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Medical Policy

Subject:	Whole Genome Sequencing, Whole Exome Sequencing, Gene Panels, and Molecular Profiling		
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Description/Scope

This document addresses several tests including:

- Gene panel testing (for the purposes of this document, a gene panel is defined by five or more genes or gene variants tested on the same day on the same member by the same rendering provider)
  - This includes use of circulating tumor DNA panel testing (liquid biopsy), for example, as an alternative to tissue biopsy in the diagnosis of cancer, for clinical response to targeted agents of cancer treatment, for early cancer detection (that is, screening) and/or for cancer surveillance.
- Whole genome sequencing
- Whole exome sequencing
- Molecular profiling (also called comprehensive genomic profiling), including use of circulating tumor DNA panel tests (liquid biopsy) for solid tumors
- Polygenic risk score testing
- Chromosome conformation signatures

Note: This document does not address tests that include 4 or fewer genes or gene mutation variants. Please refer to:

- CG-GENE-13 Genetic Testing for Inherited Diseases
- CG-GENE-14 Gene Mutation Testing for Cancer Susceptibility and Management

Note: This document does not address circulating tumor cell (CTC) testing. Please refer to:

- LAB.00015 Detection of Circulating Tumor Cells

Note: Please see the following related documents for additional information:

- CG-GENE-10 Chromosomal Microarray Analysis (CMA) for Developmental Delay, Autism Spectrum Disorder, Intellectual Disability and Congenital Anomalies
- ~~CG-GENE-13 Genetic Testing for Inherited Diseases~~
- ~~CG-GENE-14 Gene Mutation Testing for Cancer Susceptibility and Management~~

- CG-GENE-15 Genetic Testing for Lynch Syndrome, Familial Adenomatous Polyposis (FAP), Attenuated FAP and MYH-associated Polyposis
- CG-GENE-16 BRCA Genetic Testing
- CG-GENE-19 Measurable Residual Disease Assessment in Lymphoid Cancers Using Next Generation Sequencing
- GENE.00010 Panel and other Multi-Gene Testing for Polymorphisms to Determine Drug-Metabolizer Status
- ~~GENE.00049 Circulating Tumor DNA Panel Testing (Liquid Biopsy)~~

Position Statement

Medically Necessary:

Gene Panel Testing for Inherited Diseases

Gene panel testing for inherited diseases is considered **medically necessary** when criteria A or B below are met:

A. Hereditary retinal disorders:
   1. Individual has suspected inherited retinal degenerative disease; **and**
   2. Results of the panel are likely to guide treatment decisions.

B. Ashkenazi Jewish associated inherited disorders (both criteria 1 and 2):
   1. Either criteria a or b:
      a. Individual has suspected genetic disease associated with Ashkenazi Jewish descent; **or**
      b. As part of preconception or prenatal genetic screening of a parent or prospective parent to determine carrier status when the parent or prospective parent is of Ashkenazi Jewish descent; **and**
   2. Genetic counseling, which encompasses **all** of the following components, has been performed:
      a. Interpretation of family and medical histories to assess the probability of disease occurrence or recurrence; **and**
      b. Education about inheritance, genetic testing, disease management, prevention, and resources; **and**
      c. Counseling to promote informed choices and adaption to the risk or presence of a genetic condition; **and**
      d. Counseling for the psychological aspects of genetic testing.

~~Testing for hereditary retinal disorders using gene panels is considered **medically necessary** for an individual with a suspected inherited retinal degenerative disease when results of the panel are likely to guide treatment decisions.~~

~~Testing for Ashkenazi Jewish associated inherited disorders using gene panels is considered **medically necessary** for an individual with suspected genetic disease or as part of preconception or prenatal genetic screening of a parent or prospective parent to determine carrier status when the parent or prospective parent is of Ashkenazi Jewish descent and when genetic counseling, which encompasses **all** of the following components, has been performed:~~

~~1. Interpretation of family and medical histories to assess the probability of disease occurrence or recurrence; **and**~~
~~2. Education about inheritance, genetic testing, disease management, prevention and resources; **and**~~
~~3. Counseling to promote informed choices and adaptation to the risk or presence of a genetic condition; **and**~~
~~4. Counseling for the psychological aspects of genetic testing.~~

Gene Panel Testing for Cancer Susceptibility ~~and Management~~

Gene panel testing for cancer susceptibility is considered **medically necessary** when criteria A or B below are met:
A. Lynch Syndrome (Hereditary Non-Polyposis Colorectal Cancer [HNPCC]):
1. When the panel contains, at a minimum, the following genes: EPCAM, MLH1, MSH2, MSH6, and PMS2; **and**
2. Individual meets criteria for Lynch Syndrome genetic testing according to CG-GENE-15.

B. Breast Cancer Susceptibility:
1. When the panel contains, at a minimum, the following genes: ATM, BARD1, BRCA1, BRCA2, CHEK2, PALB2, RAD51C, and RAD51D; **and**
2. Individual meets criteria for BRCA genetic testing according to CG-GENE-16.

Gene Panel Testing for Cancer Management

Gene panel testing for cancer management is considered **medically necessary** when criteria A, B, C, D. E, or F below are met:
A. Prostate Cancer:
1. The panel evaluates homologous recombination repair (HRR) gene alterations; **and**
2. The individual is a candidate for treatment using a poly (ADP-ribose) polymerase (PARP) inhibitor.

B. Advanced Non-Small Cell Lung Cancer:
1. Prior to initiating first-line therapy; **and**
2. When the panel contains, at a minimum, the following genes (mutations, rearrangements, fusions, or amplifications): ALK, BRAF, EGFR, ERBB2 (HER2), KRAS, MET, NTRK, RET, and ROS1.

C. Myelodysplastic Syndromes (MDS):
1. For initial evaluation of MDS; **and**
2. When the panel contains, at a minimum, the following genes: ASXL1, DNMT3A, EZH2, NRAS, RUNX1, SF3B1, SRSF2, STAG2, TET2, TP53, U2AF1, ZRSR2.

D. Acute Myeloid Leukemia (AML):
1. For initial evaluation of newly diagnosed or relapsed AML; **and**
2. When the panel contains, at a minimum, the following genes: ASXL1, BCR-ABL, c-KIT, CEBPA (biallelic), FLT3-ITD, FLT3-TKD, IDH1, IDH2, NPM1, PML-RAR alpha, RUNX1, and TP53.

E. Acute Lymphoblastic Leukemia (ALL):
1. For initial evaluation of ALL; **and**

2. When the panel contains, at a minimum, the following genes: ABL1, ABL2, CRLF2, CSF1R, FLT3, IL7R, JAK1, JAK2, JAK3, PDGFRB, and SH2B3.

F. In Vitro Companion Diagnostic Device (IVD)

1. When the test is an IVD and is used in accordance with the U.S. Food and Drug Administration (FDA) labeled indication.
   Link to the up-to-date list of FDA cleared or approved Companion Diagnostic Devices: https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools

Circulating Tumor DNA (ctDNA) Panel Testing

Use of a ctDNA panel test is considered **medically necessary** when criteria A, B, or C below are met:

A. Prostate Cancer:

1. The panel evaluates HRR gene alterations; **and**
2. The individual is a candidate for treatment using a PARP inhibitor; **and**
3. Formalin-fixed paraffin-embedded tumor tissue (FFPET) is inadequate in quality or quantity or is unavailable for testing.

B. Advanced Non-Small Cell Lung Cancer:

1. Prior to initiating first-line therapy; **and**
2. When the panel contains, at minimum, the following genes (mutations, rearrangements, fusions, or amplifications): ALK, BRAF, EGFR, ERBB2 (HER2), KRAS, MET, NTRK, RET, and ROS1; **and**
3. FFPET tissue is inadequate in quality or quantity or is unavailable for testing.

C. In Vitro Companion Diagnostic Device (IVD)

1. When the test is an IVD and is used in accordance with the U.S. Food and Drug Administration (FDA) labeled indication.
   Link to the up-to-date list of FDA cleared or approved Companion Diagnostic Devices: https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools

~~Lynch Syndrome: Testing for Lynch syndrome (Hereditary Non-Polyposis Colorectal Cancer) using gene panels (containing 5-50 genes) is considered **medically necessary** when the panel contains, at a minimum, the following genes: EPCAM, MLH1, MSH2, MSH6, and PMS2, and an individual meets criteria for Lynch syndrome (Hereditary Non-Polyposis Colorectal Cancer [HNPCC]) genetic testing according to CG-GENE-15.~~

~~Breast Cancer Susceptibility: Testing for breast cancer susceptibility using gene panels (containing 5-50 genes) is considered **medically necessary** when the panel contains, at a minimum, the following genes: ATM, BARD1, BRCA1, BRCA2, CHEK2, PALB2, RAD51C, and RAD51D, and an individual meets criteria for BRCA genetic testing according to CG-GENE-16.~~

~~Prostate Cancer: Testing for prostate cancer using gene panels is **medically necessary** when the criteria below are met:~~

~~1. The panel evaluates homologous recombination repair (HRR) gene alterations; **and**~~
~~2. The individual is a candidate for treatment using a poly (ADP-ribose)polymerase (PARP) inhibitor.~~
~~**Note:** The test should be performed using tumor tissue (not cell-free circulating tumor DNA, also known as liquid biopsy).~~

~~Advanced Non-Small Cell Lung Cancer: Testing for advanced non-small cell lung cancer using gene panels (containing 5-50 genes) is considered **medically necessary** prior to initiating first-line therapy when the panel contains, at minimum, the following genes (mutations, rearrangements, fusions, or amplifications): ALK, BRAF, EGFR, ERBB2 (HER2), KRAS, MET, NTRK, RET, and ROS1.~~
~~**Note:** The test should be performed using tumor tissue (not cell-free circulating tumor DNA, also known as liquid biopsy). For criteria relating to use of circulating tumor DNA panel testing, see GENE.00049.~~

~~Myelodysplastic Syndromes: Testing for initial evaluation of myelodysplastic syndromes (MDS) using gene panels (containing 5-50 genes) is considered **medically necessary** when the panel contains, at a minimum, the following genes: ASXL1, DNMT3A, EZH2, NRAS, RUNX1, SF3B1, SRSF2, STAG2, TET2, TP53, U2AF1, ZRSR2.~~

~~Acute Myeloid Leukemia: Testing for initial evaluation of acute myeloid leukemia (AML) using gene panels (containing 5-50 genes) is considered **medically necessary** when the panel contains, at a minimum, the following genes: ASXL1, BCR-ABL, c-KIT, CEBPA (biallelic), FLT3-ITD, FLT3-TKD, IDH1, IDH2, NPM1, PML-RAR alpha, RUNX1, and TP53.~~

~~Acute Lymphoblastic Leukemia: Testing for initial evaluation of acute lymphoblastic leukemia (ALL) using gene panels (containing 5-50 genes) is considered **medically necessary** when the panel contains, at a minimum, the following genes: ABL1, ABL2, CRLF2, CSF1R, FLT3, IL7R, JAK1, JAK2, JAK3, PDGFRB, and SH2B3.~~

Whole Exome Sequencing (WES)

Whole exome sequencing is considered **medically necessary** in the evaluation of an individual who meets **all** of the following criteria A~~1~~, ~~2~~B, and C~~3~~:

A. Meets one of the following criteria:
    1. Multiple anomalies not specific to a well-delineated genetic syndrome apparent before 1 year of age; **or**
    2. Apparently non-syndromic developmental delay/intellectual disability with onset prior to 18 years of age; **or**
    3. For the evaluation of a live fetus with abnormal fetal anatomic findings which are characteristic of a genetic abnormality; **and**
B. When the results of testing would confirm or establish a clinical diagnosis that may lead to changes in management; **and**
C. Genetic counseling, which encompasses all of the following components, has been performed:
    1. Interpretation of family and medical histories to assess the probability of disease occurrence or recurrence; **and**

2. Education about inheritance, genetic testing, disease management, prevention and resources; **and**
3. Counseling to promote informed choices and adaptation to the risk or presence of a genetic condition; **and**
4. Counseling for the psychological aspects of genetic testing.

Note: WES may include comparator WES testing of the biologic parents or sibling of the affected individual.

Molecular Profiling for the Evaluation of Malignancies

Molecular profiling is considered **medically necessary** ~~for unresectable or metastatic solid tumors~~ when all of the criteria below are met:

A. The individual has an unresectable or metastatic solid tumor; **and**
B. The test is used to assess tumor mutation burden (TMB)~~and identify candidates for checkpoint inhibition immunotherapy~~; **and**
C. The test is used to identify candidates for checkpoint inhibition immunotherapy; **and**
~~A.~~
~~B. Individual has progressed following prior treatment; **and**~~
~~C.~~D. The ~~I~~individual has no satisfactory alternative treatment options.

~~**Note:** The test should be performed using tumor tissue (not cell-free circulating tumor DNA, also known as liquid biopsy).~~

Molecular Profiling using a *circulating tumor (ctDNA)* test is considered **medically necessary** when all of the criteria below are met:

A. The individual has an unresectable or metastatic solid tumor; **and**
B. The test is used to assess TMB; **and**
C. The test is used to identify candidates for checkpoint inhibition immunotherapy; **and**
D. The individual has no satisfactory alternative treatment options; **and**
E. FFPET tissue is inadequate in quality or quantity or is unavailable for testing.

~~*Panel Testing*C tissue tissue~~
~~In Vitro Companion Diagnostic Device (IVD)~~

Not Medically Necessary:

Testing using gene panels is considered **not medically necessary** for all other indications, including when the medically necessary criteria above have not been met.

Testing using circulating tumor DNA panels is considered **not medically necessary** for all other indications, including when the medically necessary criteria above have not been met.

Testing using w~~W~~hole exome sequencing is considered **not medically necessary** for all other indications, including when the medically necessary criteria above have not been met, and for repeat sequencing.

Investigational and Not Medically Necessary:

Whole genome sequencing is considered **investigational and not medically necessary** for all indications**.**

Molecular profiling is considered **investigational and not medically necessary** for all other indications, including when the medically necessary criteria above have not been met.

Polygenic risk score testing is considered **investigational and not medically necessary** for all indications.

Chromosome conformation signature testing is considered **investigational and not medically necessary** for all indications.

Rationale

Gene Panel Testing for Inherited Diseases
The 2022~~12~~ American Academy of Ophthalmology (AAO) Clinical Statement for Inherited Retinal Degenerations recommends genetic testing be ordered at the initial visit for individuals with a suspected inherited retinal degenerative disease. The causative mutation can be identified in up to 60-80% of affected individuals, which can guide treatment decisions. The scope of genetic testing recommended varies, multi-gene testing may be necessary when there are multiple causative genes, while single gene analysis might be more appropriate for certain conditions. For diseases such as Leber congenital amaurosis (LCA), which is caused by multiple different genes, it can be more efficient to order a single test which has been designed to specifically evaluate for all of the known causative genes (AAO, 2022~~Stone, 2012~~).

Advances in genetic testing technologies have led to the development and use of large-scale DNA sequencing, including but not limited to expanded carrier panels. Generally, carrier screening guidelines have focused on the assessment of individual conditions and ancestry. However, the effectiveness of this approach can be impacted by limited or inaccurate knowledge of ancestry and an increasingly multiethnic society. Approaches to screening have also been influenced by the recognition that while some genetic conditions occur more frequently in certain populations, genetic disorders are not limited to specific ethnic groups (Edwards, 2015).

Due to limited knowledge about ancestry, individuals may be unaware of their reproductive risk of transmitting disorders to offspring. Expanded carrier screening panels may lead to prevention of disease in offspring or avoidance of unnecessary treatments. However, currently there is no data which demonstrates improved reproductive outcome. There is also no uniform or standardized process for best practice.

According to the American College of Medical Genetics (ACMG):

> The completion of the full human genome sequence, followed by dramatic improvement in the speed and cost of DNA sequencing and microarray hybridization analysis, has enabled the ascertainment of an unprecedented quantity of disease-specific genetic variants in a time frame suited to prenatal/preconception screening and diagnosis. Now it is possible, using new technologies, to screen for mutations in many genes for approximately the same cost as previously required to detect mutations in a single gene or a relatively small number of population-specific mutations in several genes. Commercial laboratories have begun to offer such expanded carrier screening panels to physicians and the public, but there has been no professional guidance on which disease genes and mutations to include (Grody, 2013).

The American College of Medical Genetics recommend carrier screening in individuals of Ashkenazi Jewish descent (Gross, 2008).

Genetic testing for cardiac ion channel mutations in persons with suspected channelopathies, such as long QT syndrome (LQTS) or hereditary cardiomyopathies, including hypertrophic cardiomyopathy is complicated by varying penetrance and genotype-phenotypic profiles. Testing often seeks to permit cascade screening of families; however, genetic testing using panels is not appropriate for an individual when a genetic mutation with strong evidence for pathogenicity has already been identified in a first-degree relative (proband) with a clinical diagnosis. Data supporting the clinical utility of gene panel testing for LQTS or hereditary cardiomyopathies is limited. Furthermore, a substantial proportion of individuals with hereditary cardiomyopathies or LQTS may have a variant of uncertain significance identified if a genetic testing panel is used.

Gene Panel Testing for Cancer Susceptibility and Management
Until recently, genetic testing for cancer susceptibility was generally carried out by direct sequencing (Sanger) which analyzes a specific gene for a particular mutation. However, next generation sequencing, (including but not limited to massively parallel sequencing and microarray testing) has made it possible to conduct panel testing which involves the analysis of multiple genes for multiple mutations simultaneously. Panel testing has the potential benefit of analyzing multiple genes more rapidly and thereby providing the results of the genetic work-up in a more timely fashion. However, the newer sequencing techniques may be associated with a higher error rate and lower diagnostic accuracy than direct sequencing which could affect the clinical validity of testing. Another potential drawback of the newer technologies is that they may provide information on genetic mutations which is of uncertain clinical significance. In assessing the value of a specific genetic testing panel for susceptibility to a particular malignant condition, consideration should be given to the peer-reviewed, published literature addressing the analytical validity, clinical validity, and clinical utility of the test. Evidence demonstrating a positive impact of the panel on the care of individuals with, or at risk for, a specific cancer should be considered. Use of gene panels is considered in accordance with generally accepted standards of medical practice to assess individuals at risk for Lynch syndrome (hereditary non-polyposis colorectal cancer) and breast cancer, and to evaluate certain individuals with prostate cancer (testing for homologous recombination repair [HRR] gene alterations), advanced non-small cell lung cancer, myelodysplastic syndrome, acute myeloid leukemia, and acute lymphoblastic leukemia.

The American Society of Clinical Oncology (ASCO) last issued a policy statement update regarding genetic and genomic testing for cancer susceptibility in 2015. The findings and conclusions regarding the state of the technology are summarized as follows:

- ASCO recognizes that concurrent multigene testing (i.e., panel testing) may be efficient in circumstances that require evaluation of multiple high-penetrance genes of established clinical utility as possible explanations for a patient's personal or family history of cancer. Depending on the specific genes included on the panel employed, panel testing may also identify mutations in genes associated with moderate or low cancer risks and mutations in high-penetrance genes that would not have been evaluated on the basis of the presenting personal or family history. Multigene panel testing will also identify variants of uncertain significance (VUSs) in a substantial proportion of patient cases, simply as a result of the multiplicity of genes tested. ASCO affirms that it is sufficient for cancer risk assessment to evaluate genes of established clinical utility that are suggested by the patient's personal and/or family history. Because of the current uncertainties and knowledge gaps, providers with particular expertise in cancer risk assessment should be involved in the ordering and interpretation of multigene panels that include genes of uncertain clinical utility and genes not suggested by the patient's personal and/or family history.
- All of the challenges described here raise the possibility of harm to the individual undergoing panel-based testing, including the potential for inappropriate medical intervention and psychological stress resulting from the incidental identification of a mutation in a gene that was not suggested by family history or from aggressive management of moderate-penetrance mutations (or VUSs) that is not yet supported by evidence.
- There remains an urgent need for more research into the implications of unexpected mutations in high-penetrance genes and mutations in moderate-penetrance genes. Continued research is also necessary to resolve VUSs. ASCO recognizes the complexity of the analysis and interpretation of genetic tests. ASCO supports high-quality standards to help providers and patients understand the accuracy, benefits, and limitation of genetic tests from individual laboratories. ASCO believes that current regulation of tests to detect inherited genetic variants is insufficient. Where tests are considered laboratory-developed or commercial tests, ASCO supports a risk-based approach to US Food and Drug Administration (FDA) regulation. High-risk tests used to identify patients who are at increased risk for cancer should be subject to regulatory review. ASCO also recognizes that regulation must be designed in a manner that does not compromise innovation or limit patient access to testing.

- ASCO supports the development of a rapid approval pathway for tests that address an unmet medical need, with the understanding that more than one test should be available before such a need is considered to have been met (Robson, 2015).

Colorectal Cancer Susceptibility
Various laboratories offer next-generation sequencing panels (including but not limited to massively parallel sequencing, and microarray testing), making it possible to conduct panel testing which involves the analysis of multiple genes for multiple mutations simultaneously. The ColoNext™ test (manufactured by Ambry Genetics), which tests for variants in 17 genes, is one such example. Of the 17 genes tested, 12 are considered by the 2023 National Comprehensive Cancer Network© (NCCN) guideline on genetic/familial high-risk assessment for colorectal cancer to have well-established evidence of association with colorectal risk. The guideline notes that evidence is well-established for the following colorectal genes that are commonly included in gene panels: APC, BMPR1A, EPCAM, MLH1, MSH2, MSH6, MUTYH biallelic pathogenic variants, PMS2, PTEN, SMAD4, STK11 and TP53.

Lynch syndrome is an autosomal dominant disorder that is caused by a germline mutation in one of several DNA mismatch repair genes or loss of expression of MSH2 due to deletion in the EPCAM gene (previously called TACSTD1). The mismatch repair (MMR) genes that are associated with Lynch syndrome include:

- MLH1 (MutL homolog 1), which is located on chromosome 3p22.2
- MSH2 (MutS homolog 2), which is located on chromosome 2p21-16
- MSH6 (MutS homolog 6), which is located on chromosome 2p16.3
- PMS2 (postmeiotic segregation 2), which are located on chromosome 7p22.1

The 2023 NCCN guideline on genetic/familial high-risk assessment for colorectal cancer recommends that testing for Lynch syndrome (EPCAM, MLH1, MSH2, MSH6, and PMS2 sequence analysis) includes individuals who meet the Bethesda guidelines, the Amsterdam II criteria, who have a cancer diagnosis prior to age 50, or have a predicted risk for Lynch syndrome greater than 5% on one of the following prediction models: MMRpredict, MMRpro or PREMM5. Use of targeted gene panels ~~(containing 5-50 genes)~~ that include EPCAM, MLH1, MSH2, MSH6, and PMS2 is considered in accordance with generally accepted standards of medical practice.

Breast Cancer Susceptibility
Multi-gene testing for hereditary forms of cancer can analyze a set of genes which are associated with a specific family cancer type. Multi-gene panel testing can impact medical management and can provide an association for prediction of risk of breast cancer. However, not all genes tested show a strong association for breast cancer. It's important to define which genes are most useful clinically as not all genes available on multi-gene tests will change risk management based on other risk factors such as family history.

In the 2023 ~~National Comprehensive Cancer Network®~~ NCCN guidelines ~~Clinical Practice Guidelines in Oncology (NCCN Guidelines®)~~ for genetic/familial high-risk assessment: breast, ovarian, and pancreatic, recommendations

are made for genetic panel testing using these genes ATM, BARD1, BRCA1, BRCA2, CHEK2, PALB2, and CDH1.

Study among cancer susceptibility genes and breast cancer risk continues. Two case-control studies have been published which analyzed various genes which are susceptible for breast cancer risk. A 2021 study by Dorling and colleagues looked at a panel of 34 susceptible genes from samples of 60,466 individuals with breast cancer and 53,461 controls from 25 countries. The objective was the estimated odds ratios for breast cancer overall and tumor subtypes. Using the Cancer Risk Estimates Related to Susceptibility (CARRIERS) population-based studies of breast cancer in the United States, Hu and colleagues (2021) reported on 17 studies and analyzed 28 genes (predisposed to cancer) in 32,247 participants (case group) with breast cancer compared to 32,544 unaffected participants (control group). The objective was the association between variants in each gene and risk of breast cancer. Significant associations between breast cancer and variants in 8 genes: ATM, BARD1, BRCA1, BRCA2, CHEK2, PALB2, RAD51C, and RAD51D were found in both studies. Of note, several genes regarded as having strong evidence of an association with breast cancer risk, for example, CDH1, PTEN, STK11, and TP53, are very rare and did not show a significant association, presumably given their low prevalence. The majority of mutations among case subjects were BRCA1, BRCA2, and PALB2, and among controls, CHEK2 and ATM, reflecting the higher and lower penetrance of the genes respectively. BRCA1, BRCA2, and PALB2 are associated with a high risk of breast cancer (with odds ratios ranging from 5.0 to 10.6 in the study by Dorling et al.), and mutations in CHEK2 and ATM are associated with a moderate risk (with odds ratios ranging from 2.1 to 2.5). Use of targeted gene panels ~~(containing 5-50 genes~~) is considered in accordance with generally accepted standards of medical practice.

Management of HPV-related Cancers
Human papillomavirus (HPV) infection is a sexually transmitted virus that is associated with condyloma acuminatum, squamous intraepithelial lesions, as well as malignancy, including anogenital malignancies (cervical, vaginal, vulval, penile, and anal carcinoma) and oropharyngeal squamous cell carcinoma (OPSCC) of the head and neck. HPV-associated head and neck cancers occur primarily in the tonsils, soft palate, or base of tongue.

The NavDx test is a commercially available circulating tumor HPV DNA (ctHPVDNA) test designed to aid in the detection of HPV-related cancer. Chera and colleagues (2020) reported the results of a prospective study in which 115 participants with nonmetastatic HPV-associated (p16-positive) OPSCC were treated with definitive chemoradiation therapy (CRT). The participants underwent a 3-month post-CRT positron emission tomography (PET)/computed tomography (CT) scan and were thereafter clinically evaluated every 2-4 months (years 1-2), then every 6 months (years 3-5). Chest imaging was carried out every 6 months. Blood specimens were drawn every 6-9 months for analysis of plasma ctHPVDNA using a multianalyte digital polymerase chain reaction assay. The primary endpoint was to estimate the positive predictive value (PPV) and negative predictive value (NPV) of ctHPVDNA surveillance. After a median follow-up time of 23 months (range, 6.1-54.7 months), 15 subjects (13%) developed disease recurrence. Eighty-seven participants had undetectable ctHPVDNA at all post-treatment time points, and none developed recurrence (NPV, 100%; 95% CI, 96% to 100%). A total of 28 subjects developed a positive ctHPVDNA during post-treatment surveillance, 15 of whom were diagnosed with biopsy-proven recurrence. Sixteen subjects had two consecutively positive ctHPVDNA blood tests, 15 of whom developed biopsy-

proven recurrence. The ~~negative predictive value~~NPV of ctHPVDNA for detecting disease recurrence was 100%; the ~~positive predictive value~~PPV for recurrence of two consecutive positive tests was 94% (95% CI, 70% to 99%).

O'Boyle and colleagues (2022) conducted a prospective observational study to assess whether the clearance kinetics of ctHPVDNA is associated with postoperative disease status. The study included a total of 33 subjects with HPV+OPSCC undergoing surgery. Blood was collected prior to surgery, on postoperative days 1 (POD 1), 7, and 30 and with follow-up. A subcohort of 12 participants underwent frequent blood collections in the first 24 hours after surgery to define early clearance kinetics. Plasma was analyzed using custom droplet digital polymerase chain reaction (ddPCR) assays for HPV genotypes 16, 18, 33, 35, and 45. In subjects with no pathologic risk factors for recurrence who were observed after surgery, ctHPVDNA rapidly decreased to < 1 copy/mL by POD 1 (n=8/8). In participants with risk factors for macroscopic residual disease, ctHPVDNA was markedly elevated on POD 1 (> 350 copies/mL) and remained elevated until adjuvant treatment (n=3/3). Participants with intermediate POD 1 ctHPVDNA levels (1.2-58.4 copies/mL) all possessed pathologic risk factors for microscopic residual disease (n=9/9). POD 1 ctHPVDNA levels were greater in subjects with known adverse pathologic risk factors such as extranodal extension > 1 mm (p=0.0481) and with increasing lymph nodes involved (p=0.0453) and were further associated with adjuvant treatment received (p=0.0076). One of 33 subjects had a recurrence that was detected by ctHPVDNA 2 months earlier than clinical detection.

In 2022, Berger and colleagues published a retrospective case series with 1076 individuals who had received treatment for primary HPV-driven OPSCC and were at least 3 months post-treatment. Eligibility criteria also included having undergone at least one ctHPVDNA test at least 3 months post-treatment as part of routine surveillance. A total of 80 of the 1076 (7.4%) individuals had at least one positive ctHPVDNA test during follow-up. Nearly half, 38, of the 80 individuals were tested more than 12 months after completion of therapy, 27 (34%) were tested between 6 and 12 months post-treatment and 15 (19%) were tested between 3-6 months post-treatment. Of these 80 individuals, 21 (26%) were known to have recurrent disease at the time of ctHPVDNA testing. Of the remaining 59 individuals, 55 (93%) were identified as having recurrent disease on subsequent imaging or biopsy. Thus, the PPV of the ctHPVDNA test was 76/80 (95%) for identification of recurrent disease at least 3 months post-treatment. A total of 1256 ctHPVDNA tests were negative. Ordering physicians indicated that 58 of these 1256 individuals (4.6%) had active disease at the time the test was ordered. Over half (683, 57%) of individuals with negative tests were more than 12 months post-treatment, 282 (24%) were between 6 and 12 months post-treatment and 233 (19%) were between 3 and 6 months post-treatment. Overall, the NPV of the ctHPVDNA test was 1198/1256 (95%) for identification of recurrent disease at least 3 months post-treatment. It is worth noting that ctHPVDNA were done at varying amounts of time post-treatment. Limitations of this study include that it was retrospective, uncontrolled and did not evaluate the impact of testing results on patient management or health outcomes. Additional studies are needed that demonstrate that the results of such testing results in improved measurable outcomes of patient management, compared to decisions independent of test results.

Management of Prostate Cancer

In 2020, the FDA updated the label for Lynparza (Olaparib), a poly(ADP-ribose) polymerase (PARP) inhibitor, to include individuals with deleterious or suspected deleterious germline or somatic HRR gene-mutated metastatic-resistant prostate cancer who have progressed following previous treatment and for therapy based on an FDA-

approved companion diagnostic test for Lynparza. The label was updated again in 2021 with no change to the above recommendation. This approval was based on the PROfound trial (NCT02987543). In 2020, de Bono and colleagues reported on a randomized, open-label, phase 3 trial which evaluated the use of olaparib in individuals with metastatic castration-resistant prostate cancer with disease progression while receiving a hormonal agent. All participants had a tumor mutation in one of the genes involved in the homologous recombinant repair (HRR) pathway. Participants were divided into two cohorts; cohort A included 245 participants who had at least one alteration in BRCA1, BRCA2, or ATM. Cohort B included 142 participants who had alterations in any of the other 12 prespecified genes (BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, PPP2R2A,RAD51B, RAD51C, RAD51D and RAD54L). Primary endpoint was progression-free survival in cohort A. Participants were randomized in a 2:1 fashion to receive either olaparib or hormonal agent (control). The authors report that in cohort A, progression-free survival was a median of 7.4 months for those taking olaparib compared to a median of 3.6 months in the control group. Median overall survival in cohort A was 18.5 months for those taking olaparib compared to a median overall survival of 15.1 months in the control group. The final analysis of overall survival was reported by Hussain and colleagues (2020). In cohort A, median duration of overall survival was 19.1 months with olaparib and was 14.7 months in the control group. In cohort B, median duration of overall survival was 14.1 months with olaparib and 11.5 months in the control group. The overall population (cohorts A and B) had a median duration of overall survival of 17.3 months for those taking olaparib and 14.0 months for those in the control group. The study authors note that the role of PPP2R2A could not be validated as a homologous recombination repair gene based on preclinical data and there was no benefit of overall survival with treatment of olaparib over control therapy in the individuals who had alterations in PPP2R2A. The FDA label also notes that while individuals with gene mutations for PPP2R2A were enrolled in the trial, Lynparza is not indicated for those with this gene mutation due to unfavorable risk-benefit.

In addition to Olaparib, several other PARP inhibitors have been evaluated in treating men with metastatic prostate cancer and a pathogenic variant in an HRR gene (or genes involving DNA damage response pathways), including Rucaparib, Niraparib, and Talazoparib.

Management of Non-Small Cell Lung Cancer
Gene alterations have been identified which can impact selection of therapy. Testing of specimens for gene alterations can help identify potentially effective targeted therapy and avoid therapy unlikely to provide clinical benefit. In the 2023~~2~~ NCCN guideline ~~Clinical Practice Guidelines in Oncology~~ for non-small cell lung cancer, ~~they recommend~~ molecular testing is recommended for actionable biomarkers (with these specified genes ALK, BRAF, EGFR, ERBB2 (HER2), KRAS, MET, NTRK, RET and ROS1) prior to administering first-line therapy. It is also recommended that when feasible, "testing be performed via a broad, panel-based approach, most typically performed by NGS." The NCCN also acknowledges that many NGS-based assays are larger than the 50-gene limit threshold, and as a result, it may be practical to follow these recommendations. Use of targeted gene panels ~~(containing 5-50 genes)~~ is considered in accordance with generally accepted standards of medical practice.

Guardant360 Panel Tests
In a single-center observational study, Thompson and colleagues (2016) examined the concordance between tissue biopsy samples and Guardant360 blood samples for individuals with non-small cell lung cancer (NSCLC). A total

of 102 subjects with a diagnosis of NSCLC or suspected NSCLC were included in the study. Tissue samples (n=50) were processed using the Illumina TruSeq Amplicon 47 gene cancer panel (n=38) or the 20 gene Penn Precision Panel (n=12). For the 50 subjects who had both blood and tissue tests, the overall concordance was 60%. For EGFR mutations, the overall concordance was 79%. The authors concluded that ctDNA testing has potential for real-time molecular monitoring for individuals with advanced cancer.

McCoach and colleagues (2018) performed a retrospective cohort study to determine the clinical utility of Guardant360 for detecting anaplastic lymphoma kinase (ALK) fusions in NSCLC during diagnosis or during treatment with ALK inhibitors. The researchers included 88 subjects with 96 plasma-detected ALK fusions from the Guardant360 de-identified database. Subjects were separated into 4 cohorts: cohort 1 (n=42) contained subjects with a newly discovered ALK fusion, cohort 2 (n=31) contained subjects with a known or presumed ALK fusion and whose cell-free DNA (cfDNA) was obtained at progression, cohort 3 (n=13) contained subjects without additional clinical information, and cohort 4 (n=6) contained subjects who had been treated with anti-EGFR targeted therapy and found to have an ALK fusion by cfDNA. In cohort 1, the Guardant360 test found an ALK fusion in 16 subjects who had been reported as tissue-negative or tissue insufficient. Of the 42 subjects in the cohort, 10 had tissue samples available (5 ALK-positive, 5 ALK-negative), 11 had insufficient samples, and 21 did not have ALK information available. For the 5 subjects who were identified by Guardant360 as ALK-positive despite negative tissue biopsies, 3 eventually responded to ALK inhibitor therapy while clinical data was not available for the other 2 subjects. For cohort 2, 16 samples contained 1-3 ALK resistant mutations. For 5 samples, an ALK kinase domain mutation was identified in cfDNA despite the ALK fusion not detected in cfDNA and the prior tissue sample showing an ALK fusion. For cohort 3, the clinical status was unknown and no resistance mutations or bypass pathways were identified. For cohort 4, 6 subjects were found to have ALK fusions. The authors concluded that cfDNA NGS testing is an "additional tool" for detecting alterations, resistance mutations, and bypass pathways. Limitations of the study included the retrospective design and lack of clinical data for some subjects. The authors noted that tissue evaluation was at the providers' discretion and the testing method was not available for all subjects. Furthermore, no sensitivity or specificity information was provided.

Aggarwal and colleagues (2018) conducted a single-center, prospective study to assess mutation detection using Guardant360 for individuals with stage IV NSCLC. A total of 323 participants had Guardant360 plasma testing as part of clinical management. The primary outcomes were targetable alterations detected with plasma and tissue next-generation sequencing, the association between allele fractions of mutations detected in tissue and plasma, and the association of response rate with the plasma allele fractions of the targeted mutations. For 113 individuals, therapeutically targetable mutations were detected in EGFR, ALK, MET, BRCA1, ROS1, RET, ERBB2, or BRAF. Of 94 participants who had plasma testing alone, 31 had a targetable mutation detected and were considered to not need tissue biopsy. For the 229 participants who had concurrent plasma and tissue testing or were not able to have tissue testing, an additional 35 targetable mutations were detected. For those who received targeted therapy based on the plasma result, 36 out of 42 participants had complete/partial response or stable disease. Of the 128 subjects with concurrent plasma and tissue next generation sequencing results, 8 therapeutically relevant mutations were found in plasma only, 31 were detected in both plasma and tissue, and 16 were detected in tissue only, with an overall concordance of 81.3%. Therapeutically targetable mutation detection was highest for individuals with liver metastases (100% concordance with tissue [n=13]) compared with individuals with M1a disease (46.2%

concordance). Based on the level of discordance found in the study, the authors note that "a tissue biopsy remains essential for initial cancer diagnosis"; however, in the setting of inadequate tissue DNA, "plasma NGS can be an adequate surrogate for molecular profiling." The study was limited by a single-center design, potential user bias, and the consideration of plasma testing at a single point. The study was also enriched with individuals who underwent testing after progression to detect resistance mutations, which likely increased the frequency of individuals with EGFR T790M. The long-term outcomes of employing Guardant360 plasma testing in the clinical management of stage IV NSCLC versus, or in conjunction with standard tissue biopsy remains uncertain, as does the potential risk of false-negative results.

Leighl and colleagues (2019) reported on the multicenter, prospective NILE (Non-invasive versus Invasive Lung Evaluation) study, which aimed to assess the clinical utility of Guardant360 for the identification of eight genomic biomarkers (EGFR, ALK, ROS1, BRAF V600E, RET, MET, MET exon 14, ERBB2 [HER2]) in individuals with newly diagnosed metastatic NSCLC. A total of 307 individuals were enrolled with biopsy-confirmed, previously untreated non-squamous NSCLC (stage IIIB/IV) and tissue genotyping (genomic testing and PD-L1 expression analysis using next generation sequencing polymerase chain reaction "hotspot" testing, FISH and/or IHC, or Sanger sequencing). Participants submitted a pre-treatment blood sample for Guardant360 testing. A total of 282 individuals met all inclusion criteria and were included in the final analysis. Tissue genotyping for all eight biomarkers was completed in 51 individuals (18.1%) (the majority of individuals had sequential individual biomarker testing, and did not undergo physician-directed sequencing of all eight genomic biomarkers), and Guardant360 testing for all eight biomarkers was completed in 268 individuals. One of eight biomarkers was identified in tissue samples in 60 individuals compared to 77 individuals with Guardant360 ($p<0.0001$). For 60 individuals with tissue-positive results, one of the eight biomarkers was identified in tissue alone (n=12) but not with Guardant360, a false-negative rate of 20%. In regards to these 12 individuals, the researchers note: "the lack of full genomic assessment obtained by comprehensive cfDNA genomic profiling may have led to the patient being treated with a less efficacious therapy." While the primary objective to demonstrate non-inferiority of Guardant360 compared to tissue-based genotyping was achieved, the study was limited in that only 18% of participants received comprehensive tissue genomic profiling. As with other research on the topic, a substantial number of false-negative results were obtained by cfDNA, which can lead to undertreatment.

Zugazagoitia and colleagues (2019) evaluated the ability of the Guardant360 test to identify individuals with NSCLC in routine clinical practice who have tyrosine-kinase inhibitor (TKI) resistance. This was a prospective study that included 53 individuals with EGFR, ALK or ROS1-altered advanced stage NSCLC who experienced progression (clinical or radiological) on prior TKI therapy. The sample was divided into 3 subgroups; 1) EGFR-mutant NSCLC with resistance to first/second-generation EGFR TKI (cohort 1, n=31); 2) EGFR T790 + NSCLC with osimertinib resistance (cohort 2, n=15) and ALK/ROS1-rearranged NSCLC with resistance to crizotinib and/or next generation ALK/ROS1 TKI (cohort 3, n=7). Individuals with sufficient tumor DNA shedding such that plasma findings could be adequately interpreted were classified as "shedders". In cohort 1, 20 individuals (65%) were classified as shedders and 9 (29%) were found to have EGFR T790 M mutations with Guardant360 testing. In 2 additional individuals, EGFR T790 M mutations were identified by another method; these 11 individuals received subsequent osimertinib therapy. In cohort 2, Guardant360 testing in 10 individuals were classified as shedders and, in 9 of these, at least 1 pathologic alteration in addition to the EGFR sensitizing and/or T790M mutation was

detected. None of the individuals in cohort 2 received subsequent targeted therapies. In cohort 3, which included only 7 individuals, 4 individuals were shedders and were found to have actionable alterations. Two individuals in cohort 3 received subsequent treatment informed by Guardant360 testing. A substantial number of individuals in the study were not considered to be tumor DNA shedders and this study did not compare outcomes in individuals managed with and without the Guardant360 test.

The correlation between tissue-based tumor mutation burden (tTMB) testing and blood-based TMB (bTMB) testing was examined using data from the MYSTIC phase 3 randomized trial of first-line treatment with durvalumab, with or without tremelimumab versus platinum-based chemotherapy (Rizvi, 2020). tTMB testing was done using the FoundationOne tissue platform and bTMB testing used the Guardant OMNI platform. Among 352 study participants with matched tissue and blood samples (31.5% of individuals in the randomized study), results of the two types of samples were correlated (Spearman p=0.6, Pearson r=0.7). A bTMB of at least 20 mutations/megabase (mut/Mb) was associated with improved OS for durvalumab plus tremelimumab versus chemotherapy. A tTMB of at least 10 mut/MB was associated with longer OS in both of the immunotherapy groups versus chemotherapy.

Myelodysplastic Syndromes
Myelodysplastic syndromes are conditions that can occur when the cells in bone marrow are abnormal and have problems making new blood cells. It is considered to be a type of cancer. Researchers have found that mutations in certain genes are disease-related and can be presumptive of myelodysplastic syndromes. The 2023 NCCN guideline for myelodysplastic syndromes notes the following genes are frequently somatically mutated in myelodysplastic syndromes: ASXL1, DNMT3A, EZH2, NRAS, RUNX1, SF3B1, SRSF2, STAG2, TET2, TP53, U2AF1, and ZRSR2. The NCCN does not address panel testing for relapsed myelodysplastic syndromes. Use of targeted gene panels ~~(containing 5-50 genes)~~ is considered in accordance with generally accepted standards of medical practice.

Acute Myeloid Leukemia
Acute myeloid leukemia is a type of cancer that starts in the bone marrow. It can move into the bloodstream and spread to other parts of the body including the lymph nodes, liver, spleen, central nervous system, and testicles. There are several gene variants which are associated with specific prognosis for AML. The 2023~~2~~ NCCN guidelines for acute myeloid leukemia recommend testing for ASXL1, BCR-ABL, c-KIT, CEBPA (biallelic), FLT3-ITD, FLT3-TKD, IDH1, IDH2, NPM1, PML-RAR alpha, RUNX1, and TP53. The NCCN guidelines also recommend all individuals "should be tested for mutations in these genes and multiplex gene panels and comprehensive next-generation sequencing (NGS) analysis are recommended for the ongoing management of AML and various phases of treatment" (NCCN, 2023). Use of targeted gene panels ~~(containing 5-50 genes~~) is considered in accordance with generally accepted standards of medical practice.

Acute Lymphoblastic Leukemia
Acute lymphoblastic leukemia is a type of cancer that starts in the bone marrow. It can progress quickly and develops from immature forms of white blood cells. It can move into the bloodstream and spread to other parts of the body including the lymph nodes, liver, spleen, central nervous system, and testicles. The 2022 NCCN

guidelines for acute lymphoblastic leukemia recommend testing for ABL1, ABL2, CRLF2, CSF1R, FLT3, IL7R, JAK1, JAK2, JAK3, PDGFRB, and SH2B3. Information regarding these gene variants may aid in risk stratification. The NCCN does not address panel testing for relapsed ALL. Use of targeted gene panels ~~(containing 5-50 genes)~~ is considered in accordance with generally accepted standards of medical practice.

Unselected Population Screening

As part of a population health study targeting Nevada's diverse demographics (the Healthy Nevada Project), Grzymski and colleagues (2020) reported on the genetic risk and disease manifestation of three inherited autosomal dominant conditions: BRCA-related hereditary breast and ovarian cancer, Lynch syndrome, and familial hypercholesterolemia. With a cohort of 26,906 participants, the authors identified 214 unique pathogenic or likely pathogenic variants carried by 358 individuals (1.33%). Of the 273 carriers with medical records available for review, 60 participants were identified as having clinical disease relevant to the underlying carrier status (21.9%). There were 135 individuals with hereditary breast and ovarian cancer with records available which revealed 28 individuals with disease who were also carriers (20.7%) compared with 523 individuals with disease who were not carriers (2.6%). Records were available for 66 individuals who were carriers of Lynch syndrome. A diagnosis of colon or other cancer was found in 19 participants (28.8%). The prevalence in non-carriers was 0.5% (92 individuals with disease). The records of 73 individuals with familial hypercholesterolemia were reviewed. The prevalence of hyperlipidemia in carriers was 53.4% compared to 25.7% in non-carriers. Net health outcomes were not assessed. While these results suggest genetic screening for certain conditions has potential in identifying at-risk carriers not detected in medical practice, a population health screening approach could underestimate the impact of preventive screening in larger populations with diverse cohorts. There is potential for overinterpretation of disease risk along with ethical and social factors. The risk of benefits of population-based screening programs need to be carefully assessed with long-term studies; at this time, application is not considered in accordance with generally accepted standards of medical practice.

High-risk Population Screening

The Galleri test (Grail, Inc., Menlo Park, CA) is a liquid biopsy test designed to detect over 50 types of cancer. The test is intended to be used with existing screening tools to improve cancer detection in individuals at increased risk of cancer. Because its intended use is as a screening test, the developers were primarily interested in creating a test with high specificity and thus a low false-positive rate.

The Galleri test development was informed by findings from the Circulating Cell-Free Atlas (CCGA) study, a prospective observational study to develop and validate a multi-cancer detection test. The CCGA enrolled 15,254 participants, 8584 with cancer and 6670 without cancer. In 2020, Liu and colleagues published results of a sub-study of the CCGA evaluating the sensitivity and specificity of a multi-cancer ctDNA test (the current version of which is known as the Galleri test). The analysis included 6689 participants from the CCGA who did not have cancer (n=4207) or whose cancer was previously untreated (n=2482). The sample was divided into training and validation sets. The test had high specificity in both the training set (99.8%, 95% confidence interval [CI], 99.4 to 99.9) and the validation set (99.3%, 95% CI, 98.3 to 99.8%), indicating a false positive rate of less than 1%. The sensitivity of the test for stage I-III cancers was 44.2% (95% CI, 31.4 to 47.2%) in the training set and 43.9% (95% CI, 39.4 to 48.5%) in the validation set. For the pre-specified group of 12 "high-signal cancers", stage I-III

sensitivity was 69.8% (95% CI, 65.6 to 73.7%) in the training set and 67.3% (95% CI, 60.7 to 73.3%) in the validation set. High-signal cancers, identified in preliminary research studies, include the following types of cancer: "anus, bladder, colon/rectum, esophagus, head and neck, liver/bile-duct, lung, lymphoma, ovary, pancreas, plasma cell neoplasm, stomach".

Another sub-analysis of the CCGA was published by Klein and colleagues in 2021 and evaluated the test with an independent validation set of 5309 CCGA participants (n=3237 with cancer and n=2069 without cancer). Specificity was 99.5% (95% CI, 99.0 to 99.8%), indicating a false positive rate of 0.5% and overall sensitivity was 51.5% (95% CI, 49.6% to 53.3%). Sensitivity for the 12 high-signal cancers, discussed above, was 76.3% (95% CI, 74.0 to 78.5%).

The test has not been evaluated in a large, prospective setting to assess the population-based implications of screening, including the potential for overdiagnosis and overtreatment, as well the sequelae of false-positive results (including associated follow-up testing, both invasive and non-invasive), and false-negative results (which may include obviation of recommended screening test such as colonoscopy or mammography).

Whole Exome Sequencing (WES)
It is estimated that most disease-causing mutations (around 85%) of clinically important sequence variants occur within the regions of the genome that encode proteins. While similar to whole genome sequencing (WGS), WES reads only the parts of the human genome that encode proteins, leaving the other regions of the genome unread (Choi, 2009). Since most of the errors that occur in DNA sequences that then lead to genetic disorders are located in the exons, sequencing of the exome is being explored as a more efficient method of analyzing an individual's DNA to discover the genetic cause of diseases or disabilities. It has been theorized that sequencing of the human exome can be used to identify genetic variants in individuals to diagnose diseases.

A potential major indication of WES is the establishment of a molecular diagnosis in individuals with a phenotype that is suspicious for a genetic disorder or for individuals with known genetic disorders that have a large degree of genetic heterogeneity involving substantial gene complexity. Such individuals may be left without a clinical diagnosis of their disorder, despite a lengthy diagnostic work-up involving a variety of traditional molecular and other types of conventional diagnostic tests. For some of these individuals, WES, after initial conventional testing has failed to make the diagnosis, may return a likely pathogenic variant. Results of WES testing are intended to guide treatment decisions including confirming or establishing a clinical diagnosis that may lead to changes in management (which may in some cases, may obviate the need for further testing, and/or end the diagnostic odyssey).

The 2021 Practice Guideline by the ACMG provides exome sequencing and genome sequencing recommendations for children with congenital anomalies or intellectual disability (Manickam, 2021) based on an assessment of 167 studies, 36 of which had a participant population greater than 20 individuals. The guidelines strongly recommend whole exome/genome sequencing as a first-tier or second-tier test (guided by clinical judgment and often clinician–member/family shared decision making after CMA or focused testing) for individuals with one or more congenital

anomalies prior to one year of age or for individuals with developmental delay (DD) or intellectual disability with onset prior to 18 years of age:

> The literature supports the clinical utility and desirable effects of whole exome/genome sequencing on active and long-term clinical management of patients with congenital anomalies, or developmental delay or intellectual disability, and on family-focused and reproductive outcomes with relatively few harms. Compared with standard genetic testing, whole exome/genome sequencing has a higher diagnostic yield.

The guidelines also note that WES, which only evaluates the coding regions of the genome, is widely available, with extensive experience interpreting and comparing test results. At this time, WGS, which provides additional assessment of non-coding regions of the genome is limited to small number of clinics and labs. The ACMG includes WES in their guideline statement merely with the expectation that WES will become more commonly used and available.

For prenatal testing, recommendations are made in a 2022 -position statement from the International Society for Prenatal Diagnosis on the use of genome-wide sequencing. For prenatal diagnosis, the authors recommend prenatal sequencing can be beneficial in current pregnancies with a fetus with a major single anomaly or multiple organ system anomalies. Sequencing can also be beneficial with a maternal or paternal personal history of a prior undiagnosed fetus who was affected by a major single or multiple anomalies.

Historically, prenatal diagnosis has been performed using G-banded karyotyping to detect chromosomal abnormalities. The yield in this approach results in a diagnosis in 9-19% of fetal anomalies. The use of CMA provides an additional 6% yield. Cause of the majority of fetal anomalies is unknown. Identifying the cause of fetal anomalies can help determine prognosis, inform recurrence risk, and guide clinical management. Prior studies of use of exome sequencing to diagnose unexplained fetal anomalies showed diagnostic yields of 8.5% and 10% (Petrovski, 2019; Lord, 2019). The relatively low yields might be explained by the wide range of structural anomalies which were included. There is limited data regarding the usefulness of exome sequencing for diagnosis of specific, severe prenatal phenotypes. In a 2020 study by Sparks and colleagues the authors reported on the diagnostic yield of exome sequencing in detecting pathogenic or pathogenic variants in 127 participants with unexplained cases of nonimmune hydrops fetalis (NIHF). The presence of NIHF was defined by fetal ascites, pleural or pericardial effusions, skin edema, cystic hygroma, increased nuchal translucency, or combination of the conditions. There were 37/127 cases in which the authors identified diagnostic genetic variants. Overall there were 25/37 cases in which diagnostic variants were autosomal dominant (12% of those were inherited and 88% were de novo). Autosomal recessive diagnostic variants were found in 10/37 cases (95% inherited and 5% de novo). Potentially diagnostic variants were identified in 12 additional cases.

WES presents ethical questions about informing individuals about incidental findings that have clinical significance. Ongoing discussions continue to explore whether or not, and how to inform individuals about medically relevant mutations in genes unrelated to the diagnostic question (that is, mutations of unknown significance, non-paternity and sex chromosome abnormalities). This type of information may not only affect the individual being tested, but may also implicate family members.

The 2021 Practice Guideline by the ACMG (Manickam, 2021) notes:

> ES is available widely as a clinical tool with a number of commercial and academic laboratories offering this testing. Best practice includes familial comparators ("trio") if available to help contextualize rare variants, but also can be effectively performed as proband only or duo, with diagnostic yield being slightly reduced compared with trio testing.

While some of the potential advantages of WES include the fact that it can be carried out more quickly than traditional genetic testing, it is not without limitations. WES typically covers only 85-95% of the exome and has no, or limited coverage of other areas of the genome. Areas of concern with this technology include: (1) gaps in the identification of exons prior to sequencing; (2) the need to narrow the large initial number of variants to manageable numbers without losing the likely candidate mutation; (3) difficulty identifying the potential causative variant when large numbers of variants of unknown significance are generated for each individual. It is more difficult to detect chromosomal changes, duplications, large deletions, rearrangements, epigenetic changes or nucleotide repeats from WES data compared with other genomic technologies (ACMG, 2012; Teer, 2010[a]; Teer, 2010[b]). Other uses of WES are not considered in accordance with generally accepted standards of medical practice.

Whole Genome Sequencing

WGS, also known as full genome sequencing (FGS), complete genome sequencing, or entire genome sequencing, is a laboratory procedure which seeks to determine an individual's entire DNA sequence, specifying the order of every base pair within the genome at a single time. WGS allows researchers to study the 98% of the genome that does not generally contain protein-coding genes. In the clinical setting, this process frequently involves obtaining a DNA sample from the individual (typically from blood, saliva, or bone marrow) and sequencing an individual's entire chromosomal and mitochondrial DNA. Because of the large volume of genomic data involved in this process, the genomic information is processed by and stored on microprocessors and computers.

A 2021 randomized trial by Krantz and colleagues reported on the effect of WGS in the clinical management of 354 acutely ill infants. Participants included acutely ill infants in pediatric intensive care units, aged between 0 and 120 days with a clinical suspicion of a genetic disorder. Participants were randomized to receive WGS test results either 15 days (the early group, n=176) or 60 days (the delayed group, n=178) after testing with a total 90-day observation window. Primary outcome was the difference in the number of participants who had a change in management in the early and delayed groups at 60 days. Change in management was defined as having no change in care, a condition-specific intervention, condition-specific supportive care, palliative care, or a combination of the latter. Secondary outcome measures included diagnostic efficacy of WGS, change of management at 90 days, length of hospital stay, and mortality. At 60 days, in the early group, diagnostic efficacy was found in 55/176 infants and a change in management was noted in 34/161 infants. At 60 days in the delayed group, diagnostic efficacy was found in 27/178 infants with a change in management in 17/165 infants. At 90 days, in the early group, diagnostic efficacy was noted in 55/176 infants with a change in management in 38/159 infants. At 90 days, in the delayed group, diagnostic efficacy was noted in 56/178 infants with a change in management noted in 45/161 infants. The most frequent changes in management at 60 days were condition-supportive care and included subspecialty referrals and

medication changes. There were no significant differences regarding mortality and length of hospital stay between the early and delayed groups. Given the 90-day observation window, it is likely other changes in management may not have been captured. There is also a lack of validated instruments in testing individual- and family-reported outcomes.

Researchers continue to explore the relationship between mutations in the genomic material and the development or presence of disease. The clinical role of WGS has yet to be established. Research is still being done to determine if WGS can be used to accurately identify the presence of a disease, predict the development of a particular disease in asymptomatic individuals as well as how an individual might respond to pharmacological therapy. It has been theorized that WGS might eventually improve clinical outcomes by preventing the development of disease.

Cytogenomic Microarray Analysis
Cytogenomic microarray analysis collectively describes two different laboratory techniques: array comparative genomic hybridization (aCGH) and single nucleotide polymorphism (SNP) arrays. While both of these techniques detect copy number variants (CNVs), they identify different types of genetic variation. aCGH allows the detection of gains and losses in DNA copy number across the entire genome without prior knowledge of specific chromosomal abnormalities. SNP arrays allow genotyping based on allele frequency. SNP arrays have additional oligonucleotide probes which analyze thousands of SNPs throughout the genome in order to identify deletions and duplications. The use of cytogenomic microarray analysis as a diagnostic tool for congenital anomalies as well as for individuals with unexplained developmental delay (DD), autism spectrum disorder (ASD) or intellectual disability (intellectual developmental delay) is specifically addressed by CG-GENE-10 Chromosomal Microarray Analysis (CMA) for Developmental Delay, Autism Spectrum Disorder, Intellectual Disability and Congenital Anomalies.

Molecular Profiling
Molecular profiling, also called comprehensive genomic profiling, is a method for identifying multiple biomarkers in the malignant tumors of persons who have cancer. The biomarker information can be used to identify treatment options. The personalized tumor molecular profiling services or test panels addressed in this document are similar in that they all ~~evaluate tumor tissue and, from it,~~ produce a molecular profile of the tumor and a list of potential therapies. However, their individual testing methods vary from matching over expressed genes with drugs to more complex systems biology approaches. Large multi-biomarker panels test a variety of markers. It is often the case that not every test in these panels has a proven benefit.

Some commercially available molecular profile panels are listed below:
FoundationOne
FoundationOne uses next generation sequencing (NGS) "to interrogate the entire coding sequence of 236 cancer-related genes (3769 exons) plus 47 introns from 19 genes frequently altered or rearranged in cancer." FoundationOne helps match the genomic alterations present in a tumor with specific targeted therapies or clinical trials. Recent small studies (Drilon, 2013; Lipson, 2012; Vignot, 2013) have investigated next generation sequencing in individuals with lung cancer. Others have used next generation sequencing in those with breast cancer (Ross, 2013a); colorectal and other gastrointestinal cancers (Dhir, 2017; Gong, 2017; Lipson, 2012), ovarian

cancer (Ross, 2013b), and prostate cancer (Beltran, 2013). Limitations of these studies include small sample sizes and lack of randomization.

FoundationOne CDx
On November 30, 2017, the FDA approved the FoundationOne CDx NGS sequencing test as a companion diagnostic for several drugs including: Gilotrif® (afatinib), Iressa® (gefitinib), Tarceva® (erlotinib), Tagrisso® (osimertinib), Alecensa® (alectinib), Xalkori® (crizotinib), Zykadia® (ceritinib), Tafinlar® (dabrafenib) in combination with Mekinist® (trametinib), Tafinlar® (dabrafenib), Zelboraf® (vemurafenib), Mekinist® (trametinib), Cotellic® (cobimetinib) in combination with Zelboraf® (vemurafenib), Herceptin® (trastuzumab), Kadcyla® (ado-trastuzumabemtansine), Perjeta® (pertuzumab), Erbitux® (cetuximab), Vectibix® (panitumumab), and Rubraca® (rucaparib). In addition, the test detects substitutions and alterations in 324 genes and is indicated to provide general tumor mutation profiling of solid malignant neoplasms in accordance with professional guidelines in oncology.

The FDA approval was based on concordance studies that compared the Foundation One CDx test to approved specific companion diagnostic tests including the cobas® EGFR Mutation Test (EGFR exon 19 deletions, L858R, EGFR T790M), Ventana ALK CDx Assay (ALK), Vysis ALK Break-Apart FISH Probe Kit (ALK), therascreen® KRAS RGQ PCR Kit (KRAS), Dako HER2 FISH pharmDx® Kit (ERBB2 [HER2]), cobas® BRAF V600 Mutation Test (BRAF V600), THxID™ BRAF kit (BRAF V600), and FoundationFocus CDx_{BRCA} (BRCA1 and BRCA2). The sample size for each biomarker comparison study ranged from 175 to 342, the positive percent agreement ranged from 89.4% to 100%, and the negative percent agreement ranged from 86.1% to 100%. For the BRCA1 and BRCA2 mutation, the FoundationOne CDx was considered concordant based on the previous approval of the FoundationFocus CDx_{BRCA} test. The FDA states, "The clinical concordance studies, with the exception of *ALK* and *EGFR* T790M, were subject to pre-screening bias, therefore the concordance results may be overestimated and the failure rate may be underestimated." For the T790M mutation, there is ongoing research to determine why a subset population with a mutant allele frequency < 5% tested negative with the cobas EGFR Mutation Test v2 but tested positive with the FoundationOne CDx test. The FDA concluded that, overall, the FoundationOne CDx test demonstrated non-inferiority to the corresponding specific companion diagnostic tests (FDA, 2017a). On March 16, 2018, the Centers for Medicare and Medicaid Services (CMS) approved NGS-based in vitro companion diagnostic laboratory tests for national coverage after an FDA-CMS parallel review.

In 2018, Hellmann and colleagues reported results from the CheckMate 227 study, an open-label, phase 3 trial (NCT02477826) designed to evaluate the efficacy of nivolumab or nivolumab-based regimens as first-line therapy in participants with stage IV or recurrent non-small cell lung cancer (NSCLC) that have not previously received chemotherapy as primary therapy. Trial participants were stratified into PD-L1 expression levels (at least 1% or less than 1%). In addition, tumor mutation burden was determined using the FoundationOne CDx assay. At 1 year, the progression-free survival (PFS) rate for participants with a high tumor mutation burden that received nivolumab in combination with ipilimumab was 42.6% versus 13.2% for the chemotherapy group. The median PFS was 7.2 months (95% confidence interval [CI], 5.5 to 13.2) for participants that received nivolumab in combination with ipilimumab versus 5.5 months for the chemotherapy group (95% CI, 4.4 to 5.8) (HR for disease progression or death, 0.58; 97.5% CI, 0.41 to 0.81; P<0.001). The authors concluded:

> Progression-free survival was significantly longer with first-line nivolumab plus ipilimumab than with chemotherapy alone among patients with NSCLC and a high tumor mutational burden, irrespective of PD-L1 expression level. The results validate the benefit of nivolumab plus ipilimumab in NSCLC and the role of tumor mutational burden as a biomarker for patient selection.

Additional data regarding the CheckMate 227 study was published by Hellmann and colleagues in 2019. The authors reported on the overall survival with nivolumab plus ipilimumab compared to chemotherapy in participants with a tumor PD-L1 expression level of 1% or greater. There were 679 participants who had evaluation of tumor mutation burden which showed a similar degree of overall survival regardless of whether they had a high tumor mutation burden or a low tumor mutation burden. The authors conclude:

> …although absolute survival with nivolumab plus ipilimumab was greatest in patients with a high tumor mutational burden, a similar relative benefit of nivolumab plus ipilimumab, as compared with chemotherapy, was seen in patients regardless of tumor mutational burden.

Based on this data showing no difference in survival outcomes between individuals whose tumors had high or low levels of tumor mutation burden, Bristol-Myers Squibb announced its decision in January 2019 to withdraw the supplemental biologics license application with the FDA seeking approval for the combination of nivolumab and ipilimumab for individuals with advanced NSCLC with tumor mutational burden greater than or equal to 10 mutations per megabase.

The 2023~~2~~ NCCN guideline for NSCLC notes that the emerging biomarker tumor mutation burden may be helpful to identify eligibility of first-line therapy with nivolumab with or without ipilimumab for those with NSCLC, however there is no consensus regarding how to measure tumor mutation burden.

In June 2020, the FDA updated the label for pembrolizumab (Keytruda® [Merck, Kenilworth, NJ]) to include treatment for individuals with unresectable or metastatic solid tumors with tumor mutation burden-high (defined as greater than or equal to 10 mutations per megabase) when confirmed by an FDA-approved test following progression after prior treatment and no satisfactory alternative treatment options. According to the FDA label, the accelerated approval was based on the Keynote-158 trial (NCT02628067), a multicenter, non-randomized, open-label trial. Efficacy outcomes were tumor response rate and duration of response. Tumor mutation burden was assessed by the Foundation One CDx assay. Of the 1050 subjects enrolled in the efficacy analysis population, tumor mutation burden was analyzed in 790 subjects. There were 102 subjects who had tumors identified as tumor mutation burden-high. With a median follow-up time of 11.1 months, 29% of participants reached an objective response rate, 4% reached a complete response, and 25% reached a partial response. Duration of response was assessed at 57% with a duration of greater than or equal to 12 months and 50% with a duration of greater than or equal to 24 months. Continuation of approval may be contingent on verification and description of clinical benefit in confirmatory trials.

FoundationOne Liquid CDx

In 2020, Woodhouse and colleagues published data on the performance of the FoundationOne Liquid CDX test. The authors retrospectively tested plasma samples from 375 individuals with hormone receptor (HR)-positive, HER2-negative breast cancer to evaluate the clinical validity of the assay as an aid in identifying individuals with PIK3CA alterations. In comparing the FoundationOne Liquid CDx test and the tumor tissue polymerase chain reaction (PCR)-based clinical trial assay (CTA), the positive percent agreement (PPA) was 71.7% and the negative percent agreement (NPA) was 100%. In addition, to evaluate the clinical validity of the assay as an aid in identifying individuals with advanced NSCLC who might be eligible for treatment with an EGFR tyrosine kinase inhibitor, the authors used samples collected for another clinical trial that had been evaluated with a different test (the EGRF Mutation Test v2). They conducted a non-inferiority analysis with 177 samples to evaluate the non-inferiority of the FoundationOne test to two replications of the reference test. The analysis found that the Foundation One Liquid CDx test was non-inferior to the reference test. In the first replication, the PPA between the FoundationOne Liquid CDx test and the reference test was 97.7% and the NPA was 95.6%. In the second replication, the PPA was 97.7% and the NPA was 95.4%.

In 2021, Takeda and colleagues reported on a prospective cohort study to evaluate the feasibility and utility of using the FoundationOne CDx test with individuals who have advanced or recurrent solid tumors. A total of 181 samples were processed and 175 of these yielded gene profiling data, for a success rate of 96.7%. A total of 174 of the 175 tested individuals had at least one known or likely pathogenic gene alteration, and 24 individuals (14%) received targeted therapy. Results of Kaplan-Meier analysis found that the median progression-free survival (PFS) was 12.1 (95% CI, 6.9 to 17.4) months for the individuals who received targeted therapy. The authors did not report PFS in individuals who did not receive targeted therapy.

Yang and colleagues (2022) conducted a multivariate analysis of factors associated with survival in 185 individuals with newly diagnosed glioblastoma who underwent FoundationOne CDx testing. In the full model that controlled for potential confounding variables, the presence of three of nine variants, CDKN2B, EGFR and PTEN were significantly associated with overall survival (OS) (CDKN3B and EGFR with reduced survival and PTEN with higher survival). None of the nine variants assessed were significantly associated with progression-free survival.

LiquidHALLMARK

Poh and colleagues (2022) reported on the diagnostic performance of the LiquidHallmark test, an 80-gene panel consisting of an amplicon-based NGS assay for genomic profiling of cfDNA. The authors examined 1592 samples submitted to their laboratory between January 2018 and May 2021. A total of 52% of samples were from individuals with lung cancer. The test is designed for individuals with advanced cancer; however, 9% of samples were from individuals with localized tumors and 4.5% were from healthy individuals undergoing screening. A total of 73.6% (1120/1521) cancer samples were ctDNA positive, including in 40.6% of localized tumor samples and 78.5% of metastatic tumor samples. The most commonly altered genes among the ctDNA positive samples were TP53, KRAS, PIK3CA, APC, SMAD4, and PTEN. EGFR alterations were detected in 36.1% of the ctDNA positive samples and in 62.8% of the lung cancer samples.

Ravi and colleagues (2022) evaluated the ability of the LiquidHallmark test to identify resistance mechanisms to immune checkpoint inhibitors. The study included 39 individuals with advanced urothelial carcinoma and involved

serial testing with the LiquidHallmark test. At least one genomic alteration was detected in the ctDNA samples of 37 individuals (95%) pre-therapy and 39 individuals (100%) post-therapy. There was a median of three unique genomic alterations per individual in both the pre- and post-immune checkpoint inhibitor therapy samples. The most common genomic alterations were in TP53 (54% pre- and post-therapy), TERT (49% pre-therapy and 59% post-therapy, respectively) and BRCA1/BRCA2 (33% pre-therapy and 33% post-therapy, respectively). At the time the samples were taken post-therapy, 9 of 36 evaluable individuals (25%) had a complete or partial response to treatment. Among these 9 individuals, 7 (78%) had clearance of one or more of their genomic alterations according to ctDNA. Four individuals had clearance of TP53 variants and these individuals had a significantly higher rate of response to immune checkpoint inhibitor therapy compared to individuals who had TP53 variants after therapy (50% versus 12.5%; p=0.046).

No published studies were available comparing patient management or health outcomes in individuals who were managed with or without use of the LiquidHallmark test.

Other Tests

Other tests are becoming available on the market. One such example is the Oncotype MAP™ PanCancer Tissue Test (Paradigm Diagnostics, Inc., Phoenix, AZ) in which next-generation sequencing is used to identify genetic alterations among 257 genes to match appropriate targeted therapy for tumor mutation burden of solid tumors.

Whole transcriptome testing can assist in determining how cells normally function and how changes in gene activity can contribute to disease by showing what genes are active in which cells. DNA is the molecule which contains instructions needed to build and maintain cells. In order for the instructions to be read and completed, the DNA has to be read and transcribed (that is, copied into RNA). The testing involves the presence and amount of RNA. By analyzing the RNA, it is possible to count the transcripts to determine the amount of gene activity.

Molecular Intelligence Service or Target Now

A widely used tumor molecular profile has been the Target Now Molecular Profiling Service. According to the Caris Life Sciences website, their tumor profiling service is now being promoted as the Molecular Intelligence™ Service. The published literature addressing these services is limited. Von Hoff and colleagues (2010) evaluated 86 individuals with refractory metastatic cancer. PFS using a treatment regimen selected by Target Now molecular profiling of a malignant tumor was compared with the PFS of the most recent treatment regimen on which the individual experienced progression. A molecular target was detected in 84 of 86 (98%) participants. A total of 66 (78.6%) individuals were treated according to the molecular profile results with 18 of the 66 (27%) having a PFS ratio (defined as PFS on molecular profile–selected therapy or PFS on prior therapy) of greater than or equal to 1.3 (95% CI, 17% to 38%; p=0.007).

An editorial (Doroshow, 2010) accompanying the study reported that the trial had a number of significant limitations, including uncertainty surrounding the achievement of time to progression (the study's primary

endpoint), and a lack of a randomized design. Additional limitations include a small number of participants and lack of duplication of study results by an independent dataset.

Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT)
Cheng and colleagues (2015) developed and evaluated the MSK-IMPACT, "a hybridization capture-based assay targeting all coding regions of 341 oncogenes and tumor suppressors." The ability of the assay to detect single nucleotide variants (SNVs) and short insertions and deletions (indels) was assessed in 284 known positive solid tumor samples. Of these, 75 had a matched normal sample available. The authors reported successful detection of known variants in all 284 cases, and ability to achieve high degrees of resolution and levels of coverage to > 500x in tumor samples that allows low-frequency mutations to be detected. On November 15, 2017, the FDA granted marketing authorization for MSK-IMPACT based on a *de novo* request (FDA 2017b).

Other Molecular Profiling
Other molecular profiling such as, GeneKey, GeneTrails Solid Tumor Panel, MatePair, MyAML, OmniSeq, OnkoMatch, OncInsights, and SmartGenomics have less published validation. To date, there is insufficient peer-reviewed evidence specifically validating these tests.

In 2012, Tsimberidou and colleagues developed a personalized medicine program at a single facility in the context of early clinical trials. Their goal was to observe whether molecular analysis of advanced cancer and use of targeted therapy to counteract the effects of specific aberrations would be associated with improved clinical outcomes. Participants with advanced or metastatic cancer refractory to standard therapy underwent molecular profiling. A total of 175 subjects were treated with matched therapy, and the overall response rate was 27%. Of the 116 subjects treated with non-matched therapy, the response rate was 5%. The median time-to-failure was 5.2 months for those on matched therapy versus 2.2 months on non-matched therapy. At a median of 15 months follow-up, median survival was 13.4 months versus 9.0 months in favor of matched therapy.

Jameson and colleagues (2013) performed a small pilot study investigating multi-omic molecular profiling (MMP) for the selection of breast cancer treatment. MMP treatment recommendations were selected in 25 cases and original treatment plans were revised accordingly. Partial responses were reported in 5/25 (20%), stable disease in 8/25 (32%) and 9/25 had no disease progression at 4 months. This study was limited by its small size and non-randomization. A large randomized prospective trial is needed for further evaluation.

Primarily marketed to researchers, Life Technologies Inc. offers several variations of their Ion Torrent™ Next Generation Sequencing Ion AmpliSeq™ panels, according to the company website. The Ion AmpliSeq Comprehensive Cancer Panel analyzes more than 400 cancer-related genes and tumor suppressor genes. The Ion AmpliSeq Cancer Hotspot Panel v2 analyzes the "hotspot" regions of 50 cancer-related and tumor suppressor genes.

Studies on Molecular Profiling Therapy
LeTourneau and colleagues (2012, 2015) reported on an open-label, randomized controlled phase II trial of treatment of refractory metastatic solid tumors directed by molecular profiling versus standard of care treatment

(SHIVA trial). A total of 195 adults, consisting of 99 in the experimental group and 96 in the control group, were enrolled from eight academic centers in France. The primary outcome was progression-free survival (PFS) analyzed by intention-to-treat. Randomization was stratified by three molecular pathways (hormone receptor pathway, PI3K/AKT/mTOR pathway, and RAF/MEK pathway). Molecular analysis included targeted NGS, gene copy number alterations and hormone expression by immunohistochemistry. The molecularly targeted drugs used in the experimental group were approved for clinical use in France, but were outside their indications. The control group received standard treatment chosen by the physician. Median follow-up was 11.3 months for both the experimental and control groups at the time of primary analysis of PFS. Median PFS was 2.3 months (95% CI, 1.7-3.8) in the experimental group versus 2.0 months (95% CI, 1.7-2.7 months) in the control group (hazard ratio, 0.88; 95% CI, 0.65-1.19; p=0.41). Upon subgroup analysis, there was no statistically significant difference in PFS between the two groups. Objective responses were reported for 4 of 98 (4.1%) assessable subjects in the targeted treatment group versus 3 of 89 (3.4%) assessable subjects in the standard care group. Among the safety population, grade 3-4 adverse events were reported for 43 of the 100 subjects (43%) who received a molecularly targeted agent and 32 (35%) of 91 subjects treated in the control group. The authors suggested that "off-label use of molecularly targeted agents should be discouraged and enrollment in clinical trials should be encouraged to help identify predictive biomarkers of efficacy."

Presley and colleagues (2018) conducted a multicenter, retrospective, cohort study to compare broad-based genomic sequencing to routine EGFR and ALK biomarker testing in individuals with advanced NSCLC (stage IIIB/IV or unresectable nonsquamous). The primary outcomes were the 12-month mortality and overall survival from the start of first-line treatment. The researchers examined the Flatiron Health Database records of 5688 individuals (median age 67 years) who received care for advanced NSCLC between January 1, 2011 and July 31, 2016: 875 received broad-based genomic sequencing (multigene panel testing assay of more than 30 genes) and 4813 received routine EGFR/ALK testing. Subjects were required to have documented broad-based genomic sequencing testing or EGFR testing; if EGFR was negative, ALK testing was required. All subjects received at least one line of systemic antineoplastic treatment. At 12 months, the unadjusted mortality rates were 49.2% for the broad-based group and 35.9% for the EGFR/ALK group. Of the subjects in the broad-based group, 4.5% received targeted treatment based on test results, 9.8% received EGFR/ALK targeted treatment, and 85.1% received no targeted treatment. When using an instrumental variable analysis, no significant association was found between broad-based genomic sequencing and 12-month mortality (difference in the predicted probability of death at 12 months between the groups: −3.6%; 95% CI, −18.4% to 11.1%; p=0.63). The predicted probability of 12-month mortality was 44.4% (95% CI, 42.9% to 45.9%) in the EGFR/ALK group and 41.1% (95% CI, 27.7% to 54.5%) in the broad-based group. For the propensity score-matched sample, the overall survival was not significantly different between the groups (42.0% vs. 45.1%; 0.92 HR; 95% CI, 0.73 to 1.11; p=0.40). The researchers concluded that "among patients receiving care for advanced NSCLC in the community oncology setting, broad-based genomic sequencing directly informed treatment in a minority of patients and was not independently associated with better survival." Limitations of the study included a relatively small and homogenous sample for the broad-based group and the possible inaccuracy of the electronic health records.

In an industry-sponsored study by Conroy and colleagues (2021) the authors present the initial results of their Illumina TruSight Oncology 500 High-Throughput assay as a scalable comprehensive genomic profiling way to

detect and deliver biomarker information regarding precision therapeutics in oncology. The TruSight Oncology assay is a next-generation sequence-based in vitro diagnostic assay to detect genomic variants and signatures. The assay analyzes 523 cancer-relevant genes from RNA and DNA from routine formalin-fixed paraffin-embedded tissue specimens. In this study, there were 717 samples selected from an inventory of banked RNA and DNA. These samples represented 31 tumor types. While the assay detected small variants, copy number alterations, MSI, TMB, and gene fusions, this retrospective study shows no association with improved clinical outcomes.

Other Considerations
The 2022~~2~~3 NCCN Guidelines do not contain recommendations for the general strategy of testing a tumor for a wide range of biomarkers. However, the guidelines do contain recommendations for specific genetic testing for individual cancers, when there is a known drug-biomarker combination that has demonstrated benefits for that particular type of tumor, such as colon or NSCLC. In order to conserve tissue, the current NSCLC guidelines support an FDA approved NGS companion diagnostic test that can simultaneously test for EGFR mutations, BRAF mutations, ROS1 rearrangements, and ALK rearrangements.

A 2018 joint guideline (Lindeman, 2018), *Updated Molecular Testing Guideline for the Selection of Lung Cancer Patients for Treatment with Targeted Tyrosine Kinase Inhibitors*, from the CAP, International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP) states that "multiplexed genetic sequencing panels are preferred over multiple single-gene tests to identify other treatment options beyond EGFR, ALK, and ROS1" (level of evidence rating: expert consensus opinion - serious limitations in quality of evidence). However, the authors note that "the strength of evidence is inadequate supporting the use of multiplexed genetic sequencing panels compared with single-gene tests."

Polygenic Risk Score
Polygenic risk score testing measures multiple single nucleotide polymorphisms which have been proposed as being associated with a specific disease or condition. Using an algorithm, a number or score is created that is intended to provide an estimated prediction of the risk of some future health outcome. Polygenic risk scores have been proposed to estimate an individual's lifetime genetic risk of disease. Polygenic risk score tests are being developed for a number of conditions such as heart disease, diabetes, cancer, obesity, and schizophrenia.

In a 2020 study by Damask and colleagues, the authors sought to determine whether individuals with a high polygenic risk score for coronary artery disease had a higher incidence of major adverse cardiovascular events (MACE) and whether those individuals had greater risk reduction of events following treatment with alirocumab (given for hyperlipidemia). In this post-hoc analysis of participants from the ODYSSEY OUTCOMES trial (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab), there were 11,953 individuals who had available DNA samples. In this study the authors defined high genetic risk as those with greater than 90th percentile polygenic risk score. Those with less than or equal to 90th percentile were considered lower genetic risk. MACE risk analysis was performed in the placebo arm while treatment benefit analysis was performed in all participants. In the placebo group, the incidence of MACE related to polygenic risk score for coronary artery disease was 17.0% for those with high genetic risk and 11.4% for those considered to be low genetic risk. In the group who received treatment (alirocumab), the absolute reduction in those with high

polygenic risk score was 6.0% and 1.5% in the low polygenic risk score group. The relative risk reduction by alirocumab was 37% in the high polygenic risk score group and 13% in the low polygenic risk score group. With this ad-hoc analysis, further validation is necessary. The authors also used a top threshold (defined in this study as greater than 90th percentile). Lack of consistent threshold for polygenic risk scores across studies make it difficult to generalize these results. Furthermore, given that participants enrolled into the ODYSSEY OUTCOMES trial were already candidates for intensive lipid lowering therapy, the added clinical utility of polygenic risk scoring is uncertain.

Marston and colleagues (2020) also reported on an ad-hoc analysis of 14,298 participants (7163 in the evolocumab arm and 7135 in the placebo arm) from the FOURIER trial (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk). The FOURIER trial was a multinational, randomized, double-blind, placebo-controlled trial which looked at the efficacy of evolocumab in individuals with atherosclerotic cardiovascular disease. In the Marston study, the authors sought to determine whether genetic risk score could risk-stratify individuals with atherosclerotic cardiovascular disease and predict benefit from evolocumab treatment. The authors looked at two outcomes; major coronary events (defined as coronary heart death, myocardial infarction, and coronary revascularization) and major vascular events (defined as major coronary events plus stroke). Those in the genetic cohort were followed for a median of 2.3 years. Genetic risk categories were measured as low, intermediate, or high. There were 1235 participants who had a major vascular event with 1074 of those being major coronary events. In the placebo arm, there were 774 participants who had a major vascular event, with 673 of those being major coronary events. Major vascular event rates in the low-genetic-risk category were 10.1%, 11.3% in the intermediate-genetic-risk category, and 13.8% in the high-genetic-risk category. Major coronary event rates in the low-genetic-risk category were 8.0%, 9.7% in the intermediate-genetic-risk category, and 13.2% in the high-genetic-risk category. In the entire study cohort, there were 1446 participants with a major vascular event, 1269 of which were major coronary events. In assessing the benefit of evolocumab by genetic risk categories, the hazard ratios (95% CI) for major vascular events in the low-, intermediate-, and high-genetic-risk categories were 0.92, 0.91, and 0.69, respectively. For those individuals without multiple clinical risk factors or high genetic risk, there was no benefit noted over a median of 2.3 years. In individuals with multiple clinical risk factors but without high genetic risk, there was a 13% relative risk reduction and 1.4% absolute risk reduction in major vascular events. For those with high genetic risk (irrespective of major clinical risk factors) there was a 31% relative risk reduction and 4.0% absolute risk reduction. There was no significant difference for the ARR across clinical risk factor burden in the high-genetic-risk category for either major vascular events or major coronary events. Study participants were divided into categories based on percentile relative to the study population, not a healthy reference population, which may have led to individuals with higher genetic risk moved into lower risk categories. Given that participants enrolled into the FOURIER trial were already candidates for intensive lipid lowering therapy, the added clinical utility of polygenic risk scoring is uncertain.

There is disagreement in the literature about whether adding polygenic risk score testing for coronary heart disease risk prediction adds value and improves net health outcomes. A 2022 review by Groenendyk and colleagues looked at five studies for coronary heart disease risk prediction and reported on the additive value of any new test for risk prediction. While polygenic risk score was associated with a risk of coronary heart disease in all studies, the addition did not lead to improvements in clinical decision-making or improved net health outcomes. Positive

predictive values ranged from 1.8% to 16.6% and false-positives ranged from 77.1% to 85.7%. The authors concluded there were no meaningful improvements when polygenic risk scores were added to traditional risk scores for coronary heart disease.

A 2022 cohort study by Joo and colleagues looked at whether genome-wide polygenic scores for psychiatric disorders and common traits were associated with the risk of suicidal thoughts among preadolescent children (age 9-10 years old). The authors analyzed data from the cohort of the Adolescent Brain and Cognitive Development (ABCD) study. In order to generate genome-wide polygenic scores, the authors used 24 psychiatric and common traits known to be associated with suicidal thoughts and behaviors. There were 6592 children included in the primary analysis (5374 of whom had only European ancestry). There were 935 children with suicidal thoughts or behaviors and 5657 children without suicidal thoughts or behaviors (the control group). Overall, the authors found genome-wide polygenic scores for attention-deficit hyperactivity disorder (ADHD) had the most significant association with phenotypes for suicidal thoughts and behaviors, with associations also found for schizophrenia and general happiness. For those in the European ancestry only group, three additional genome-wide polygenic scores were found to be associated with suicidal thoughts and behaviors: autism spectrum disorder, major depressive disorder, and posttraumatic stress disorder. While this cohort study results highlight the potential utility of genome-wide polygenic scores, further development of screening methods and intervention strategies for children at risk of suicide are necessary.

In a 2022 study by Bigdeli and colleagues, the authors reported on the penetrance of polygenic risk scores for schizophrenia, bipolar disorder, and major depression of participants in the Veterans Health Administration. The billing codes were compared to in-person clinical interviews and in this retrospective review there were 707,299 study participants with 9378 confirmed with a diagnosis of schizophrenia or bipolar 1 disorder. Among those with confirmed diagnosis, 8962 were also correctly identified using billing codes. Of the 707,299 total study participants, 84,806 were genotyped as African ancestry and 314,909 were of European ancestry. Polygenic risk scores were associated with a diagnosis of schizophrenia (odds ratio [OR], 1.81 [95%CI, 1.76-1.87]) and bipolar disorder (OR, 1.42 [95%CI, 1.39-1.44]. For those of African ancestry, the corresponding effect sizes in participants were smaller for schizophrenia (OR, 1.35 [95%CI, 1.29-1.42]) and bipolar disorder (OR, 1.16 [95%CI, 1.11-1.12]). There is no evidence of improved net health outcomes or change in medical management.

While polygenic risk scores can explain relative risk for a disease, prospective data is needed to assess whether risk identification resulting in therapeutic decision-making leads to net health outcomes. Current studies also lack generalizability.

Gene Panel Testing for Age-Related Macular Degeneration (AMD)
The leading cause of blindness in the elderly population is AMD, a complex disease. There are two major types of AMD, a dry form and wet form. The dry form is associated with slowly progressive vision loss, and the wet form may lead to rapidly progressive and severe vision loss. The risk of AMD and the risk for development of the wet form are associated with genetic factors and also non-genetic influences, such as smoking and obesity.

Gene panel testing for AMD is aimed at identifying individuals at risk of developing advanced AMD. Genetic variants associated with AMD account for approximately 70% of the risk for the condition (Gorin, 2012). Over 25 genes have been reported to influence the risk of developing AMD, discovered originally through family-based linkage studies, and then through large genome-wide association studies. Genes influencing several biological pathways, including genetic loci associated with the regulation of complement, lipid, angiogenic and extracellular matrix pathways, have been associated with the onset, progression and involvement of early, intermediate and advanced stages of AMD.

Loci based on common single nucleotide polymorphisms (SNPs) contribute to the greatest AMD risk. Major AMD loci identified in different populations include complement factor H (CFH) and age-related maculopathy susceptibility 2 (ARMS2)/HtrA serine peptidase 1 (HTRA1). Although changes in both ARMS2 and HTRA1 have been studied as potential AMD risk factors, the two genes are located very close together, making it difficult to determine which one is associated with AMD risk, or whether both genes cause increased risk. Other genes in the complement pathway shown to be associated with AMD include complement 2 (C2), complement 3 (C3), complement factor B (CFB), and complement factor 1 (CF1).

Large genome-wide association studies have implicated high-density lipoprotein (HDL) cholesterol pathway genes, including cholesterylester transfer protein (CETP) and hepatic lipase (LIPC), and possibly lipoprotein lipase (LPL) and ATP-binding cassette (ABCA1) (Lim, 2012). The collagen matrix pathway genes COL10A1 and COL8A1, the extracellular matrix pathway gene known as tissue inhibitor of metalloproteinase 3 (TIMP3) and genes in the angiogenesis pathway, vascular endothelial growth factor A (VEGFA) have been associated with AMD.

Models for predicting AMD risk include various combinations of epidemiologic, clinical and genetic factors, and report areas under the curve (AUC) of approximately 0.8 (Hageman, 2011; Jakobsdottir, 2009). A multi-center prospective evaluation of 1446 participants by Seddon and colleagues (2009) demonstrated that a model of AMD risk that included age, gender, education, baseline AMD grade, smoking and body mass index gave an AUC of 0.757. The addition of the genetic factors, SNPs in CFH, ARMS2, C2, C3 and CFB, increased the AUC to 0.821. Klein and colleagues (2011) evaluated longitudinal data from 2846 study participants and showed that an individual's macular phenotype, as represented by the Age-Related Eye Disease Study (AREDS) Simple Scale score, which rates the severity of AMD based on the presence of large drusen and pigment changes to predict the rate of advanced AMD, has the greatest predictive value. The predictive model used in the Klein analysis included age, family history, smoking, the AREDS Simple Scale score, presence of very large drusen, presence of advanced AMD in one eye, and genetic factors (CFH and ARMS2). The AUC was 0.865 without genetic factors included and 0.872 with genetic factors included. These risk models suggest a small increase in the ability to assess risk of developing advanced AMD based on genetic factors. In a 2015 analysis, Seddon and colleagues included 10 rare and common genetic variants in their risk prediction model, resulting in an AUC of 0.911 for progression to advanced AMD.

The potential clinical utility of gene panel testing for AMD consists of prevention and monitoring of disease, and therapy guidance. Currently, the only preventive measures available for the disease are good health practices (for example, smoking cessation) and high-dose antioxidants and zinc supplements. The impact of more frequent

monitoring for those at risk for developing AMD is unknown. In regard to therapy guidance, there have been no consistent associations between response to therapy and specific genotypes. Additionally, there is a lack of a consistent association between response to vitamin supplements or anti-VEGF (vascular endothelial growth factor) therapy and VEGF gene polymorphisms (Awh, 2013; Chew, 2014; Fauser, 2015; Hagstrom, 2014, Hagstrom, 2015).

In 2015, Awh and colleagues performed a retrospective subgroup analysis of subjects from the 2001 Age-Related Eye Disease Study (AREDS). DNA was not collected from all AREDS subjects and the analysis was based on DNA from white AREDS subjects with category 3 or 4 AMD. The analysis was restricted to white subjects "because AMD genetics has been studied best in this group." Four genotype groups based on CFH and ARM2 risks alleles were defined. The benefit of treatment with the AREDS formulation seemed to be the result of a positive response by subjects in only one genotype group, and neutral or unfavorable responses in three genotype groups. Subjects with two CFH alleles and no ARMS2 risk alleles showed more of a progression with treatment containing zinc as compared to placebo. Subjects with zero or one CFH risk alleles and one or two ARMS2 risk alleles benefited with treatment containing zinc as compared to placebo. For subjects with zero or one CFH risk alleles and no ARMS2 risk alleles, zinc containing treatment did not alter progression as compared with placebo, but antioxidant treatment decreased progression. For subjects with two CFH risk alleles and one or two AMRS2 risks alleles, no treatment was better than placebo. The authors concluded that "validation by an independent data set would be helpful, but no such data exists, and a replication trial would take many years." In reference to this analysis, Odaibo (2015) indicated that very different conclusions were drawn by Awh as compared to AREDS and pending a larger study specifically testing their hypothesis, no final conclusions can yet be drawn.

Seddon and colleagues (2016) also retrospectively analyzed data from AREDS and similarly reported that the effectiveness of antioxidant and zinc supplementation appeared to vary by genotype and that genetic factors may become relevant when selecting specific treatments. However, the authors concluded that "additional studies are needed to determine the biological mechanism for this interaction and its implications for the comprehensive management of AMD."

The American Academy of Ophthalmology (AAO) Task Force on Genetic Testing (2022~~12~~) includes the following recommendation for testing of inherited eye diseases:

> Avoid routine genetic testing for genetically complex disorders like age-related macular degeneration and late-onset primary open-angle glaucoma until specific treatment or surveillance strategies have been shown in 1 or more published clinical trials to be of benefit to individuals with specific disease associated genotypes. In the meantime, confine the genotyping of such patients to research studies.

Stone (2015) re-emphasized the AAO recommendations and indicated that the clinical utility of genetic testing for AMD needs to be evaluated in a prospective randomized manner.

The 2015 AAO Preferred Practice Pattern for AMD does not recommend the routine use of genetic testing for AMD and specifically states "One or more prospectively designed clinical trials will need to demonstrate the value of genetic testing in AMD. Thus, the routine use of genetic testing is not supported by the existing literature and is not recommended at this time."

Similarly, the 2020 AAO Age-Related Macular Degeneration Preferred Practice Pattern® document states:

> The primary risk factors for the development of advanced AMD include increasing age, northern European ancestry, and genetic factors. Cigarette smoking is the main modifiable risk factor that has been consistently identified in numerous studies. Smoking cessation is strongly recommended when advising patients who have AMD or are at risk for AMD. The routine use of genetic testing is not recommended at this time (Flaxel, 2020).

The clinical validity of gene panel testing for AMD may provide a small, incremental benefit to risk stratification based on non-genetic risk factors. However, the clinical utility of genetic testing for AMD is currently limited and any association with specific genotypes and specific therapies needs to be evaluated with additional study in a prospective manner.

Commercially available gene panel tests for AMD include but are not necessarily limited to:

- Macula Risk® PGx (Artic Medical Laboratory, Grand Rapids, MI)
- RetnaGene™ AMD (Nicox for Sequenom, San Diego, CA)

Chromosome Conformation Signature
This refers to a type of genomic testing that looks at the regulation of an individual's genes at the level of 3D conformation. It may provide information about how a person will or will not respond to therapy. Chromosome conformation signatures analyze changes in the regulation of a genome before the results of epigenetic changes are known to be obvious abnormalities. Using whole blood, the test genetically profiles 8 epigenetic markers by qPCR which are then reported as either high or low probability of responding to immune checkpoint-inhibitor therapy. There is a paucity of peer-reviewed published literature evaluating clinical outcomes for chromosome conformation signatures in evaluating checkpoint-inhibitor therapy.

Background/Overview

Genetic Testing Using Panels of Genes
NGS addresses any of the technologies that allow rapid sequencing of large numbers of segments of DNA, up to and including entire genomes. NGS is not a specific sequencing technology or a test in itself. Instead, the term emphasizes the difference between the earlier testing methods that involved the sequencing of one DNA strand at a time. NGS includes but is not limited to massively parallel sequencing and microarray analysis.

NGS has led to the development of genetic testing incorporating panels which analyze multiple genes for multiple mutations simultaneously. Genetic testing using panels of genes may identify numerous genetic mutations that may contribute to the development of hereditary cancers.

Commercially available genetic testing panels for breast and/or ovarian cancers include, but are not limited to: BreastNext® (Ambry Genetics®); OvaNext® (Ambry Genetics®); BREVAGen (Phenogen Sciences); and myRisk Hereditary Cancer test (Myriad Genetics).

- The BreastNext genetic panel evaluates select genes that may be associated with a lifetime risk of breast cancer for individuals who, based on personal and family history, are at high risk for breast cancer and have tested negative for BRCA1 and 2 mutations.
- The OvaNext genetic panel simultaneously analyzes 23 genes that contribute to an increased risk for breast, ovarian and/or uterine cancers.
- The BREVAGen genetic panel assesses the risk for sporadic breast cancer by combining a woman's individual clinical risk factors (Gail score) with seven specific genetic markers.
- The myRisk Hereditary Cancer panel uses next-generation sequencing to examine genes associated with 8 cancer syndromes (breast, colorectal, endometrial, melanoma, pancreatic, gastric, and prostate).

The ColoNext™ test (manufactured by Ambry Genetics) is an example that tests for variants in 14 genes that have been associated with hereditary colorectal cancer, including the genes that cause Lynch syndrome (MLH1, MSH2, MSH6, PMS2 and EPCAM) as well as the gene that causes FAP (APC).

Circulating Tumor DNA Testing (Liquid Biopsy)

Liquid biopsy is proposed as a less-invasive method for cancer identification, surveillance, and treatment guidance. The National Cancer Institute (NCI) defines liquid biopsy as "a test done on a sample of blood to look for cancer cells from a tumor that are circulating in the blood or for pieces of DNA from tumor cells that are in the blood." ctDNA tests detect small fragments of mutated DNA that are released from tumors into blood, presumably by apoptosis and/or necrosis. Some ctDNA liquid biopsy tests are targeted for specific gene mutations. For example, in the instance of non-small cell lung cancer, a targeted liquid biopsy may be used to identify the presence of the EGFR mutation and determine if individuals may benefit from kinase inhibitor medication. Other liquid biopsy tests analyze multiple biomarkers and are purported to detect various cancers or treatments (Perakis, 2017).

There are several limitations of liquid biopsies. In regard to cancer management, many cancers do not have specific DNA mutations that can be identified and, when present, can be different in individuals with the same cancer. The DNA found in the fluid sample may not fully represent the tumor and mislead treatment decisions. The mutations found may not be "driver" mutations and may not provide useful information about the cancer. In regard to cancer detection, liquid biopsies can test positive for cancer when no cancer is present (false-positive) or test negative when cancer is present (false-negative). Because cancer cells release more mutated DNA fragments in later cancer stages, the test may not identify early cancer. Likewise, a liquid biopsy can detect cancerous cells that may never

actually cause harm, leading to overtreatment (NCI, 2018). While liquid biopsies are promising, a great deal of research is still needed to determine if these tests improve outcomes for individuals with cancer.

Whole Genome Sequencing
WGS, also known as full genome sequencing (FGS), complete genome sequencing, or entire genome sequencing, is a laboratory procedure which seeks to determine an individual's entire DNA sequence, specifying the order of every base pair within the genome at a single time. The role of WGS in the clinical setting has yet to be established.

Whole Exome Sequencing
While similar to WGS, WES reads only the parts of the human genome that encode proteins. Since most of the errors that occur in DNA sequences that then lead to genetic disorders are located in the exons, sequencing of the exome is being explored as a more efficient method of analyzing an individual's DNA to discover the genetic cause of diseases or disabilities. Various applications of WES are being explored including but not limited to determining if sequencing of the human exome can be used to identify genetic variants in individuals in order to diagnose diseases in individuals without the processing complexity associated with WGS.

Molecular Profiling
The rationale for molecular profiling is that more complete knowledge of molecular marker status may alter treatment and possibly improve individual outcomes. Molecular profiling refers to the analysis of DNA, RNA and/or proteins within the tumor cells. The term “molecular profiling” was initially limited to DNA analysis, but has now expanded to include analyses of RNA and proteins as well. Examples of commercially available multiple molecular testing panels are listed above. At this, only use of molecular profiling as a means of assessing tumor mutation burden has been established as a means of identifying candidates for targeted drug therapy.

Polygenic Risk Score
A polygenic risk score is a way for individuals to learn about their risk of developing a disease based on the total number of changes related to the disease. Some diseases can be traced to a variant in a single gene, while other diseases can occur due to variants in multiple genes. These variants can be identified by comparing the genomes of individuals with and without the disease. Using a computerized algorithm and statistics, a number or score is created to estimated how the collection of an individual’s variants affect risk for a certain disease.

Gene Panel Testing for Age-Related Macular Degeneration (AMD)
AMD, a global disease that causes blindness, is becoming increasingly prevalent and has no effective cure (Jager, 2008). AMD affects the macula located in the center of the retina. The macula has the highest photoreceptor concentration and is where visual detail is discerned. Wet AMD occurs with the pathological formation of new blood vessels (angiogenesis) behind the retina. These new blood vessels often leak blood and fluid displacing the macula from its normal position at the back of the eye and distorting central vision as a result. Wet AMD is also known as advanced AMD.

According to the American Academy of Ophthalmology, the risk of AMD increases as an individual ages. AMD is most common among older white Americans, affecting more than 14% of white Americans 80 years of age and

older. Caucasian Americans have the greatest likelihood of developing AMD (in 2010 affecting 2.5% of white adults age 50). By comparison, AMD affected 0.9% each of African-Americans, Hispanics and people of other races (AAO, 2019).

Commercially available gene panel tests for AMD are aimed at identifying those individuals who are at risk of developing advanced AMD. Examples of these tests include but are not necessarily limited to the following:

> Arctic Medical Laboratories offers Macula Risk PGx which uses 15 associated biomarkers in an algorithm to determine an individual's risk of progression to advanced AMD and aid in the selection of eye vitamin formulations for AMD based on his or her individual genetic risk profile. The Vita Risk™ pharmacogenetic result is provided as part of the Macula Risk PGx laboratory report.
>
> Nicox offers Sequenom's RetnaGene AMD in North America, which evaluates the risk of an individual with early or intermediate AMD progressing to advanced choroidal neovascular disease (wet AMD). The RetnaGene AMD test assesses the impact of 12 genetic variants (single nucleotide polymorphisms or SNPs) located on genes that are collectively associated with the risk of progressing to advanced disease in patients with early- or intermediate-stage disease (CFH/CFH region, C2, CRFB, ARMS2, C3). A risk score is generated, and the individual is categorized into a low, moderate, or high risk group.

Chromosome Conformation Signature
An example of the new platform to evaluate epigenetic biomarkers (chromosome conformation signatures) is the EpiSwitch™ (Oxford BioDynamics, Gaithersburg, MD).

In Vitro Companion Diagnostic Devices (IVD)
According to the FDA, a companion diagnostic is defined as:

> "..a medical device, often an in vitro device, which provides information that is essential for the safe and effective use of a corresponding drug or biological product. The test helps a health care professional determine whether a particular therapeutic product's benefits to patients will outweigh any potential serious side effects or risks.
>
> Companion diagnostics can:

- identify patients who are most likely to benefit from a particular therapeutic product;
- identify patients likely to be at increased risk for serious side effects as a result of treatment with a particular therapeutic product; or
- monitor response to treatment with a particular therapeutic product for the purpose of adjusting treatment to achieve improved safety or effectiveness".

To date, the Guardant360 CDx test has received FDA-approval as a companion diagnostic test in the following situations:

- Testing for EGFR exon 19 deletions, EGFR exon 21 L858R and T790M for individuals with NSCLC considering treatment with osimertinib
- Testing for EGRF exon 20 insertions for individuals with NSCLC considering treatment with amivantamb
- Testing for G12C in individuals with NSCLC considering treatment with sotorasib
- Testing for ERBB2 activating mutations in individuals with NSCLC considering treatment with fam-trastuzumab deruxtecan-nxki
- Testing for ESR1 missense mutations between codons 310 and 547 in individuals with breast cancer considering treatment with elacestrant.

There are numerous companion diagnostic indications for the Foundation One CDx test (tissue-based) and the blood-based Foundation One Liquid CDx test.

Companion diagnostic indications for the Foundation One CDx test (March 21, 2023) are listed below:

Tumor Type	**Biomarker(s) Detected**	**Therapy**
Non-small cell lung cancer (NSCLC)	EGFR exon 19 deletions and EGFR exon 21 L858R alterations	Gilotrif® (afatinib), Iressa® (gefitinib), Tagrisso® (osimertinib), or Tarceva® (erlotinib)
	EGFR exon 20 T790M alterations	Tagrisso® (osimertinib)
	ALK rearrangements	Alecensa® (alectinib), Xalkori® (crizotinib), or Zykadia® (ceritinib)
	BRAF V600E	Tafinlar® (dabrafenib) in combination with Mekinist® (trametinib)
	MET single nucleotide variants (SNVs) and indels that lead to MET exon 14 skipping	Tabrecta™ (capmatinib)
	ROS1 fusions	Rozlytrek® (entrectinib)
Melanoma	BRAF V600E	Tafinlar® (dabrafenib) or Zelboraf® (vemurafenib)
	BRAF V600E and V600K	Mekinist® (trametinib) or Tecentriq® (atezolizumab) in combination with Cotellic® (cobimetinib) and Zelboraf® (vemurafenib)

	BRAF V600 mutation-positive	Tecentriq® (atezolizumab) in combination with Cotellic® (cobimetinib) and Zelboraf® (vemurafenib)
Breast cancer	ERBB2 (HER2) amplification	Herceptin® (trastuzumab), Kadcyla® (ado-trastuzumabemtansine), or Perjeta® (pertuzumab)
	PIK3CA alterations	Pigray® (alpelisib)
Colorectal cancer	KRAS wild-type (absence of mutations in codons 12 and 13)	Erbitux® (cetuximab)
	KRAS wild-type (absence of mutations in exons 2, 3, and 4) and NRAS wild type (absence of mutations in exons 2, 3, and 4)	Vectibix® (panitumumab)
Ovarian cancer	BRCA1/2 alterations	Lynparza® (olaparib) or Rubraca® (rucaparib)
Cholangiocarcinoma	FGFR2 fusion or other select rearrangements	Pemazyre® (pemigatinib) or Truseltiq fgv™ (infigratinib)
Prostate cancer	Homologous Recombination Repair (HRR) gene alterations	Lynparza® (olaparib)
Solid Tumors	Tumor mutational burden >10 mutations per megabase	Keytruda® (pembrolizumab)
	Microsatellite instability-high (MSI-H)	Keytruda® (pembrolizumab)
	NTRK1/2/3 fusions	lVitrakvi® (larotrectinib) or Rozlytrek® (entrectinib)

Companion diagnostic indications for the Foundation One Liquid CDx test (March 21, 2023) are listed below:

Tumor Type	**Biomarker(s) Detected**	**Therapy**
Non-small cell lung cancer (NSCLC)	EGFR exon 19 deletions and EGFR exon 21 L858R substitution	EGRF tyrosine kinase inhibitors approved by FDA
	ALK rearrangements	Alecensa® (alectinib),

	MET single nucleotide variants (SNVs) and indels that lead to MET exon 14 skipping	Tabrecta™ (capmatinib)
	ROS1 fusions	Rozlytrek® (entrectinib)
Breast cancer	PIK3CA alterations	Piqray® (alpelisib)
Prostate cancer	BRCA1, BRCA2, ATM alterations	Lynparza® (olaparib)
	BRCA1, BRCA2 alerations	Rubraca® (rucaparib)
Solid Tumors	NTRK1/2/3 fusions	Rozlytrek® (entrectinib)

Definitions

Ashkenazi Jewish: Persons related to Jewish settlers of the Rhine Valley in Germany and France in the middle ages.

Cancer Moonshot: A collaborative effort between the public and private sectors (including but not limited to the governments, researchers, healthcare providers, data and technology experts, patients, families, and patient advocates) to make a decade's worth of advances in the understanding, prevention, diagnosis, treatment, and care of cancer.

Cell-free DNA (cfDNA): DNA that is circulating freely in body fluids, such as blood plasma, and is released from all types of cells.

Checkpoint Inhibition Immunotherapy (or Checkpoint Inhibitors): A type of drug (monoclonal antibody) that blocks certain proteins produced by immune T cells and cancer cells that keep the immune system in check and

prevent the T cells from attacking cancer cells. By blocking these proteins, checkpoint inhibitors thus unleash the immune T cells to kill the cancer cells. The following is a list of FDA-approved checkpoint inhibitor drugs.

- Pembrolizumab (Keytruda®)
- Nivolumab (Opdivo®)
- Atezolizumab (Tecentriq®)
- Avelumab (Bavencio®)
- Durvalumab (Imfinzi®)
- Ipilimumab (Yervoy®)

Circulating tumor DNA (ctDNA): Fragments of DNA that are released from a tumor and migrate into bodily fluids, such as blood plasma. Examples of ctDNA panel tests include, but are not limited to:

- Cancer Intercept® (Pathway Genomics, San Diego, CA)
- CellMax-LBx (CellMax Life, Sunnyvale, CA)
- Circulogene Comprehensive Lung, Gastrointestinal and Liver and Pancreatic Cancer Panels; Hereditary Cancer Gene Panel (Circulogene, Birmingham, AL)
- ClearID® Solid Tumor Cancer Panel (Cynvenio Biosystems, Westlake Village, CA)
- FoundationOne® Liquid CDx (Foundation Medicine, Cambridge, MA)
- Galleri™ Multi-Cancer Detection Test (Grail Inc., Menlo Park, CA)
- GeneStrat® (Biodesix, Boulder, CO)
- Guardant360® CDx, Guardant360® and Guardant Reveal™ (Guardant Health, Redwood, CA)
- LiquidHALLMARK® Test (Lucence Health)NavDx® (Navaris™, Natick, MA)
- OncoGxOne™ NGS Solid Tumor Panel (Admera Health, South Plainfield, NJ)
- OncoBEAM™ Lung2 Panel and OncoBEAM™ EGFR V2 Assay (Sysmex Inostics, Baltimore, MD)
- elio Plasma Complete (Personal Genome Diagnostics, Baltimore, MD)
- Resolution ctDx Lung™ (Resolution Bioscience, Kirkland, WA)
- Target Selector™ Breast Cancer, Non-Small Cell Lung Cancer, Squamous Cell Lung Cancer and Prostate Cancer Profiles (Biocept, San Diego, CA)

Copy number variant: An alteration of the DNA of a genome that results in the cell having an abnormal number of copies of one or more sections of the DNA.

Drusen: Pale whitish-yellow deposits of extracellular material formed in a layer of the retina.

Exome: All the exons in a genome.

First-degree relative: Any relative who is a parent, sibling, or offspring to another.

Gene panel: When five or more genes are tested on the same day on the same member by the same rendering provider.

Genetic testing: A type of test that is used to determine the presence or absence of a specific gene or set of genes to help diagnose a disease, screen for specific health conditions, and for other purposes.

Genome: An organism's entire set of DNA.

Genomic data: Information derived from the sequencing of DNA or RNA fragments.

Genotype: The genetic structure (constitution) of an organism or cell.

Immunohistochemistry: The process of detecting proteins in the cells of a tissue section.

Indel: A genomic insertion or deletion.

Messenger ribonucleic acid (mRNA): A molecule that results when a cell "reads" a DNA strand.

Molecular profiling services: Laboratory services which catalogue a number of genetic markers in an attempt to select optimal therapy.

Mutation: A permanent, transmissible change in genetic material.

Next-generation sequencing: Any of the technologies that allow rapid sequencing of large numbers of segments of DNA, up to and including entire genomes.

Panel testing: Involves the analysis of multiple genes for multiple mutations simultaneously.

Polygenic risk score: A way to learn about the risk of developing a disease based on the total number of changes related to the disease.

Tumor mutation burden: A biomarker used to assess responsiveness to immunotherapy by measuring the total number of mutations per coding area of a tumor genome. Tumor mutation burden is typically determined by molecular (genomic) profiling with a large multigene assay/panel.

Whole-exome sequencing: Reads only the parts of the human genome that encode proteins, leaving the other regions of the genome unread.

Whole genome sequencing: A laboratory procedure which seeks to determine an individual's entire DNA sequence, specifying the order of every base pair within the genome at a single time.

Coding

The following codes for treatments and procedures applicable to this document are included below for informational purposes. Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement policy. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

Gene panel testing for inherited diseases
When services may be Medically Necessary when criteria are met:

CPT

81412	Ashkenazi Jewish associated disorders (eg, Bloom syndrome, Canavan disease, cystic fibrosis, familial dysautonomia, Fanconi anemia group C, Gaucher disease, Tay-Sachs disease), genomic sequence analysis panel, must include sequencing of at least 9 genes, including *ASPA, BLM, CFTR, FANCC, GBA, HEXA, IKBKAP, MCOLN1, and SMPD1*
81434	Hereditary retinal disorders (eg, retinitis pigmentosa, Leber congenital amaurosis, cone-rod dystrophy), genomic sequence analysis panel, must include sequencing of at least 15 genes, including *ABCA4, CNGA1, CRB1, EYS, PDE6A, PDE6B, PRPF31, PRPH2, RDH12, RHO, RP1, RP2, RPE65, RPGR,* and *USH2A*

ICD-10 Diagnosis

All diagnoses

When services are Not Medically Necessary
For the procedure codes listed above when criteria are not met, for the following codes, or when the code describes a procedure indicated in the Position Statement section as not medically necessary.

CPT

81410	Aortic dysfunction or dilation (eg, Marfan syndrome, Loeys Dietz syndrome, Ehler Danlos syndrome type IV, arterial tortuosity syndrome); genomic sequence analysis panel, must include sequencing of at least 9 genes, including *FBN1, TGFBR1, TGFBR2, COL3A1, MYH11, ACTA2, SLC2A10, SMAD3,* and *MYLK*
81411	Aortic dysfunction or dilation (eg, Marfan syndrome, Loeys Dietz syndrome, Ehler Danlos syndrome type IV, arterial tortuosity syndrome); duplication/deletion analysis panel, must include analyses for *TGFBR1, TGFBR2, MYH11,* and *COL3A1*
81413	Cardiac ion channelopathies (eg, Brugada syndrome, long QT syndrome, short QT syndrome, catecholaminergic polymorphic ventricular tachycardia); genomic sequence analysis panel, must include sequencing of at least 10 genes, including *ANK2, CASQ2, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNQ1, RYR2,* and *SCN5A*
81419	Epilepsy genomic sequence analysis panel, must include analyses for *ALDH7A1, CACNA1A, CDKL5, CHD2, GABRG2, GRIN2A, KCNQ2, MECP2, PCDH19, POLG,*

	PRRT2, *SCN1A*, *SCN1B*, *SCN2A*, *SCN8A*, *SLC2A1*, *SLC9A6*, *STXBP1*, *SYNGAP1*, *TCF4*, *TPP1*, *TSC1*, *TSC2*, and *ZEB2*
81430	Hearing loss (eg, nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); genomic sequence analysis panel, must include sequencing of at least 60 genes, including *CDH23*, *CLRN1*, *GJB2*, *GPR98*, *MTRNR1*, *MYO7A*, *MYO15A*, *PCDH15*, *OTOF*, *SLC26A4*, *TMC1*, *TMPRSS3*, *USH1C*, *USH1G*, *USH2A*, and *WFS1*
81431	Hearing loss (eg, nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); duplication/deletion analysis panel, must include copy number analyses for *STRC* and *DFNB1* deletions in *GJB2* and *GJB6* genes
81439	Hereditary cardiomyopathy (eg, hypertrophic cardiomyopathy, dilated cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy) genomic sequence analysis panel, must include sequencing of at least 5 cardiomyopathy-related genes (eg, DSG2, MYBPC3, MYH7, PKP2, TTN)
81440	Nuclear encoded mitochondrial genes (eg, neurologic or myopathic phenotypes), genomic sequence panel, must include analysis of at least 100 genes, including *BCS1L*, *C10orf2*, *COQ2*, *COX10*, *DGUOK*, *MPV17*, *OPA1*, *PDSS2*, *POLG*, *POLG2*, *RRM2B*, *SCO1*, *SCO2*, *SLC25A4*, *SUCLA2*, *SUCLG1*, *TAZ*, *TK2*, and *TYMP*
81441	Inherited bone marrow failure syndromes (IBMFS) (eg, Fanconi anemia, dyskeratosis congenita, Diamond-Blackfan anemia, Shwachman-Diamond syndrome, GATA2 deficiency syndrome, congenital amegakaryocytic thrombocytopenia) sequence analysis panel, must include sequencing of at least 30 genes, including BRCA2, BRIP1, DKC1, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, GATA1, GATA2, MPL, NHP2, NOP10, PALB2, RAD51C, RPL11, RPL35A, RPL5, RPS10, RPS19, RPS24, RPS26, RPS7, SBDS, TERT, and TINF2
81442	Noonan spectrum disorders (eg, Noonan syndrome, cardio-facio-cutaneous syndrome, Costello syndrome, LEOPARD syndrome, Noonan-like syndrome), genomic sequence analysis panel, must include sequencing of at least 12 genes, including *BRAF*, *CBL*, *HRAS*, *KRAS*, *MAP2K1*, *MAP2K2*, *NRAS*, *PTPN11*, *RAF1*, *RIT1*, *SHOC2*, and *SOS1*
81443	Genetic testing for severe inherited conditions (eg, cystic fibrosis, Ashkenazi Jewish-associated disorders [eg, Bloom syndrome, Canavan disease, Fanconi anemia type C, mucolipidosis type VI, Gaucher disease, Tay-Sachs disease], beta hemoglobinopathies, phenylketonuria, galactosemia), genomic sequence analysis panel, must include sequencing of at least 15 genes (eg, *ACADM*, *ARSA*, *ASPA*, *ATP7B*, *BCKDHA*, *BCKDHB*, *BLM*, *CFTR*, *DHCR7*, *FANCC*, *G6PC*, *GAA*, *GALT*, *GBA*, *GBE1*, *HBB*, *HEXA*, *IKBKAP*, *MCOLN1*, *PAH*)
81448	Hereditary peripheral neuropathies (eg, Charcot-Marie-Tooth, spastic paraplegia), genomic sequence analysis panel, must include sequencing of at least 5 peripheral neuropathy-related genes (eg, *BSCL2*, *GJB1*, *MFN2*, *MPZ*, *REEP1*, *SPAST*, *SPG11*, *SPTLC1*)
81470	X-linked intellectual disability (XLID) (eg, syndromic and non-syndromic XLID); genomic sequence analysis panel, must include sequencing of at least 60 genes,

	including *ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL, RPS6KA3,* and *SLC16A2*
81471	X-linked intellectual disability (XLID) (eg, syndromic and non-syndromic XLID); duplication/deletion gene analysis, must include analysis of at least 60 genes, including *ARX, ATRX, CDKL5, FGD1, FMR1, HUWE1, IL1RAPL, KDM5C, L1CAM, MECP2, MED12, MID1, OCRL, RPS6KA3,* and *SLC16A2*
81479	Unlisted molecular pathology procedure [when specified as an inherited disease gene panel that does not meet the medically necessary criteria, such as the following: Counsyl, GeneVu, GoodStart Select, Inherigen, Inheritest Carrier Screen, Natera Horizon]
81599	Unlisted multianalyte assay with algorithmic analysis [when specified as a gene panel for inherited disease other than those listed as medically necessary, including but not limited to Macula Risk® PGx. RetnaGene™ AMD]
0205U	Ophthalmology (age-related macular degeneration), analysis of 3 gene variants (2 CFH gene, 1 ARMS2 gene), using PCR and MALDI-TOF, buccal swab, reported as positive or negative for neovascular age-related macular-degeneration risk associated with zinc supplements Vita Risk®, Arctic Medical Laboratories, Arctic Medical Laboratories
0216U	Neurology (inherited ataxias), genomic DNA sequence analysis of 12 common genes including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants Genomic Unity® Ataxia Repeat Expansion and Sequence Analysis, Variantyx Inc, Variantyx Inc
0217U	Neurology (inherited ataxias), genomic DNA sequence analysis of 51 genes including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants Genomic Unity® Comprehensive Ataxia Repeat Expansion and Sequence Analysis, Variantyx Inc, Variantyx Inc
0237U	Cardiac ion channelopathies (eg, Brugada syndrome, long QT syndrome, short QT syndrome, catecholaminergic polymorphic ventricular tachycardia), genomic sequence analysis panel including *ANK2, CASQ2, CAV3, KCNE1, KCNE2, KCNH2, KCNJ2, KCNQ1, RYR2,* and *SCN5A,* including small sequence changes in exonic and intronic regions, deletions, duplications, mobile element insertions, and variants in non-uniquely mappable regions Genomic Unity® Cardiac Ion Channelopathies Analysis, Variantyx Inc, Variantyx Inc
0268U	Hematology (atypical hemolytic uremic syndrome [aHUS]), genomic sequence analysis of 15 genes, blood, buccal swab, or amniotic fluid Versiti™ aHUS Genetic Evaluation, Versiti™ Diagnostic Laboratories, Versiti™

0269U	Hematology (autosomal dominant congenital thrombocytopenia), genomic sequence analysis of 14 genes, blood, buccal swab, or amniotic fluid Versiti™ Autosomal Dominant Thrombocytopenia Panel, Versiti™ Diagnostic Laboratories, Versiti™
0270U	Hematology (congenital coagulation disorders), genomic sequence analysis of 20 genes, blood, buccal swab, or amniotic fluid Versiti™ Coagulation Disorder Panel, Versiti™ Diagnostic Laboratories, Versiti™
0271U	Hematology (congenital neutropenia), genomic sequence analysis of 23 genes, blood, buccal swab, or amniotic fluid Versiti™ Congenital Neutropenia Panel, Versiti™ Diagnostic Laboratories, Versiti™
0272U	Hematology (genetic bleeding disorders), genomic sequence analysis of 51 genes, blood, buccal swab, or amniotic fluid, comprehensive Versiti™ Comprehensive Bleeding Disorder Panel, Versiti™ Diagnostic Laboratories, Versiti™
0273U	Hematology (genetic hyperfibrinolysis, delayed bleeding), genomic sequence analysis of 8 genes (F13A1, F13B, FGA, FGB, FGG, SERPINA1, SERPINE1, SERPINF2, PLAU), blood, buccal swab, or amniotic fluid Versiti™ Fibrinolytic Disorder Panel, Versiti™ Diagnostic Laboratories, Versiti™
0274U	Hematology (genetic platelet disorders), genomic sequence analysis of 43 genes, blood, buccal swab, or amniotic fluid Versiti™ Comprehensive Platelet Disorder Panel, Versiti™ Diagnostic Laboratories, Versiti™
0276U	Hematology (inherited thrombocytopenia), genomic sequence analysis of 42 genes, blood, buccal swab, or amniotic fluid Versiti™ Inherited Thrombocytopenia Panel, Versiti™ Diagnostic Laboratories, Versiti™
0277U	Hematology (genetic platelet function disorder), genomic sequence analysis of 31 genes, blood, buccal swab, or amniotic fluid Versiti™ Platelet Function Disorder Panel, Versiti™ Diagnostic Laboratories, Versiti™
0278U	Hematology (genetic thrombosis), genomic sequence analysis of 12 genes, blood, buccal swab, or amniotic fluid Versiti™ Thrombosis Panel, Versiti™ Diagnostic Laboratories, Versiti™
0400U	Obstetrics (expanded carrier screening), 145 genes by next-generation sequencing, fragment analysis and multiplex ligation-dependent probe amplification, DNA, reported as carrier positive or negative Genesys Carrier Panel, Genesys Diagnostics, Inc [Note: code is effective 07/01/2023]

ICD-10 Diagnosis

All diagnoses

Gene Panel Testing for Cancer Susceptibility and Management

When services may be Medically Necessary when criteria are met:

CPT	
	For cancer susceptibility:
81432	Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer); genomic sequence analysis panel, must include sequencing of at least 10 genes, always including *BRCA1*, *BRCA2*, *CDH1*, *MLH1*, *MSH2*, *MSH6*, *PALB2*, *PTEN*, *STK11*, and *TP53* [for breast cancer testing ~~of less than 51 genes and~~ when genes *ATM*, *BARD1*, *CHEK2*, *RAD51C*, and *RAD51D* are also included]
81433	Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer); duplication/deletion analysis panel, must include analyses for *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, and *STK11* [for breast cancer testing ~~of less than 51 genes and~~ when genes *ATM*, *BARD1*, *CHEK2*, *PALB2*, *RAD51C*, and *RAD51D* are also included]
81435	Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis); genomic sequence analysis panel, must include sequencing of at least 10 genes, including *APC*, *BMPR1A*, *CDH1*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *PTEN*, *SMAD4*, and *STK11* [for Lynch syndrome testing ~~of less than 51 genes and~~ when genes *EPCAM* and *PMS2* are also included]
81436	Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis); duplication/deletion analysis panel, must include analysis of at least 5 genes including *MLH1*, *MSH2*, *EPCAM*, *SMAD4*, and *STK11* [for Lynch syndrome testing ~~of less than 51 genes and~~ when genes *MSH6* and *PMS2* are also included]
0101U	Hereditary colon cancer disorders (eg, Lynch syndrome, *PTEN* hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with MRNA analytics to resolve variants of unknown significance when indicated (15 genes [sequencing and deletion/duplication], *EPCAM* and *GREM1* [deletion/duplication only]) ColoNext®, Ambry Genetics®, Ambry Genetics®
0102U	Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with MRNA analytics to resolve variants of unknown significance when indicated (17 genes [sequencing and deletion/duplication]) BreastNext®, Ambry Genetics®, Ambry Genetics®
0103U	Hereditary ovarian cancer (eg, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with MRNA analytics to resolve variants of unknown significance when indicated (24 genes [sequencing and deletion/duplication], *EPCAM* [deletion/duplication

	only]) OvaNext®, Ambry Genetics®, Ambry Genetics®
0238U	Oncology (Lynch syndrome), genomic DNA sequence analysis of *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM*, including small sequence changes in exonic and intronic regions, deletions, duplications, mobile element insertions, and variants in non-uniquely mappable regions Genomic Unity® Lynch Syndrome Analysis, Variantyx Inc, Variantyx Inc **For cancer susceptibility** (breast, Lynch syndrome) **or management** (NSCLC, prostate cancer, ALL, AML, MDS, IVD)
81445	Targeted genomic sequence analysis panel, solid organ neoplasm, 5-50 genes (eg, *ALK, BRAF, CDKN2A, EGFR, ERBB2, KIT, KRAS, NRAS, MET, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET*), interrogation for sequence variants and copy number variants or rearrangements, if performed; DNA analysis or combined DNA and RNA analysis [when specified as one of the following]: • Breast cancer panel test including at a minimum *ATM, BARD1, BRCA1, BRCA2, CHEK2, PALB2, RAD51C*, and *RAD51D* genes • Lynch Syndrome panel test including at a minimum *EPCAM, MLH1, MSH2, MSH6*, and *PMS2* genes • NSCLC panel test including at a minimum *ALK, BRAF, EGFR, ERBB2* (*HER2*), *KRAS, MET, NTRK, RET* and *ROS1* genes • Prostate cancer panel to evaluate deleterious germline or somatic homologous recombination repair (HRR) genes (eg, *ATM, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, PPP2R2A, RAD51B, RAD51C, RAD51D, RAD54L)*
81450	Targeted genomic sequence analysis panel, hematolymphoid neoplasm or disorder, 5-50 genes (eg, *BRAF, CEBPA, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KRAS, KIT, MLL, NRAS, NPM1, NOTCH1*), interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis [when specified as one of the following]: • Acute lymphoblastic leukemia (ALL) panel test including at a minimum *ABL1, ABL2, CRLF2, CSF1R, FLT3, IL7R, JAK1, JAK2, JAK3, PDGFRB*, and *SH2B3* genes • Acute myeloid leukemia (AML) panel test including at a minimum *ASXL1, BCR-ABL, c-KIT, CEBPA* (biallelic), *FLT3-ITD, FLT3-TKD, IDH1, IDH2, NPM1, PML-RAR alpha, RUNX1*, and *TP53* genes • Myelodysplastic syndrome (MDS) panel test including at a minimum *ASXL1, DNMT3A, EZH2, NRAS, RUNX1, SF3B1, SRSF2, STAG2, TET2, TP53, U2AF1*, and *ZRSR2* genes
81455	Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm, 51 or greater genes (eg, *ALK, BRAF, CDKN2A, CEBPA, DNMT3A, EGFR, ERBB2, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MLL, NPM1, NRAS, MET, NOTCH1, PDGFRA,*

PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis [when specified as one of the following:

- Acute lymphoblastic leukemia (ALL) gene panel, including at a minimum *ABL1, ABL2, CRLF2, CSF1R, FLT3, IL7R, JAK1, JAK2, JAK3, PDGFRB*, and *SH2B3* genes
- Acute myeloid leukemia (AML) gene panel, including at a minimum *ASXL1, BCR-ABL, c-KIT, CEBPA* (biallelic), *FLT3-ITD, FLT3-TKD, IDH1, IDH2, NPM1, PML-RAR alpha, RUNX1*, and *TP53* genes
- Breast cancer gene panel, including at a minimum *ATM, BARD1, BRCA1, BRCA2, CHEK2, PALB2, RAD51C*, and *RAD51D* genes
- Lynch Syndrome gene panel, including at a minimum *EPCAM, MLH1, MSH2, MSH6*, and *PMS2* genes
- Myelodysplastic syndrome (MDS) gene panel, including at a minimum *ASXL1, DNMT3A, EZH2, NRAS, RUNX1, SF3B1, SRSF2, STAG2, TET2, TP53, U2AF1*, and *ZRSR2* genes
- NSCLC gene panel, including at a minimum *ALK, BRAF, EGFR, ERBB2 (HER2), KRAS, MET, NTRK, RET* and *ROS1* genes
- Prostate cancer gene panel to evaluate deleterious germline or somatic homologous recombination repair (HRR) genes (eg, *ATM, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, PPP2R2A, RAD51B, RAD51C, RAD51D, RAD54L)*
- An In Vitro Companion Diagnostic Device

81479 Unlisted molecular pathology procedure [when specified as one of the following panels]:

- Acute lymphoblastic leukemia (ALL) ~~5-50~~ gene panel, including at a minimum *ABL1, ABL2, CRLF2, CSF1R, FLT3, IL7R, JAK1, JAK2, JAK3, PDGFRB*, and *SH2B3* genes
- Acute myeloid leukemia (AML) ~~5-50~~ gene panel, including at a minimum *ASXL1, BCR-ABL, c-KIT, CEBPA* (biallelic), *FLT3-ITD, FLT3-TKD, IDH1, IDH2, NPM1, PML-RAR alpha, RUNX1*, and *TP53* genes
- Breast cancer ~~5-50~~ gene panel, including at a minimum *ATM, BARD1, BRCA1, BRCA2, CHEK2, PALB2, RAD51C*, and *RAD51D* genes
- Lynch Syndrome ~~5-50~~ gene panel, including at a minimum *EPCAM, MLH1, MSH2, MSH6*, and *PMS2* genes
- Myelodysplastic syndrome (MDS) ~~5-50~~ gene panel, including at a minimum *ASXL1, DNMT3A, EZH2, NRAS, RUNX1, SF3B1, SRSF2, STAG2, TET2, TP53, U2AF1*, and *ZRSR2* genes
- NSCLC ~~5-50~~ gene panel, including at a minimum *ALK, BRAF, EGFR, ERBB2* (*HER2*), *KRAS, MET, NTRK, RET* and *ROS1* genes
- Prostate cancer ~~5-50~~ gene panel to evaluate deleterious germline or somatic homologous recombination repair (HRR) genes (eg, *ATM, BARD1, BRCA1, BRCA2,*

BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, PPP2R2A, RAD51B, RAD51C, RAD51D, RAD54L)

- An In Vitro Companion Diagnostic Device

~~0101U~~ ~~Hereditary colon cancer disorders (eg, Lynch syndrome, *PTEN* hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with MRNA analytics to resolve variants of unknown significance when indicated (15 genes [sequencing and deletion/duplication], *EPCAM* and *GREM1* [deletion/duplication only])~~
~~ColoNext®, Ambry Genetics®, Ambry Genetics®~~

~~0102U~~ ~~Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with MRNA analytics to resolve variants of unknown significance when indicated (17 genes [sequencing and deletion/duplication])~~
~~BreastNext®, Ambry Genetics®, Ambry Genetics®~~

~~0103U~~ ~~Hereditary ovarian cancer (eg, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with MRNA analytics to resolve variants of unknown significance when indicated (24 genes [sequencing and deletion/duplication], *EPCAM* [deletion/duplication only])~~
~~OvaNext®, Ambry Genetics®, Ambry Genetics®~~

~~0238U~~ ~~Oncology (Lynch syndrome), genomic DNA sequence analysis of *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM*, including small sequence changes in exonic and intronic regions, deletions, duplications, mobile element insertions, and variants in non-uniquely mappable regions~~
~~Genomic Unity® Lynch Syndrome Analysis, Variantyx Inc, Variantyx Inc~~

0022U Targeted genomic sequence analysis panel, non-small cell lung neoplasia, DNA and RNA analysis, 23 genes, interrogation for sequence variants and rearrangements, reported as presence or absence of variants and associated therapy(ies) to consider
Oncomine™ Dx Target Test, Thermo Fisher Scientific, Thermo Fisher Scientific

0050U Targeted genomic sequence analysis panel, acute myelogenous leukemia, DNA analysis, 194 genes, interrogation for sequence variants, copy number variants or rearrangements
MyAML NGS Panel; LabPMM LLC, an Invivoscribe Technologies, Inc. Company

ICD-10 Diagnosis

All diagnoses

When services are Not Medically Necessary

For the procedure codes listed above when criteria are not met, for the following codes, or when the code describes a procedure indicated in the Position Statement section as not medically necessary.

CPT	
81437	Hereditary neuroendocrine tumor disorders (eg, medullary thyroid carcinoma, parathyroid carcinoma, malignant pheochromocytoma or paraganglioma); genomic sequence analysis panel, must include sequencing of at least 6 genes, including *MAX*, *SDHB*, *SDHC*, *SDHD*, *TMEM127*, and *VHL*
81438	Hereditary neuroendocrine tumor disorders (eg, medullary thyroid carcinoma, parathyroid carcinoma, malignant pheochromocytoma or paraganglioma); duplication/deletion analysis panel, must include analyses for *SDHB*, *SDHC*, *SDHD*, and *VHL*
81449	Targeted genomic sequence analysis panel, solid organ neoplasm, 5-50 genes (eg, ALK, BRAF, CDKN2A, EGFR, ERBB2, KIT, KRAS, MET, NRAS, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed; RNA analysis
81451	Targeted genomic sequence analysis panel, hematolymphoid neoplasm or disorder, 5-50 genes (eg, BRAF, CEBPA, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MLL, NOTCH1, NPM1, NRAS), interrogation for sequence variants, and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; RNA analysis
~~81455~~	~~Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm, 51 or greater genes (eg, *ALK, BRAF, CDKN2A, CEBPA, DNMT3A, EGFR, ERBB2, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MLL, NPM1, NRAS, MET, NOTCH1, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET*), interrogation for sequence variants and copy number variants or rearrangements or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis~~
81456	Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes (eg, ALK, BRAF, CDKN2A, CEBPA, DNMT3A, EGFR, ERBB2, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MET, MLL, NOTCH1, NPM1, NRAS, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; RNA analysis
81479	Unlisted molecular pathology procedure [when specified as a gene panel that does not meet medically necessary criteria]
81599	Unlisted multianalyte assay with algorithmic analysis [when specified as a gene panel that does not meet medically necessary criteria]
~~0050U~~	~~Targeted genomic sequence analysis panel, acute myelogenous leukemia, DNA analysis, 194 genes, interrogation for sequence variants, copy number variants or rearrangements MyAML NGS Panel; LabPMM LLC, an Invivoscribe Technologies, Inc. Company~~
0129U	Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis and deletion/duplication analysis panel (ATM, BRCA1, BRCA2, CDH1, CHEK2, PALB2, PTEN, and TP53) BRCAplus, Ambry Genetics

0343U	Oncology (prostate), exosome-based analysis of 442 small noncoding RNAs (sncRNAs) by quantitative reverse transcription polymerase chain reaction (RT-qPCR), urine, reported as molecular evidence of no-, low-, intermediate- or high-risk of prostate cancer miR Sentinel™ Prostate Cancer Test, miR Scientific, LLC, miR Scientific, LLC

ICD-10 Diagnosis

All diagnoses

Whole Exome Sequencing

When services may be Medically Necessary when criteria are met:

CPT

81415	Exome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis
81416	Exome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator exome (eg, parents, siblings)
81417	Exome (eg, unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained exome sequence (eg, updated knowledge or unrelated condition/syndrome)
0214U	Rare diseases (constitutional/heritable disorders), whole exome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, proband Genomic Unity® Exome Plus Analysis - Proband, Variantyx Inc, Variantyx Inc
0215U	Rare diseases (constitutional/heritable disorders), whole exome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, each comparator exome (eg, parent, sibling) Genomic Unity® Exome Plus Analysis - Comparator, Variantyx Inc, Variantyx Inc

ICD-10 Diagnosis

All diagnoses

When services are Not Medically Necessary

For the procedure codes listed above when criteria are not met, for the following procedure code, or when the code describes a procedure indicated in the Position Statement section as not medically necessary.

CPT

0036U	Exome (ie, somatic mutations), paired formalin-fixed paraffin-embedded tumor tissue and normal specimen, sequence analyses

EXaCT-1 Whole Exome Testing; Lab of Oncology-Molecular Detection, Weill Cornell Medicine Clinical Genomics Laboratory

ICD-10-Diagnosis

All diagnoses

Molecular profiling

When services may be Medically Necessary when criteria are met:

CPT

Including, but not limited to, the following:

81479	Unlisted molecular pathology procedure [when specified as a molecular profiling panel test using plasma specimen, for example the LiquidHallmark test]
0037U	Targeted genomic sequence analysis, solid organ neoplasm, DNA analysis of 324 genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability and tumor mutational burden FoundationOne CDx™ (F1CDx); Foundation Medicine, Inc.
0048U	Oncology (solid organ neoplasia), DNA, targeted sequencing of protein-coding exons of 468 cancer-associated genes, including interrogation for somatic mutations and microsatellite instability, matched with normal specimens, utilizing formalin-fixed paraffin-embedded tumor tissue, report of clinically significant mutation(s) MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets); Memorial Sloan Kettering Cancer Center
0211U	Oncology (pan-tumor), DNA and RNA by next-generation sequencing, utilizing formalin-fixed paraffin-embedded tissue, interpretative report for single nucleotide variants, copy number alterations, tumor mutational burden, and microsatellite instability, with therapy association MI Cancer Seek™ - NGS Analysis, Caris MPI d/b/a Caris Life Sciences, Caris MPI d/b/a Caris Life Sciences
0244U	Oncology (solid organ), DNA, comprehensive genomic profiling, 257 genes, interrogation for single-nucleotide variants, insertions/deletions, copy number alterations, gene rearrangements, tumor-mutational burden and microsatellite instability, utilizing formalin-fixed paraffin-embedded tumor tissue Oncotype MAP™ PanCancer Tissue Test, Paradigm Diagnostics, Inc, Paradigm Diagnostics, Inc
0250U	Oncology (solid organ neoplasm), targeted genomic sequence DNA analysis of 505 genes, interrogation for somatic alterations (SNVs [single nucleotide variant], small insertions and deletions, one amplification, and four translocations), microsatellite instability and tumor-mutation burden PGDx elio™ tissue complete, Personal Genome Diagnostics, Inc, Personal Genome Diagnostics, Inc

0326U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free circulating DNA analysis of 83 or more genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability and tumor mutational burden Guardant360, Guardant Health Inc.
0329U	Oncology (neoplasia), exome and transcriptome sequence analysis for sequence variants, gene copy number amplifications and deletions, gene rearrangements, microsatellite instability and tumor mutational burden utilizing DNA and RNA from tumor with DNA from normal blood or saliva for subtraction, report of clinically significant mutation(s) with therapy associations Oncomap™ ExTra; Exact Sciences; Genomic Health, Inc.
0334U	Oncology (solid organ), targeted genomic sequence analysis, formalin-fixed paraffin-embedded (FFPE) tumor tissue, DNA analysis, 84 or more genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability and tumor mutational burden Guardant360 TissueNext™, Guardant Health, Inc, Guardant Health, Inc
0379U	Targeted genomic sequence analysis panel, solid organ neoplasm, DNA (523 genes) and RNA (55 genes) by nextgeneration sequencing, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability, and tumor mutational burden Solid Tumor Expanded Panel, Quest Diagnostics®, Quest Diagnostics®
0391U	Oncology (solid tumor), DNA and RNA by next-generation sequencing, utilizing formalin-fixed paraffin-embedded (FFPE) tissue, 437 genes, interpretive report for single nucleotide variants, splice-site variants, insertions/deletions, copy number alterations, gene fusions, tumor mutational burden, and microsatellite instability, with algorithm quantifying immunotherapy response score Strata Select™, Strata Oncology, Inc, Strata Oncology, Inc [Note: code is effective 07/01/2023]

ICD-10 Diagnosis

C00.0-C80.2	Malignant neoplasms

When services are Investigational and Not Medically Necessary:
For the procedure and diagnosis codes listed above when criteria are not met or for all other diagnoses not listed, for the following procedure codes, or when the code describes a procedure indicated in the Position Statement section as investigational and not medically necessary.

CPT

81479	Unlisted molecular pathology procedure [when specified as a molecular profiling panel that does not meet medically necessary criteria]

81599	Unlisted multianalyte assay with algorithmic analysis [when specified as a molecular profiling panel that does not meet medically necessary criteria]

ICD-10 Diagnosis

All diagnoses

Circulating Tumor DNA (ctDNA) Panel Testing

When services may be Medically Necessary when criteria are met:

CPT

81479	Unlisted molecular pathology procedure [when specified as one of the following ctDNA panels]: • NSCLC cell-free DNA panel, including at a minimum *ALK*, *BRAF*, *EGFR*, *ERBB2* (*HER2*), *KRAS*, *MET*, *NTRK*, *RET* and *ROS1* genes • Prostate cancer cell-free DNA panel to evaluate deleterious germline or somatic homologous recombination repair (HRR) genes (eg, *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDK12*, *CHEK1*, *CHEK2*, *FANCL*, *PALB2*, *PPP2R2A*, *RAD51B*, *RAD51C*, *RAD51D*, *RAD54L)* • An In Vitro Companion Diagnostic Device
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™, Resolution Bioscience, Resolution Bioscience, Inc
0239U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free DNA, analysis of 311 or more genes, interrogation for sequence variants, including substitutions, insertions, deletions, select rearrangements, and copy number variations FoundationOne® Liquid CDx, FOUNDATION MEDICINE, INC, FOUNDATION MEDICINE, INC
0242U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free circulating DNA analysis of 55-74 genes, interrogation for sequence variants, gene copy number amplifications, and gene rearrangements Guardant360® CDx, Guardant Health Inc, Guardant Health Inc
0388U	Oncology (non-small cell lung cancer), next-generation sequencing with identification of single nucleotide variants, copy number variants, insertions and deletions, and structural variants in 37 cancer-related genes, plasma, with report for alteration detection InVisionFirst®-Lung Liquid Biopsy, Inivata, Inc, Inivata, Inc [Note: code is effective 07/01/2023]
0397U	Oncology (non-small cell lung cancer), cell-free DNA from plasma, targeted sequence analysis of at least 109 genes, including sequence variants, substitutions, insertions, deletions, select rearrangements, and copy number variations

Agilent Resolution ctDx FIRST, Resolution Bioscience, Inc, Resolution Bioscience, Inc [Note: code is effective 07/01/2023]

ICD-10 Diagnosis

All diagnoses

When services are Not Medically Necessary:

For the procedure codes listed above when criteria are not met, for the following procedure codes, or when the code describes a procedure indicated in the Position Statement section as not medically necessary.

CPT

81479	Unlisted molecular pathology procedure [when specified as a liquid biopsy panel using plasma specimen that does not meet medically necessary criteria]
0306U	Oncology (minimal residual disease [MRD]), next-generation targeted sequencing analysis, cell-free DNA, initial (baseline) assessment to determine a patient-specific panel for future comparisons to evaluate for MRD Invitae PCM Tissue Profiling and MRD Baseline Assay, Invitae Corporation, Invitae Corporation
0307U	Oncology (minimal residual disease [MRD]), next-generation targeted sequencing analysis of a patient-specific panel, cell-free DNA, subsequent assessment with comparison to previously analyzed patient specimens to evaluate for MRD Invitae PCM MRD Monitoring, Invitae Corporation, Invitae Corporation
0333U	Oncology (liver), surveillance for hepatocellular carcinoma (HCC) in high-risk patients, analysis of methylation patterns on circulating cell-free DNA (cfDNA) plus measurement of serum of AFP/AFP-L3 and oncoprotein des-gamma-carboxy-prothrombin (DCP), algorithm reported as normal or abnormal result HelioLiver™ Test, Fulgent Genetics, LLC, Helio Health, Inc
0356U	Oncology (oropharyngeal), evaluation of 17 DNA biomarkers using droplet digital PCR (ddPCR), cell-free DNA, algorithm reported as a prognostic risk score for cancer recurrence NavDx®, Naveris, Inc, Naveris, Inc
0368U	Oncology (colorectal cancer), evaluation for mutations of APC, BRAF, CTNNB1, KRAS, NRAS, PIK3CA, SMAD4, and TP53, and methylation markers (MYO1G, KCNQ5, C9ORF50, FLI1, CLIP4, ZNF132 and TWIST1), multiplex quantitative polymerase chain reaction (qPCR), circulating cell-free DNA (cfDNA), plasma, report of risk score for advanced adenoma or colorectal cancer ColoScape™ Colorectal Cancer Detection, DiaCarta Clinical Lab, DiaCarta, Inc

ICD-10 Diagnosis

All diagnoses

Other panels (Whole Genome, Whole Transcriptome, Polygenic Risk Scoring, Chromosome conformation signatures)

When services are Investigational and Not Medically Necessary:

For the following codes, or when the code describes a procedure indicated in the Position Statement section as investigational and not medically necessary.

CPT	
81425	Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis
81426	Genome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator exome (eg, parents, siblings)
81427	Genome (eg, unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained genome sequence (eg, updated knowledge or unrelated condition/syndrome)
81460	Whole mitochondrial genome (eg, Leigh syndrome, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes [MELAS], myoclonic epilepsy with ragged-red fibers [MERFF], neuropathy, ataxia, and retinitis pigmentosa [NARP], Leber hereditary optic neuropathy [LHON]), genomic sequence, must include sequence analysis of entire mitochondrial genome with heteroplasmy detection
81465	Whole mitochondrial genome large deletion analysis panel (eg, Kearns-Sayre syndrome, chronic progressive external ophthalmoplegia), including heteroplasmy detection if performed
81479	Unlisted molecular pathology procedure [when specified as a whole genome, whole transcriptome or polygenic risk score test]
81599	Unlisted multianalyte assay with algorithmic analysis [when specified as a whole genome, whole transcriptome or polygenic risk score test]
0094U	Genome (eg, unexplained constitutional or heritable disorder or syndrome), rapid sequence analysis RCIGM Rapid Whole Genome Sequencing, Rady Children's Institute for Genomic Medicine (RCIGM)
0212U	Rare diseases (constitutional/heritable disorders), whole genome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, proband Genomic Unity® Whole Genome Analysis - Proband, Variantyx Inc, Variantyx Inc
0213U	Rare diseases (constitutional/heritable disorders), whole genome and mitochondrial DNA sequence analysis, including small sequence changes, deletions, duplications, short tandem repeat gene expansions, and variants in non-uniquely mappable regions, blood or saliva, identification and categorization of genetic variants, each comparator genome (eg,

	parent, sibling) Genomic Unity® Whole Genome Analysis - Comparator, Variantyx Inc, Variantyx Inc
0260U	Rare diseases (constitutional/heritable disorders), identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping Augusta Optical Genome Mapping, Georgia Esoteric and Molecular (GEM) Laboratory, LLC, Bionano Genomics Inc
0264U	Rare diseases (constitutional/heritable disorders), identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping Praxis Optical Genome Mapping, Praxis Genomics LLC
0265U	Rare constitutional and other heritable disorders, whole genome and mitochondrial DNA sequence analysis, blood, frozen and formalin-fixed paraffin embedded (FFPE) tissue, saliva, buccal swabs or cell lines, identification of single nucleotide and copy number variants Praxis Whole Genome Sequencing, Praxis Genomics LLC
0266U	Unexplained constitutional or other heritable disorders or syndromes, tissue specific gene expression by whole transcriptome and next-generation sequencing, blood, formalin-fixed paraffin embedded (FFPE) tissue or fresh frozen tissue, reported as presence or absence of splicing or expression changes Praxis Transcriptome, Praxis Genomics LLC
0267U	Rare constitutional and other heritable disorders, identification of copy number variations, inversions, insertions, translocations, and other structural variants by optical genome mapping and whole genome sequencing Praxis Combined Whole Genome Sequencing and Optical Genome Mapping, Praxis Genomics LLC
0297U	Oncology (pan tumor), whole genome sequencing of paired malignant and normal DNA specimens, fresh or formalin-fixed paraffin-embedded (FFPE) tissue, blood or bone marrow, comparative sequence analyses and variant identification Praxis Somatic Whole Genome Sequencing, Praxis Genomics LLC
0298U	Oncology (pan tumor), whole transcriptome sequencing of paired malignant and normal RNA specimens, fresh or formalin-fixed paraffin-embedded (FFPE) tissue, blood or bone marrow, comparative sequence analyses and expression level and chimeric transcript identification Praxis Somatic Transcriptome, Praxis Genomics LLC
0299U	Oncology (pan tumor), whole genome optical genome mapping of paired malignant and normal DNA specimens, fresh frozen tissue, blood, or bone marrow, comparative structural variant identification Praxis Somatic Optical Genome Mapping, Praxis Genomics LLC
0300U	Oncology (pan tumor), whole genome sequencing and optical genome mapping of paired malignant and normal DNA specimens, fresh tissue, blood, or bone marrow, comparative

	sequence analyses and variant identification Praxis Somatic Combined Whole Genome Sequencing and Optical Genome Mapping, Praxis Genomics LLC
0331U	Oncology (hematolymphoid neoplasia), optical genome mapping for copy number alterations and gene rearrangements utilizing DNA from blood or bone marrow, report of clinically significant alternations Augusta Hematology Optical Genome Mapping, Georgia Esoteric and Molecular Labs, Augusta University,~~;~~ Bionano ~~Genomics~~
0332U	Oncology (pan-tumor), genetic profiling of 8 DNA-regulatory (epigenetic) markers by quantitative polymerase chain reaction (qPCR), whole blood, reported as a high or low probability of responding to immune checkpoint–inhibitor therapy EpiSwitch® CiRT (Checkpoint-inhibitor Response Test), Next Bio-Research Services, LLC, Oxford BioDynamics, PLC
0335U	Rare diseases (constitutional/heritable disorders), whole genome sequence analysis, including small sequence changes, copy number variants, deletions, duplications, mobile element insertions, uniparental disomy (UPD), inversions, aneuploidy, mitochondrial genome sequence analysis with heteroplasmy and large deletions, short tandem repeat (STR) gene expansions, fetal sample, identification and categorization of genetic variants IriSight™ Prenatal Analysis – Proband, Variantyx, Inc, Variantyx, Inc
0336U	Rare diseases (constitutional/heritable disorders), whole genome sequence analysis, including small sequence changes, copy number variants, deletions, duplications, mobile element insertions, uniparental disomy (UPD), inversions, aneuploidy, mitochondrial genome sequence analysis with heteroplasmy and large deletions, short tandem repeat (STR) gene expansions, blood or saliva, identification and categorization of genetic variants, each comparator genome (eg, parent) IriSight™ Prenatal Analysis – Comparator, Variantyx, Inc, Variantyx, Inc
0401U	Cardiology (coronary heart disease [CAD]), 9 genes (12 variants), targeted variant genotyping, blood, saliva, or buccal swab, algorithm reported as a genetic risk score for a coronary event CARDIO inCode-Score (CIC-SCORE), GENinCode U.S. Inc, GENinCode U.S. Inc [Note: code is effective 07/01/2023]

ICD-10 Diagnosis

All diagnoses

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Caris Test
Circulogene
ClearID
elio Plasma Complete
EXaCT-1 Whole Exome Sequencing
FoundationOne
FoundationOne CDx
FoundationOne Liquid CDx
Galleri
GeneKey
GeneStrat
Genetic testing panels
Genetic testing using panels
Guardant360
Guardant 360 CDx
Guardant Reveal
LiquidHALLMARK
Liquid Biopsy
Ion Torrent Next Generation Sequencing Ion AmpliSeq
Macula Risk
Macula Risk PGx
MatePair
Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT)
Multi-Omic Molecular Profiling (MMP)
MyAML
myRisk Hereditary Cancer test
Navaris
NavDx
OmniSeq
OncInsights
OncoBEAM
Oncotype MAP™ PanCancer Tissue Test
OvaNext Test
SmartGenomics
RetnaGene AMD
Target Now Molecular Profiling Service
Target Selector
Tumor Mutation Burden
Tumor Portrait Test
23andMe Age-Related Macular Degeneration Genetic Health Risk Report
Vita Risk

The use of specific product names is illustrative only. It is not intended to be a recommendation of one product over another, and is not intended to represent a complete listing of all products available.

Document History

Status	Date	Action
Revised	05/11/2023	Medical Policy & Technology Assessment Committee (MPTAC) review. Reformatted hierarchy for gene panel testing for inherited diseases, testing for cancer susceptibility, testing for cancer management, and molecular profiling for the evaluation of malignancies. Revised panel testing criteria to remove 5-50 gene parameters. Revised AML MN statement to include "newly diagnosed or relapsed." Added circulating tumor DNA to scope of document (moved content from GENE.00049 into this document and added new criteria for prostate cancer and advance non-small cell lung cancer). Revised molecular profiling criteria to remove "progressed following prior treatment" language. Revised NMN statement for Whole Exome Sequencing to address repeat testing. Added In Vitro Companion Diagnostic Devices (IVD) to scope of document. Updated Description/Scope, Rationale, Background/Overview, Definitions, References, and Index sections. Updated Coding section to include 07/01/2023 CPT changes, added 0388U, 0391U, 0397U, 0400U, 0401U; added codes 0179U, 0239U, 0242U, 0306U, 0307U, 0326U, 0333U, 0356U, and 0368U previously addressed in GENE.00049.
	03/29/2023	Updated Coding section with 04/01/2023 CPT changes, added 0379U; also removed codes 0130U, 0131U, 0132U, 0134U, 0135U now addressed in GENE.00054.
Revised	11/10/2022	~~Medical Policy & Technology Assessment Committee (~~MPTAC~~)~~ review. Content from GENE.00037 Genetic Testing for Macular Degeneration transferred into this document. Updated Description/Scope, Rationale, Background/Overview, Definitions, References, and Index sections. Added Chromosome conformation signatures to scope of document and added to INV/NMN statement. Updated Coding section to add 0205U and AMD gene panels previously addressed in GENE.00037, and 81439 previously addressed in CG-GENE-23; also added code 0332U and updated with 01/01/2023 CPT changes to add 81441, 81449, 81451 and 81456 and descriptor changes for 81445, 81450, 81455.
	09/28/2022	Updated Coding section with 10/01/2022 CPT changes; added 0334U, 0335U, 0336U, 0343U; revised descriptor for 0276U; removed 0012U, 0013U, 0014U and 0056U deleted 09/30/2022.
	06/29/2022	Updated Coding section with 07/01/2022 CPT changes; added 0329U, 0331U.

Revised	02/17/2022	MPTAC review. Added polygenic risk score testing to the scope as investigational and not medically necessary. Clarified criteria for Lynch syndrome to add "containing 5-50 genes" and "at a minimum." Added MN statements for gene panel testing for initial evaluation of myelodysplastic syndromes, acute myeloid leukemia, and acute lymphoblastic leukemia. Clarified criteria for WES to clarify "live" fetus. Revised MN criteria for gene panel testing for prostate cancer to remove "Lynparza" and add "a poly (ADP-ribose)polymerase (PARP) inhibitor." Revised INV/NMN statement for testing for gene panels and whole exome sequencing to NMN only. Updated Description/Scope, Rationale, Background/Overview, Definitions, and References sections. Updated Coding section, including removing 0171U now addressed in CG-GENE-19.
Revised	11/11/2021	MPTAC review. Added MN criteria for breast cancer susceptibility using gene panels. Added MN criteria for advanced non-small cell lung cancer using gene panels. Added MN criteria for whole exome sequencing. Updated Description/Scope, Rationale, References, and Websites for Additional Information sections. Updated Coding section to include 01/01/2022 CPT changes, added 0297U, 0298U, 0299U, 0300U.
	10/01/2021	Updated Coding section with 10/01/2021 CPT changes; added 0260U, 0264U-0274U, 0276U-0278U.
	07/01/2021	Updated Coding section with 07/01/2021 CPT changes; added 0250U.
Reviewed	02/11/2021	MPTAC review. Updated Description/Scope, Rationale, References, and Index sections. Updated Coding section with 04/01/2021 CPT changes; added 0244U.
Revised	11/05/2020	MPTAC review. Added MN criteria for prostate cancer using gene panels when the panel evaluates HRR repair gene alterations and an individual is a candidate for treatment with Lynparza (olaparib). Updated Rationale and Reference sections. Updated Coding section to include 01/01/2021 CPT changes to add 81419, 0237U, 0238U.
Revised	08/13/2020	MPTAC review. Removed MN indication for molecular profiling for NSCLC. Added MN indication for molecular profiling for unresectable or metastatic solid tumors. Updated Rationale and References sections. Updated Coding section to include 10/01/2020 CPT changes, added 0211U-0217U; added 81448 previously addressed in GENE.00033.
	07/08/2020	Updated Coding section; added 81413 previously addressed in GENE.00007.
	04/01/2020	Updated Coding section with 04/01/2020 CPT changes; added 0171U.
Revised	01/13/2020	MPTAC review. Addition to Position Statement regarding gene panel testing for Lynch Syndrome. Updated Rationale and Coding sections.
New	11/07/2019	MPTAC review. Initial document development. Moved content regarding whole genome sequencing, whole exome sequencing, gene panel tests and molecular profiling from GENE.00001 Genetic Testing for Cancer Susceptibility, GENE.00012 Preconception or Prenatal Genetic Testing of a

Parent or Prospective Parent, GENE.00025 Molecular Profiling and Proteogenomic Testing for the Evaluation of Malignancies, GENE.00028 Genetic Testing for Colorectal Cancer Susceptibility, GENE.00029 Genetic Testing for Breast and/or Ovarian Cancer Syndrome, GENE.00030 Genetic Testing for Endocrine Gland Cancer Susceptibility, GENE.00035 Genetic Testing for TP53 Mutations, and GENE.00043 Genetic Testing of an Individual's Genome for Inherited Diseases to this new medical policy document. Updated Coding section to remove 81506, not applicable.