD). Rare Diseases. Hereditary spastic paraplegia. 2017c. Available at: https://rarediseases.org/rare-diseases/hereditary-spastic-paraplegia/. Accessed October 23, 2024.

National Organization for Rare Disorders (NORD). Rare Diseases. Kennedy disease. 2023. Available at: https://rarediseases.org/rare-diseases/kennedy-disease/#disease-overview-main. Accessed August 7, 2024.

Nault A, Zhang C, Kim K, et al. Biopsy use in skin cancer diagnosis: Comparing dermatology physicians and advanced practice professionals. JAMA Dermatol. 2015;151(8):899-902.

Nicholson KJ, Roberts MS, McCoy KL, et al. Molecular testing versus diagnostic lobectomy in Bethesda III/IV thyroid nodules: a cost-effectiveness analysis. Thyroid. 2019;29(9):1237-1243.

Nikiforov YE, Carty SE, Chiosea SI, et al. 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. 2014;120(23):3627-3634.

Nikiforov YE, Carty SE, Chiosea SI, et al. Impact of the multi-gene ThyroSeq next-generation sequencing assay on cancer diagnosis in thyroid nodules with atypia of undetermined significance/follicular lesion of undetermined significance cytology. Thyroid. 2015;25(11):1217-1223.

Nikiforov YE, Ohori NP, Hodak SP, et al. 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. 2011;96(11):3390-3397.

Nikiforova MN, Mercurio S, Wald AI, et al. Analytical performance of the ThyroSeq v3 genomic classifier for cancer diagnosis in thyroid nodules. Cancer. 2018;124(8):1682-1690.

Nitz U, Gluz O, Christgen M, et al. Reducing chemotherapy use in clinically high-risk, genomically low-risk pN0 and pN1 early breast cancer patients: five-year data from the prospective, randomized phase 3 West German Study Group (WSG) PlanB trial. Breast Cancer Res Treat 2017;165:573-583.

Noone AM, Howlader N, Krapacho M, et al. SEER Cancer Statistics Review, 1975-2015. 2018. Available at: https://seer.cancer.gov/archive/csr/1975_2015/results_merged/sect_15_lung_bronchus.pdf. Accessed August 13, 2024.

Ohgami RS, Ma L, Merker JD, et al. Next-generation sequencing of acute myeloid leukemia identifies the significance of TP53, U2AF1, ASXL1, and TET2 mutations. Mod Pathol. 2015 May;28(5):706-14.

Ohori NP, Landau MS, Carty SE, et al. Benign call rate and molecular test result distribution of ThyroSeq v3. Cancer Cytopathol. 2019;127(3):161-168.

Olympios N, Gilard V, Marguet F, et al. TERT promoter alterations in glioblastoma: a systematic review. Cancers (Basel). 2021 Mar 8;13(5):1147.

Oncocyte™. Oncocyte closes second investment in razor genomics to complete acquisition. Available at: https://investors.oncocyte.com/news-releases/2021/02-25-2021-130231003. Accessed August 13, 2024.

Oncology SoG. Position Statement: Multiplex serum testing for women with pelvic mass. May 2013.

Palmer C, Boudreault P, Baldwin E, et al. Deaf genetic testing, and psychological well-being in deaf adults. J Genet Couns. 2013;22(4):492-507.

Papaleontiou M, Hughes DT, Guo C, et al. Population-based assessment of complications following surgery for thyroid cancer. J Clin Endocrinol Metab. 2017;102(7):2543-2551.

Pardanani AD, Levine RL, Lasho T, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. Blood. 2006;108(10):3472–3476.

Patel KN, Angell TE, Babiarz J, et al. Performance of a genomic sequencing classifier for the preoperative diagnosis of cytologically indeterminate thyroid nodules. JAMA Surg. 2018;153(9):817-824.

Patel KN, Yip L, Lubitz CC, et al. The American Association of Endocrine Surgeons guidelines for the definitive surgical management of thyroid disease in adults. Ann Surg. 2020;271(3):e21-e93.

Patel VK, Sagar KN, Naidich DP, et al. A practical algorithmic approach to the diagnosis and management of solitary pulmonary nodules. part 1: radiologic characteristics and imaging modalities. Chest. 2013a;143(3):825-839.

Patel VK, Sagar KN, Naidich DP, et al. A practical algorithmic approach to the diagnosis and management of solitary pulmonary nodules. part 2: pretest probability and algorithm. Chest. 2013b;143(3):840-846.

Paweletz CP, Sacher AG, Raymond CK, et al. Bias-corrected targeted next-generation sequencing for rapid, multiplexed detection of actionable alterations in cell-free dna from advanced lung cancer patients. Clin Cancer Res. 2016 Feb 15;22(4):915-22.

Pearlstein S, Lahouti AH, Opher E, et al. Thyroseq V3 molecular profiling for tailoring the surgical management of Hurthle cell neoplasms. Case Rep Endocrinol. 2018;2018:9329035.

Pepe MS, Feng Z, Huang Y, et al. Integrating the predictiveness of a marker with its performance as a classifier. Am J Epidemiology 2008;167:362-8.

Pérez-Oliveira S, Castilla-Silgado J, Painous C, et al. Huntingtin CAG repeats in neuropathologically confirmed tauopathies: Novel insights. Brain Pathol. 2024 Jul;34(4):e13250.

Piepkorn MW, Longton GM, Reisch LM, et al. Assessment of second-opinion strategies for diagnoses of cutaneous melanocytic lesions. JAMA Netw Open. 2019;2(10):e1912597.

Poorvu PD, Gelber SI, Rosenberg SM, et al. Prognostic impact of the 21-gene recurrence score assay among young women with node-negative and node-positive ER-positive/HER2-negative breast cancer. J Clin Oncol. 2020;38(7):725-733.

Pozzobon FC, Puig-Butille JA, Gonzalez-Alvarez T, et al. Dermoscopic criteria associated with BRAF and NRAS mutation status in primary cutaneous melanoma. Br J Dermatol. 2014;171(4):754-759.

Prior TW, Leach ME, Finanger E. Spinal Muscular Atrophy. 2000 Feb 24 [Updated 2020 Dec 3]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1352/. Accessed August 22, 2024.

Qu SQ, Qin TJ, Xu ZF, et al. Long-term outcomes of imatinib in patients with FIP1L1/ PDGFRA associated chronic eosinophilic leukemia: experience of a single center in China. Oncotarget. 2016 May 31;7(22):33229-36.

Rakovitch E, Nofech-Mozes S, Hanna W, et al. A population-based validation study of the DCIS Score predicting recurrence risk in individuals treated by breast-conserving surgery alone. Breast Cancer Res Treat. 2015;152(2):389-398.

Reddy KK, Farber MJ, Bhawan J, et al. Atypical (dysplastic) nevi: outcomes of surgical excision and association with melanoma. JAMA Dermatol. 2013;149(8):928-934.

Reilly MM. Classification and diagnosis of the inherited neuropathies. Ann Indian Acad Neurol. 2009 Apr;12(2):80-8.

Rigel DS, Russak J, Friedman R. The evolution of melanoma diagnosis: 25 years beyond the ABCDs. CA Cancer J Clin. 2010;60(5):301-316.

Rivers JK, Copley MR, Svoboda R, et al. Non-invasive gene Expression testing to rule out melanoma. Skin Therapy Lett. 2018;23(5):1-4.

Roncato R, Polesel J, Tosi F, et al. The challenge of molecular selection in liver-limited metastatic colorectal cancer for surgical resection: a systematic review and meta-analysis in the context of current and future approaches. Oncol Res. 2024 Aug 23;32(9):1407-1422.

Ronen S, Al-Rohil RN, Keiser E, et al. Discordance in diagnosis of melanocytic lesions and its impact on clinical management. Arch Pathol Lab Med. 2021;145(12):1505-1515.

Rugo HS, Lerebours F, Ciruelos E, et al. Alpelisib plus fulvestrant in PIK3CA-mutated, hormone receptor-positive advanced breast cancer after a CDK4/6 inhibitor (BYLieve): one cohort of a phase 2, multicentre, open-label, non-comparative study. Lancet Oncol. 2021 Apr;22(4):489-498. Erratum in: Lancet Oncol. 2021 May;22(5):e184.

Rumi E, Pietra D, Ferretti V, et al. JAK2 or CALR mutation status defines subtypes of essential thrombocythemia with substantially different clinical course and outcomes. Blood. 2014;123(10):1544-1551.

Sadlecki P, Walentowicz-Sadlecka M. Molecular landscape of borderline ovarian tumours: a systematic review. Open Med (Wars). 2024 Jun 7;19(1):20240976.

Scarabino D, Veneziano L, Mantuano E, et al. Leukocyte telomere length as potential biomarker of HD progression: A follow-up study. Int J Mol Sci. 2022 Nov 3;23(21):13449.

Schafer T, Merkl J, Klemm E, et al. The epidemiology of nevi and signs of skin aging in the adult general population: Results of the KORA-survey 2000. J Invest Dermatol. 2006;126(7):1490-1496.

Schnittger S, Eder C, Jeromin S, et al. ASXL1 exon 12 mutations are frequent in AML with intermediate risk karyotype and are independently associated with an adverse outcome. Leukemia. 2013 Jan;27(1):82-91.

Schoser B. Myotonic Dystrophy Type 2. 2006 Sep 21 [Updated 2020 Mar 19]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1466/. Accessed August 22, 2024.

Shain AH, Bastian BC. From melanocytes to melanomas. Nat Rev Cancer. 2016;16(6):345-358.

Shain AH, Yeh I, Kovalyshyn I, et al. The genetic evolution of melanoma from precursor lesions. N Engl J Med. 2015;373(20):1926-1936.

Shen H, Jin Y, Zhao H, et al. Potential clinical utility of liquid biopsy in early-stage non-small cell lung cancer. BMC Med. 2022 Dec 14;20(1):480.

Shoo BA, Sagebiel RW, Kashani-Sabet M. Discordance in the histopathologic diagnosis of melanoma at a melanoma referral center. J Am Acad Dermatol. 2010;62(5):751-756.

Shreeve N, Sproule C, Choy KW, et al. Incremental yield of whole genome sequencing over chromosome microarray and exome sequencing for congenital anomalies in prenatal period and infancy: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2023 Sep 19.

Silaghi CA, Lozovanu V, Georgescu CE, et al. Thyroseq v3, Afirma GSC, and microRNA panels versus previous molecular tests in the preoperative diagnosis of indeterminate thyroid nodules: a systematic review and meta-analysis. Front Endocrinol(Lausanne). 2021;12:649522.

Silvestri GA, Tanner NT, Kearnery P, et al. 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. 2018;154(3):491-500.

Smedley D, Smith KR, Martin A, et al. 100,000 Genomes Project Pilot Investigators. 100,000 genomes pilot on rare-disease diagnosis in health care - preliminary report. N Engl J Med. 2021 Nov 11;385(20):1868-1880.

Smith HS, Swint JM, Lalani SR, et al. Clinical application of genome and exome sequencing as a diagnostic tool for pediatric patients: a scoping review of the literature. Genet Med. 2019 Jan;21(1):3-16.

Society for Maternal-Fetal Medicine (SMFM). Dugoff L, Norton ME, Kuller JA. The use of chromosomal microarray for prenatal diagnosis. Am J Obstet Gynecol. 2016 Oct;215(4):B2-9.

Solin LJ, Gray R, Baehner FL, et al. A multigene expression assay to predict local recurrence risk for ductal carcinoma in situ of the breast. J Natl Cancer Inst. 2013;105(10):701-710.

Stemmer SM, Steiner M, Rizel S, et al. Clinical outcomes in ER + HER2 -node-positive breast cancer patients who were treated according to the Recurrence Score results: evidence from a large prospectively designed registry. NPJ Breast Cancer 2017;3:32.

Stevenson RE. Alpha-Thalassemia X-Linked Intellectual Disability Syndrome. 2000 Jun 19 [Updated 2020 May 28]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1449/. Accessed August 22, 2024.

Steward DL, Carty SE, Sippel RS, et al. Performance of a multigene genomic classifier in thyroid nodules with indeterminate cytology: a prospective blinded multicenter study. JAMA Oncol. 2019;5(2):204-212.

Strazzula L, Vedak P, Hoang MP, et al. The utility of re-excising mildly and moderately dysplastic nevi: a retrospective analysis. J Am Acad Dermatol. 2014;71(6):1071-1076.

Supplee J, Milan M, Lim L, et al. Sensitivity of next-generation sequencing assays detecting oncogenic fusions in plasma cell-free DNA. Lung Cancer. 2019 Aug;134:96-99.

Sutandyo N, Mulyasari R, Kosasih A, et al. Association of somatic gene mutations with risk of transformation into acute myeloid leukemia in patients with myelodysplastic syndrome: A systematic review and meta-Analysis. Asian Pac J Cancer Prev. 2022 Apr 1;23(4):1107-1116.

Swensen SJ, Silverstein MD, Ilstup DM, et al. The probability of malignancy in solitary pulmonary nodules. Application to small radiologically indeterminate nodules. Arch Intern Med. 1997;157:849-855.

Tamary H, Dgany O. Alpha-Thalassemia. 2005 Nov 1 [Updated 2024 May 23]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1435/. Accessed August 22, 2024.

Tanner NT, Aggarwal J, Gould MK, et al. Management of pulmonary nodules by community pulmonologists: a multicenter observational study. Chest. 2015;148(6):1405-1414.

Tao Y, Wang F, Shen X, et al. BRAF V600E status sharply differentiates lymph node metastasis-associated mortality risk in papillary thyroid cancer. J Clin Endocrinol Metab. 2021;106(11):3228-3238.

Taye A, Gurciullo D, Miles BA, et al. Clinical performance of a next-generation sequencing assay (ThyroSeq v2) in the evaluation of indeterminate thyroid nodules. Surgery. 2018;163(1):97-103.

Tefferi A, Thiele J, Vannucchi AM, et al. An overview on CALR and CSF3R mutations and a proposal for revision of WHO diagnostic criteria for myeloproliferative neoplasms. Leukemia. 2014;28(7):1407-1413.

Terlikowska KM, Dobrzycka B, Witkowska AM, et al. Preoperative HE4, CA125 and ROMA in the differential diagnosis of benign and malignant adnexal masses. J Ovarian Res. 2016;9(1):43.

Tosteson ANA, Tapp S, Titus LJ, et al. Association of second-opinion strategies in the histopathologic diagnosis of cutaneous melanocytic lesions with diagnostic accuracy and population-level costs. JAMA Dermatol. 2021;157(9):1102-1106.

Turner S, Chia S, Kanakamedala H, et al. Effectiveness of Alpelisib + Fulvestrant compared with real-world standard treatment among patients with HR +, HER2-, PIK3CA-mutated breast cancer. Oncologist. 2021 Jul;26(7):e1133-e1142.

Vachani A, Hammoud Z, Springmeyer S, et al. Clinical utility of a plasma protein classifier for indeterminate lung nodules. Lung. 2015b;193:1023-1027.

Vachani A, Pass HI, Rom WN, et al. Validation of a multiprotein plasma classifier to identify benign lung nodules. J Thora Oncol. 2015a;10(4):629-637.

Vachani A, Tanner NT, Aggarwal J, et al. Factors that influence physician decision making for indeterminate pulmonary nodules. Ann Am Thorac Soc. 2014;11(10):1586-1591.

Valderrabano P, Hallanger-Johnson JE, Thapa R, et al. Comparison of postmarketing findings vs the initial clinical validation findings of a thyroid nodule gene expression classifier: a systematic review and meta-analysis. JAMA Otolaryngol Head Neck Surg. 2019;145(9):783-792.

Valeriani E, Pastori D, Astorri G, et al. Factor V Leiden, prothrombin, MTHFR, and PAI-1 gene polymorphisms in patients with arterial disease: A comprehensive systematic-review and meta-analysis. Thromb Res. 2023 Oct;230:74-83.

Vargas-Salas S, Martinez JR, Urra S, et al. Genetic testing for indeterminate thyroid cytology: review and meta-analysis. Endocr Relat Cancer. 2018;25(3):R163-R177.

Veenhuizen KC, De Wit PE, Mooi WJ, et al. Quality assessment by expert opinion in melanoma pathology: Experience of the pathology panel of the dutch melanoma working party. J Path. 1997;182(3):266-272.

Wachsman W, Morhenn V, Palmer T, et al. Noninvasive genomic detection of melanoma. Br J Dermatol. 2011;164(4):797-806.

Wan Y, Wu C. SF3B1 mutations in chronic lymphocytic leukemia. Blood. 2013;121(23):4627-4634.

Wan Z, Han B. Comparison and implications of mutational profiles of myelodysplastic syndromes, myeloproliferative neoplasms, and myelodysplastic/myeloproliferative neoplasms: a meta-analysis. Front Oncol. 2020 Oct 7;10:579221.

Wang J, Gao J, Yao H, et al. Diagnostic accuracy of serum HE4, CA125 and ROMA in patients with ovarian cancer: a meta-analysis. Tumour Biol. 2014;35(6):6127-6138.

Wang M, Wu K, Zhang P, et al. The prognostic significance of the Oncotype DX Recurrence Score in T1-2N1M0 estrogen receptor-positive HER2-negative breast cancer based on the prognostic stage in the updated AJCC 8th edition. Ann Surg Oncol. 2019;26(5):1227-1235.

Wang Z, Liu W, Wang M, et al. Prognostic value of ASXL1 mutations in patients with primary myelofibrosis and its relationship with clinical features: a meta-analysis. Ann Hematol. 2021 Feb;100(2):465-479.

Ward EM, DeSantis CE, Lin CC, et al. Cancer Statistics: Breast cancer in situ. CA Cancer J Clin. 2015;65(6):481-495.

Warf MB, Flake DD II, Adams D, et al. Analytical validation of a melanoma diagnostic gene signature using formalin-fixed paraffin-embedded melanocytic lesions. Biomark Med. 2015;9(5):407-416.

Wasserstein MP, Schuchman EH. Acid Sphingomyelinase Deficiency. 2006 Dec 7 [Updated 2023 Apr 27]. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1370/. Accessed August 22, 2024.

Weiner RS, Schwartz LM, Woloshin S, et al. Population-based risk for complications following transthoracic needle lung biopsy of a pulmonary nodule. Ann Intern Med. 2011;155(3):137-144.

Weissman S. Fragile xe syndrome. Norton and Elaine Sarnoff Center for Jewish Genetics. August 2024. Available at: https://www.jewishgenetics.org/genetic-disorders/search-genetic-disorders/fragile-xe-syndrome/. Accessed November 20, 2024.

Wells SA, Jr., Asa SL, Dralle H, et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. Thyroid. 2015;25(6):567-610.

Wilson RL, Yentzer BA, Isom SP, et al. How good are US dermatologists at discriminating skin cancers? A number-needed-to-treat analysis. J Dermatolog Treat. 2012;23(1):65-69.

Wolmark N, Mamounas EP, Baehner FL, et al. Prognostic impact of the combination of recurrence score and quantitative estrogen receptor expression (ESR1) on predicting late distant recurrence risk in estrogen receptor–positive breast cancer after 5 years of tamoxifen: Results from NRG Oncology/National Surgical Adjuvant Breast and Bowel Project B-28 and B-14. J Clin Oncol. 2016 Jul 10;34(20):2350-8.

Woodard GA, Gubens MA, Jahan TM, et al. Prognostic molecular assay might improve identification of patients at risk for recurrence in early-stage non-small-cell lung cancer. Clin Lung Cancer. 2014;15(6):426-432.

Woodard GA, Wang SX, Kratz JR, et al. Adjuvant chemotherapy guided by molecular profiling and improved outcomes in early stage, non-small-cell lung cancer. Clin Lung Cancer. 2018;19(1):58-64.

Work Group; Invited Reviewers, Kim JYS, Kozlow JH, Mittal B, et al. Guidelines of care for the management of cutaneous squamous cell carcinoma. J Am Acad Dermatol. 2018;78(3):560-578.

Wylie D, Beaudenon-Huibregtse S, Haynes B, et al. Molecular classification of thyroid lesions by combined testing for miRNA gene expression and somatic gene alterations. The Journal of Pathology: Clinical Research. 2016;2(2):93-103.

Xing M, Alzahrani AS, Carson KA, et al. Association between BRAF V600E mutation and recurrence of papillary thyroid cancer. J Clin Oncol. 2015;33(1):42-50.

Xing M, Liu R, Liu X, et al. BRAF V600E and TERT promoter mutations cooperatively identify the most aggressive papillary thyroid cancer with highest recurrence. J Clin Oncol. 2014;32(25):2718-2726.

Yao Z, Allen T, Oakley M, et al. Analytical characteristics of a noninvasive gene expression assay for pigmented skin lesions. Assay Drug Dev Technol. 2016;14(6):355-363.

Yao Z, Moy R, Allen T, et al. An adhesive patch-based skin biopsy device for molecular diagnostics and skin microbiome studies. J Drugs Dermatol. 2017;16(10):979-986.

Yin L, Tang Y, Lu Q, et al. Noninvasive prenatal testing detects microdeletion abnormalities of fetal chromosome 15. J Clin Lab Anal. 2019 Jul;33(6):e22911.

Yip L. Molecular markers for thyroid cancer diagnosis, prognosis, and targeted therapy. J Surg Oncol. 2015;111(1):43-50.

Young R, Kalnicki S, Fox JL. The DCIS Score - Potential for healthcare savings? Poster presented: San Antonio Breast Cancer Symposium; December 2014; San Antonio, TX.

Zhao W, Zhang C, Li Y, et al. The prognostic value of the interaction between ASXL1 and TET2 gene mutations in patients with chronic myelomonocytic leukemia: a meta-analysis. Hematology. 2022 Dec;27(1):367-378.

Zheng W, Lin T, Chen Z, et al. The role of Fluorescence In Situ Hybridization in the surveillance of non-muscle invasive bladder cancer: An updated systematic review and meta-analysis. Diagnostics (Basel). 2022 Aug 19;12(8):2005.

Zhu CY, Sha S, Tseng CH, et al. Trends in the surgical management of known or suspected differentiated thyroid cancer at a single institution, 2010-2018. Thyroid. 2020 Nov;30(11):1639-1645.

Zujewski JA, Harlan LC, Morrell DM, et al. Ductal carcinoma in situ: trends in treatment over time in the US. Breast Cancer Res Treat. 2011;127(1):251-257.

# Policy History/Revision Information

| Date | Summary of Changes |
|---|---|
| 02/01/2025 | **Template Update**<br>• Reformatted and reorganized policy; transferred content to new template<br>• Changed policy type classification from "Policy Guideline" to "Medical Policy"<br>• Added *Clinical Evidence, FDA,* and *References* sections<br>• Updated *Instructions for Use*<br>**Related Policies**<br>• Added reference link to the UnitedHealthcare Commercial Medical Policy titled *Molecular Oncology Testing for Solid Tumor Cancer Diagnosis, Prognosis, and Treatment Decisions*<br>**Coverage Rationale**<br>• Removed content/language addressing:<br>  ○ Applicable test/assay descriptions<br>  ○ Documentation guidelines<br>  ○ A summary of nationally non-covered Indications and next generation sequencing (NGS)<br>***Guidelines***<br>• Revised language to indicate:<br>CMS National Coverage Determinations (NCDs)<br>  ○ For the tests in this policy that utilize the next generation sequencing technology, Medicare does not have a National Coverage Determination (NCD)<br>  ○ Next generation sequencing (NGS) (NCD 90.2) is applicable to diagnostic lab tests using NGS for somatic (acquired) and germline (inherited) cancer<br>  ○ For coverage guidelines of those tests, refer to next generation sequencing (NGS) (NCD 90.2)<br>  ○ Medicare Administrative Contractors (MACs) may determine coverage of diagnostic lab tests using NGS for RNA sequencing and protein analysis<br>CMS Local Coverage Determinations (LCDs) and Articles<br>  ○ Local Coverage Determinations (LCDs)/Local Coverage Articles (LCAs) exist for tests in this policy that utilize the Next Generation Sequencing technology and compliance with these policies is required where applicable<br>  ○ LCDs/LCAs also exist for molecular tests in this policy that do not utilize the next generation sequencing technology<br>  ○ For specific molecular testing LCDs/LCAs, refer to the table [in the *Centers for Medicare & Medicaid (CMS) Related Documents* section of the policy]<br>  ○ For coverage guidelines for states/territories with no LCDs/LCAs, refer to the coverage rationale [listed in the policy]<br>Covered Indications<br>*ABL1 (ABL Proto-Oncogene 1, Non-Receptor Tyrosine Kinase) Kinase Domain*<br>  ○ *ABL1* gene analysis, variants in the kinase domain is considered reasonable and necessary in patients with acute lymphoblastic leukemia (ALL) and chronic myeloid leukemia (CML) to guide therapeutic decision making<br>*ASXL1 (Additional Sex Combs Like 1, Transcriptional Regulator)*<br>  ○ *ASXL1* gene analysis is considered reasonable and necessary for prognosing patients with acute myeloid leukemia, myeloproliferative disease (MPD-essential thrombocytosis [ET], myelofibrosis & polycythemia vera [PV]), and myelodysplastic syndrome (MDS) |

*BDX-XL2 (Oncology Lung)*

- The BDX-XL2 test (Biodesix, Seattle, WA) is reasonable and necessary for the management of a lung nodule, between 8 and 30 mm in diameter, in patients 40 years or older and with a pre-test cancer risk (as assessed by the Mayo Clinic Model for Solitary Pulmonary Nodules) of 50% or less
- The intended use of the test is to assist physicians in the management of lung nodules by identifying those lung nodules with a high probability of being benign
- These lung nodules would then be candidates for non-invasive computed tomography (CT) surveillance instead of invasive procedures

*DetermaRx™ (Oncology Lung)*

- Molecular classifiers for non-small cell lung cancer (NSCLC) are considered reasonable and necessary when members meet all of the following criteria:
  - The patient has a non-squamous NSCLC with a tumor size < 5 cm, and there are no positive lymph nodes (i.e., American Joint Committee on Cancer (AJCC) Eighth Edition Stages I and IIa)
  - The patient is sufficiently healthy to tolerate chemotherapy
  - Adjuvant platinum-containing chemotherapy is being considered for the patient
  - The test is ordered by a physician who is treating the patient for NSCLC (generally a medical oncologist, surgeon, or radiation oncologist) to help in the decision of whether or not to recommend adjuvant chemotherapy

*Genomic Prostate Score® (GPS) Test (Previously Oncotype DX® Genomic Prostate Score)*

- The Genomic Prostate Score® (GPS) Test [previously Oncotype DX® Genomic Prostate Score (Genomic Health®)] is reasonable and necessary for use in very low risk, low risk, and favorable intermediate risk prostate cancer

*JAK2 (Janus Kinase 2) and MPL (MPL Proto-Oncogene, Thrombopoietin Receptor) (Myeloproliferative Disorders)*

- Genetic testing of *JAK2* exon 12 performed to identify polycythemia vera (PV) is reasonable and necessary when the following criteria are met:
  - Genetic testing impacts medical management
  - Patient would meet WHO's diagnostic criteria for PV, if *JAK2* exon 12 testing were positive
  - *JAK2 V617F* mutation analysis was previously completed and was negative
- Genetic testing of the *MPL* gene is reasonable and necessary when the following criteria are met:
  - Genetic testing impacts medical management
  - *JAK2 V617F* mutation analysis was previously completed and negative
  - Patient would meet WHO's diagnostic criteria for MPD (i.e., ET, MPF) if a clonal marker were identified

*KIT (V-Kit Hardy-Zuckerman 4 Feline Sarcoma Viral Oncogene Homolog)*

- KIT gene analysis is considered reasonable and necessary in patients who have gastrointestinal stromal tumor (GIST), acute myeloid leukemia (AML), melanoma, and myeloproliferative disease (MPD-essential thrombocytosis [ET], myelofibrosis & polycythemia vera [PV]) to guide therapeutic decision making

*MyPath Melanoma*

- The purpose of this test is to assist dermatopathologists to arrive at the correct diagnosis of melanoma versus non-melanoma when examining skin biopsies
- Molecular deoxyribonucleic acid (DNA)/ribonucleic acid (RNA) assays that aid in the diagnosis or exclusion of melanoma from a biopsy are reasonable and necessary when **all** of the following clinical conditions are met:
  - The test is ordered by a board-certified or board-eligible dermatopathologist
  - The specimen is a primary (non-metastatic, non-re-excision specimen) cutaneous melanocytic neoplasm for which the diagnosis is equivocal/uncertain (i.e., clear distinction between benign or malignant cannot be achieved using clinical and/or histopathological features alone) despite the performance of standard-of-care test procedures and relevant ancillary tests (i.e., immunohistochemical stains)
  - The specimen includes an area representative of the lesion or portion of the lesion that is suspicious for malignancy

| Date | Summary of Changes |
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| | ▪ The patient may be subjected to additional intervention, such as re-excision and/or sentinel lymph node biopsy, as a result of the diagnostic uncertainty<br>▪ The patient has not been tested with the same or similar assay for the same clinical indication<br>▪ The test is validated for use in the intended-use population and is performed according to its stated intended-use<br>*Oncotype DX® Breast Cancer Assay (Oncology Breast mRNA)*<br>o Oncology (breast), mRNA, gene expression profiling by real-time reverse transcription polymerase chain reaction (RT-PCR) of 21 genes, utilizing formalin-fixed paraffin embedded tissue, algorithm reported as recurrence score is considered reasonable and necessary to guide therapeutic decision-making in patients with the following findings:<br>▪ Estrogen-receptor positive, node-negative carcinoma of the breast<br>▪ Estrogen-receptor positive micrometastases of carcinoma of the breast<br>▪ Estrogen-receptor positive breast carcinoma with 1-3 positive nodes<br>*Oncotype DX® Breast DCIS Score™ Test (Ductal Carcinoma in Situ)*<br>o The Oncotype DX® DCIS assay (Genomic Health, Inc., Redwood City, CA) is reasonable and necessary for women diagnosed with DCIS who are planning on having breast conserving surgery and considering adjuvant radiation therapy<br>*PDGFRA (Platelet-Derived Growth Factor Receptor, Alpha Polypeptide)*<br>o *PDGFRA* gene analysis is considered reasonable and necessary in patients with PDGFRA-associated chronic eosinophilic leukemia or GIST caused by mutations in the *PDGFRA* gene to guide therapeutic decision making<br>*Pigmented Lesion Assay (PLA) (Oncology Melanoma)*<br>o The PLA is reasonable and necessary for use on melanocytic skin lesions with one or more clinical or historical characteristics suggestive of melanoma, including one or more ABCDE criteria when a clinician trained in the clinical diagnosis of skin cancer is considering the need for biopsy to rule out melanoma<br>o The PLA should not be used on clinically obvious melanoma. The PLA result is one element of the overall clinical assessment and should be used in combination with clinical and historical signs of melanoma to obtain additional information prior to a decision to biopsy<br>*PIK3CA (Phosphatidylinositol-4, 5-Biphosphate 3-Kinase, Catalytic Subunit Alpha)*<br>o The U.S. Food and Drug Administration (FDA) has approved Piqray (alpelisib) tablets, to be used in combination with the (FDA)-approved endocrine therapy fulvestrant, to treat postmenopausal women, and men, with hormone receptor (HR)-positive, human epidermal growth factor receptor 2 (HER2)-negative, *PIK3CA*-mutated, advanced, or metastatic breast cancer (as detected by an FDA-approved test) following progression on or after an endocrine-based regimen; *PIK3CA* testing is reasonable and necessary for this indication<br>o In addition to utilizing the coverage rationale referenced above in states/territories with no LCDs/LCAs, UnitedHealthcare also uses the criteria above to supplement the general Medicare criteria within the NGS jurisdiction regarding when *PIK3CA* testing is reasonable and necessary; UnitedHealthcare uses the criteria noted above in order to ensure consistency in reviewing the conditions to be met for coverage of *PIK3CA* testing<br>o Use of these criteria to supplement the coverage criteria noted above provides clinical benefits by identifying *PIK3CA*-mutated breast cancer, which has shown a clinical benefit in individuals taking alpelisib with fulvestrant for treatment of HR+, HER2-, *PIK3CA*-mutant advanced breast cancer after CDK4/6i treatment; specifically, there was a 7.9-month numeric improvement in median overall survival when alpelisib was added to fulvestrant treatment of individuals with *PIK3CA*-mutated, HR+, HER2- advanced breast cancer<br>o The added criteria will also provide numerous clinical benefits by guiding the treatment plan and medication regimen for this specific type of breast cancer<br>o The potential clinical harms of using these criteria may include denying claims in the NGS jurisdiction since there are no concrete clinical guidelines; however, with no specific clinical guidelines for this test, claims may be inappropriately allowed for indications other than the FDA approved indications for Piqray<br>o The clinical benefits of using these criteria are highly likely to outweigh any clinical harms because the criteria will ensure this test is being used when reasonable and necessary, based on the clinical studies shown in this policy including a study of individuals with PIK3CA-mutant disease with prior CDK4/6i plus hormone therapy |

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| | ○ In postmatching and unadjusted results, primary and secondary endpoints were in favor of treatment with alpelisib with fulvestrant over standard treatments<br>○ For other indications such as colorectal cancer, prostate cancer, and borderline ovarian tumors, there is insufficient evidence to support PIK3CA testing<br>*TERT (Telomerase Reverse Transcriptase)*<br>○ *TERT* gene analysis is considered reasonable and necessary in patients with malignant neoplasm of the brain<br>*Thyroseq®, ThyGeNEXT®, ThyraMIR™, and Afirma®*<br>○ ThyroSeq® is a test utilized to better define the need for thyroid surgery and the type of such surgery<br>○ ThyraMIRTM is used as a companion test to ThyGeNEXT® when ThyGeNEXT® results are inconclusive<br>○ ThyroSeq®, ThyraMIRTM, ThyGeNEXT® and Afirma® services are reasonable and necessary for patients with any of the following conditions:<br>▪ An indeterminate pathology on fine needle aspiration<br>▪ Patients with one or more thyroid nodules with a history or characteristics suggesting malignancy such as:<br>– Nodule growth over time<br>– Family history of thyroid cancer<br>– Hoarseness, difficulty swallowing or breathing<br>– History of exposure to ionizing radiation<br>– Hard nodule compared with rest of gland consistency<br>– Presence of cervical adenopathy<br>*UroVysion™ Bladder Cancer Kit*<br>○ The UroVysion™ Bladder Cancer Kit is reasonable and necessary when performed on urine specimens from persons with hematuria suspected of having bladder cancer as an aid for initial diagnosis of bladder carcinoma and subsequent monitoring for tumor recurrence in patient previously diagnosed with bladder cancer<br>○ To date, the UroVysion™ Bladder Cancer Kit is the only FDA-approved assay that is designed to detect aneuploidy for chromosomes 3, 7, and 17, and loss of the 9p21 locus via FISH<br>Non-Covered Indications<br>○ Molecular pathology tests for diseases or conditions that manifest severe signs or symptoms in newborns and in early childhood or that result in early death (e.g., Canavan disease) are not reasonable and necessary since these tests are generally not relevant to a Medicare member<br>○ The following types of genetic tests are examples of services that are not relevant to a Medicare member, are not considered a Medicare benefit (statutorily excluded), and therefore will be denied as Medicare excluded tests:<br>▪ Tests considered screening in the absence of clinical signs and symptoms of disease that are not specifically identified by the law<br>▪ Tests performed to determine carrier screening<br>▪ Prenatal diagnostic testing<br>▪ Tests performed on patients without signs or symptoms to determine risk for developing a disease or condition<br>▪ Tests without diagnosis specific indications<br>▪ Screening services such as pre-symptomatic genetic tests and services used to detect an undiagnosed disease or disease predisposition are not a Medicare benefit and are not covered<br>○ In accordance with the *Code of Federal Regulations, Title 42, Subchapter B, Part 410, Section 410.32*, the referring/ordering practitioner must have an established relationship with the patient, and the test results must be used by the ordering/referring practitioner in the management of the patient's specific medical problem<br>○ Title XVIII of the Social Security Act, Section 1862(a)(1)(A) states " ...no Medicare payment shall be made for items or services which are not reasonable and necessary for the diagnosis and treatment of illness or injury..."<br>○ Therefore, the following tests will be denied: |

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| | ▪ AFF2 (ALF transcription elongation factor 2 [FMR2]) (e.g., fragile X intellectual disability 2 [FRAXE])<br>▪ AR (androgen receptor) (e.g., spinal, and bulbar muscular atrophy, Kennedy disease, X chromosome inactivation)<br>▪ Ashkenazi Jewish Associated Disorders Carrier Screening Panel (e.g., Bloom syndrome, Canavan disease, cystic fibrosis, familial dysautonomia, Fanconi anemia group C, Gaucher disease, Tay-Sachs disease)<br>▪ ASPA (aspartoacylase) (e.g., Canavan disease)<br>▪ BCKDHB (branched-chain keto acid dehydrogenase E1, beta polypeptide) (e.g., Maple syrup urine disease)<br>▪ Cytogenomic (Genome-Wide) Analysis for Constitutional Chromosomal Abnormalities<br>▪ DMPK (DM1 protein kinase) (e.g., myotonic dystrophy type 1)<br>▪ F9 (coagulation factor IX) (e.g., hemophilia B)<br>▪ FANCC (Fanconi anemia, complementation group C) (e.g., Fanconi anemia, type C)<br>▪ Fetal Chromosomal Aneuploidy (e.g., trisomy 21, 18, and 13, monosomy X)<br>▪ Fetal Chromosomal Microdeletions (e.g., DiGeorge syndrome, Cri-du-chat syndrome)<br>▪ FMR1 (fragile X messenger ribonucleoprotein 1) (e.g., fragile X syndrome, X-linked intellectual disability [XLID])<br>▪ FXN (frataxin) (e.g., Friedreich ataxia)<br>▪ G6PD (glucose-6-phosphate dehydrogenase) (e.g., hemolytic anemia, jaundice)<br>▪ Genetic Testing for Severe Inherited Conditions Carrier Screening Panel (e.g., cystic fibrosis, Ashkenazi Jewish - associated disorders [e.g., Bloom syndrome, Canavan disease, Fanconi anemia type C, mucolipidosis type VI, Gaucher disease, Tay-Sachs disease], beta hemoglobinopathies, phenylketonuria, galactosemia)<br>▪ Genome Sequencing (e.g., unexplained constitutional or heritable disorder or syndrome)<br>▪ HBA1/HBA2 (alpha globin 1 and alpha globin 2) (e.g., alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease)<br>▪ HBB (hemoglobin, subunit beta) (e.g., sickle cell anemia, beta thalassemia, hemoglobinopathy)<br>▪ Hearing Loss Panel (e.g., non-syndromic hearing loss, Usher syndrome, Pendred syndrome)<br>▪ Hereditary Peripheral Neuropathies Panel (e.g., Charcot-Marie-Tooth, spastic paraplegia)<br>▪ Hereditary Retinal Disorders Panel (e.g., retinitis pigmentosa, Leber congenital amaurosis, cone-rod dystrophy)<br>▪ HTT (huntingtin) (e.g., Huntington disease)<br>▪ IKBKAP (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein) (e.g., familial dysautonomia)<br>▪ MCOLN1 (mucolipin 1) (e.g., Mucolipidosis, type IV)<br>▪ MECP2 (methyl CpG binding protein 2) (e.g., Rett syndrome)<br>▪ Nuclear Encoded Mitochondrial Genes Panel (e.g., neurologic or myopathic phenotypes)<br>▪ PMP22 (peripheral myelin protein 22) (e.g., Charcot-Marie-Tooth, hereditary neuropathy with liability to pressure palsies)<br>▪ SMN1 (survival of motor neuron 1, telomeric) (e.g., spinal muscular atrophy)<br>▪ SMPD1 (sphingomyelin phosphodiesterase 1, acid lysosomal) (e.g., Niemann-Pick disease, Type A)<br>▪ SNRPN/UBE3A (small nuclear ribonucleoprotein polypeptide N and ubiquitin protein ligase E3A) (e.g., Prader-Willi syndrome and/or Angelman syndrome)<br>▪ Whole Mitochondrial Genome (e.g., 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]), Kearns-Sayre syndrome, chronic progressive external ophthalmoplegia)<br>▪ X-linked Intellectual Disability (XLID) Panel (e.g., syndromic, and non-syndromic XLID)<br>○ In the instance where the tests above are used for symptomatic adults, testing is unlikely to impact therapeutic decision-making in the clinical management of the patient and therefore not reasonable and necessary |

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| | *DecisionDx®-SCC*<br>○ Current molecular biomarker tests that risk stratify individuals with cutaneous squamous cell carcinoma (cSCC) are not reasonable and necessary<br>○ Medicare does not have a NCD for DecisionDx®-SCC<br>○ LCDs/LCAs exist and compliance with these policies is required where applicable<br>○ For specific LCDs/LCAs, refer to the table [in the *Centers for Medicare & Medicaid (CMS) Related Documents* section of the policy]<br>○ For coverage guidelines for states/territories with no LCDs/LCAs, refer to the UnitedHealthcare Commercial Medical Policy titled *Molecular Oncology Testing for Solid Tumor Cancer Diagnosis, Prognosis, and Treatment Decisions*<br>*MTHFR (5,10-Methylenetetrahydrofolate Reductase)*<br>○ MTHFR genetic testing, which encodes the 5,10-methylenetetrahydrofolate reductase enzyme, for thrombophilia for all risk factors, signs, symptoms, diseases, or conditions, including cardiovascular risk assessment, is not reasonable and necessary<br>○ MTHFR is not considered to be clinically efficacious; therefore, testing is not reasonable and necessary<br>*OVERA® and ROMA™*<br>○ These multi marker serum tests related to ovarian cancer testing are not reasonable and necessary<br>*Resolution ctDX Lung™*<br>○ The Resolution ctDX Lung™ is not reasonable and necessary for non-small cell lung cancer (NSCLC)<br>**Applicable Codes**<br>***CPT Codes***<br>Non-Covered<br>• Added 0315U<br>• Revised description for 0003U and 0179U<br>• Removed 81506<br>Provisional Coverage<br>• Removed 0002M, 0002U, 0003M, 0004M, 0005U, 0006M, 0007M, 0009U, 0011M, 0012M, 0013M, 0015M, 0016M, 0016U, 0017M, 0017U, 0019M, 0019U, 0021U, 0022U, 0023U, 0036U, 0037U, 0040U, 0046U, 0048U, 0049U, 0050U, 0055U, 0060U, 0069U, 0079U, 0087U, 0088U, 0091U, 0092U, 0094U, 0095U, 0105U, 0111U, 0113U, 0114U, 0118U, 0120U, 0153U, 0154U, 0156U, 0157U, 0169U, 0170U, 0171U, 0172U, 0174U, 0177U, 0203U, 0204U, 0205U, 0206U, 0207U, 0209U, 0211U, 0212U, 0213U, 0214U, 0215U, 0216U, 0217U, 0218U, 0228U, 0229U, 0230U, 0231U, 0232U, 0233U, 0234U, 0235U, 0236U, 0239U, 0242U, 0244U, 0246U, 0249U, 0250U, 0253U, 0254U, 0258U, 0259U, 0260U, 0262U, 0263U, 0264U, 0265U, 0266U, 0267U, 0268U, 0269U, 0270U, 0271U, 0272U, 0273U, 0274U, 0276U, 0277U, 0278U, 0282U, 0285U, 0295U, 0296U, 0297U, 0298U, 0299U, 0300U, 0306U, 0307U, 0308U, 0309U, 0310U, 0312U, 0313U, 0314U, 0318U, 0319U, 0320U, 0322U, 0326U, 0327U, 0329U, 0331U, 0332U, 0333U, 0334U, 0335U, 0336U, 0339U, 0340U, 0341U, 0343U, 0344U, 0347U, 0348U, 0349U, 0350U, 0351U, 0355U, 0356U, 0359U, 0360U, 0362U, 0363U, 0364U, 0365U, 0366U, 0367U, 0375U, 0378U, 0379U, 0384U, 0385U, 0387U, 0388U, 0389U, 0391U, 0395U, 0396U, 0398U, 0400U, 0403U, 0405U, 0407U, 0409U, 0410U, 0413U, 0415U, 0417U, 0420U, 0421U, 0422U, 0424U, 0425U, 0426U, 0428U, 0433U, 0434U, 0436U, 0437U, 0438U, 81120, 81121, 81168, 81177, 81178, 81179, 81180, 81181, 81182, 81183, 81184, 81185, 81186, 81187, 81188, 81189, 81190, 81191, 81192, 81193, 81194, 81206, 81207, 81208, 81209, 81210, 81218, 81219, 81221, 81222, 81223, 81224, 81233, 81235, 81236, 81237, 81240, 81241, 81245, 81246, 81250, 81251, 81252, 81253, 81254, 81255, 81256, 81261, 81262, 81263, 81264, 81265, 81266, 81267, 81268, 81269, 81270, 81273, 81275, 81276, 81277, 81278, 81287, 81301, 81305, 81310, 81311, 81312, 81313, 81315, 81316, 81320, 81327, 81332, 81333, 81334, 81340, 81341, 81342, 81343, 81344, 81347, 81348, 81357, 81360, 81419, 81445, 81449, 81450, 81451, 81455, 81456, 81457, 81458, 81459, 81462, 81463, 81464, 81490, 81503, 81504, 81517, 81518, 81520, 81521, 81522, 81523, 81525, 81529, 81538, 81539, 81540, 81541, 81542, 81551, 81552, 81554, 81595, 86294, 86316, 86386, 88341, and 88342<br>• Reclassified/relocated 0315U (refer to list of *Non-Covered* codes) |

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| | ***HCPCS Codes***<br>• Removed G0452<br>***Diagnosis Codes***<br>• Removed C69.31, C69.32, C69.41, C69.42, C69.91, C69.92, C91.10, C91.11, C91.12, J84.10, J84.111, J84.112, J84.113, J84.116, J84.9, and R97.20<br>For CPT Code 81529<br>• Removed list of applicable codes: C43.0, C43.10, C43.111, C43.112, C43.121, C43.122, C43.20, C43.21, C43.22, C43.30, C43.31, C43.39, C43.4, C43.51, C43.52, C43.59, C43.60, C43.61, C43.62, C43.70, C43.71, C43.72, C43.8, and C43.9<br>For CPT Code 81542<br>• Removed list of applicable codes: C61<br>For CPT Codes 81175, 81176, 81279, 81338, 81339, and 0027U<br>• Added C88.80<br>• Added notation to indicate C88.8 was "deleted Sep. 30, 2024"<br>For CPT Codes 88120 and 88121<br>• Added E34.00, E34.01, and E34.09<br>• Added notation to indicate E34.0 was "deleted Sep. 30, 2024"<br>**Centers for Medicare & Medicaid (CMS) Related Documents**<br>• Updated list of documents available in the *Medicare Coverage Database* to reflect the most current information<br>• Added:<br>  ○ List of applicable *Medicare Administrative Contractors (MACs) With Corresponding States/Territories*<br>  ○ Notation to indicate:<br>  ▪ The Wisconsin Physicians Service Insurance Company (WPS) Contract Number 05901 applies only to WPS Legacy Mutual of Omaha MAC A Providers<br>  ▪ For the state of Virginia: Part B services for the city of Alexandria and the counties of Arlington and Fairfax are excluded for the Palmetto GBA jurisdiction and included within the Novitas Solutions, Inc. jurisdiction<br>  ○ Reference link to the:<br>  ▪ CMS Transmittal:<br>  – *12440, Change Request 13391, Dated 01/03/2024 (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determinations (NCDs)--April 2024 Update--CR 2 of 2)*<br>  – *12444, Change Request 13278, Dated 01/04/2024 (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determinations (NCDs)--January 2024 Update)*<br>  – *12626, Change Request 13596, Dated 05/09/2024 (International Classification of Diseases, 10th Revision (ICD-10) and Other Coding Revisions to National Coverage Determinations (NCDs)--October 2024)*<br>  ▪ *Code of Federal Regulations,Title 42 §410.32 Diagnostic x-ray tests, diagnostic laboratory tests, and other diagnostic tests: Conditions*<br>  ▪ *Social Security Act, Title XVIII Section 1862(a)(1)(A)*<br>• Removed reference link to the *PreDx® Coding and Billing Guidelines (CM00040), CGS Website*<br>**Supporting Information**<br>• Archived previous policy version MPG210.29 |

# Instructions for Use

The Medicare Advantage Policy documents are generally used to support UnitedHealthcare coverage decisions. It is expected providers retain or have access to appropriate documentation when requested to support coverage. This document may be used as a guide to help determine applicable:

- Medical necessity coverage guidelines; including documentation requirements, and/or
- Medicare coding or billing requirements.

Medicare Advantage Policies are applicable to UnitedHealthcare Medicare Advantage Plans offered by UnitedHealthcare and its affiliates. This Policy is provided for informational purposes and does not constitute medical advice. It is intended to serve only as a general reference and is not intended to address every aspect of a clinical situation. Physicians and patients should not rely on this information in making health care decisions. Physicians and patients must exercise their independent clinical discretion and judgment in determining care. Treating physicians and healthcare providers are solely responsible for determining what care to provide to their patients. Members should always consult their physician before making any decisions about medical care.

Benefit coverage for health services is determined by the member specific benefit plan document and applicable laws that may require coverage for a specific service. The member specific benefit plan document identifies which services are covered, which are excluded, and which are subject to limitations. In the event of a conflict, the member specific benefit plan document supersedes this policy. For more information on a specific member's benefit coverage, please call the customer service number on the back of the member ID card or refer to the Administrative Guide.

Medicare Advantage Policies are developed as needed, are regularly reviewed, and updated, and are subject to change. They represent a portion of the resources used to support UnitedHealthcare coverage decision making. UnitedHealthcare may modify these Policies at any time by publishing a new version on this website. Medicare source materials used to develop these policies may include, but are not limited to, CMS statutes, regulations, National Coverage Determinations (NCDs), Local Coverage Determinations (LCDs), and manuals. This document is not a replacement for the Medicare source materials that outline Medicare coverage requirements. The information presented in this Policy is believed to be accurate and current as of the date of publication. Where there is a conflict between this document and Medicare source materials, the Medicare source materials apply. Medicare Advantage Policies are the property of UnitedHealthcare. Unauthorized copying, use, and distribution of this information are strictly prohibited.

UnitedHealthcare follows Medicare coverage guidelines found in statutes, regulations, NCDs, and LCDs to determine coverage. The clinical coverage criteria governing certain items or services referenced in this Medical Policy have not been fully established in applicable Medicare guidelines because there is an absence of any applicable Medicare statutes, regulations, NCDs, or LCDs setting forth coverage criteria and/or the applicable NCDs or LCDs include flexibility that explicitly allows for coverage in circumstances beyond the specific indications that are listed in an NCD or LCD. As a result, in these circumstances, UnitedHealthcare applies internal coverage criteria as referenced in this Medical Policy. The internal coverage criteria in this Medical Policy was developed through an evaluation of the current relevant clinical evidence in acceptable clinical literature and/or widely used treatment guidelines. UnitedHealthcare evaluated the evidence to determine whether it was of sufficient quality to support a finding that the items or services discussed in the policy might, under certain circumstances, be reasonable and necessary for the diagnosis or treatment of illness or injury or to improve the functioning of a malformed body member.

Providers are responsible for submission of accurate claims. Medicare Advantage Policies are intended to ensure that coverage decisions are made accurately. UnitedHealthcare Medicare Advantage Policies use Current Procedural Terminology (CPT®), Centers for Medicare and Medicaid Services (CMS), or other coding guidelines. References to CPT® or other sources are for definitional purposes only and do not imply any right to reimbursement or guarantee claims payment.

For members in UnitedHealthcare Medicare Advantage plans where a delegate manages utilization management and prior authorization requirements, the delegate’s requirements need to be followed.