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Genetic Cancer Susceptibility Using Next Generation Sequencing

Policy Number: AHS – M2066 – Genetic Cancer Susceptibility Using Next Generation Sequencing	Prior Policy Name and Number, as applicable:
Policy Revision Date: 03/09/2022	

I. Policy Description

Next generation sequencing (NGS), also known as massively parallel sequencing, is a type of DNA sequencing technology that sequences many small fragments of DNA in parallel. The wide application of NGS has helped to identify infrequent gene alterations contributing to oncogenesis, cancer progression, metastasis, and tumor complexity (Hulick, 2020).

II. Related Policies

Policy Number	Policy Title
AHS – M2003	BRCA
AHS – M2004	Lynch Syndrome
AHS – M2032	Whole Genome and Whole Exome Sequencing
AHS – M2145	General Genetic Testing, Germline Disorders
AHS - M2146	General Genetic Testing, Somatic Disorders

III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual's benefit coverage at the time of the request. Specifications pertaining to Medicare and Medicaid can be found in Section VII of this policy document.

1) Pre-test genetic counseling with post-test follow-up counseling is required and **MEETS COVERAGE CRITERIA.**

2) Genetic cancer susceptibility panels* (see Notes 1 & 2) using next generation sequencing **MEET COVERAGE CRITERIA** when all the following criteria are met:

   a) Individual displays clinical features and/or has a family history consistent with a hereditary cancer syndrome as listed in the policies for breast cancer gene (*BRCA)* (AHS-M2003),

Lynch syndrome (AHS-M2004), and Genetic Testing for Polyposis Syndromes (AHS-M2024)

b) All genes in the panel are relevant based on the personal and family history for the individual being tested

c) Specific mutation(s) in the genes on the panel contain(s) American Medical Association (AMA) Current Procedural Terminology (CPT) coding guideline required genes at a minimum

d) The results of the genetic test will impact the medical management of the individual with surveillance as treatment and likely improve health outcomes

3) All other genetic panels **DO NOT MEET COVERAGE CRITERIA** because the current scientific evidence is not yet sufficient to establish how test results from panels which include a broad number of genes may be used to direct treatment decisions and improve health outcomes associated with all components of the panels.

Note 1: For 5 or more gene tests being run on the same platform, such as multi-gene panel next generation sequencing, please refer to AHS-R2162 Reimbursement Policy.

Note 2: Concurrent ordering of multi-gene panel tests for a specific condition **IS STRICTLY PROHIBITED**; only one multi-gene panel test may be ordered at a time for a specific condition.

IV. Table of Terminology

Term	Definition
ACMG	American College of Medical Genetics
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukemia
ASCO	American Society of Clinical Oncology
ASXL1	*ASXL transcriptional regulator 1*
ATM	*ATM serine/threonine kinase*
BARD1	*BRCA1 associated RING domain 1*
BCOR	*BCL6 corepressor*
BCR-ABL1	*BCR1/ABL1 fusion gene*
BRAF	*B-Raf proto-oncogene, serine/threonine kinase*
BRCA1/2	*Breast cancer gene 1 or 2*
BRIP1	*BRCA1 interacting helicase 1*
CALR	*Calreticulin*
CBL	*Cbl proto-oncogene*
CDH1	*Cadherin 1*
CHEK2	*Checkpoint kinase 2*
CLC	Colorectal cancer
CLIA	Clinical Laboratory Improvement Amendments
CMS	Centers For Medicare and Medicaid

CMTP	Centre For Medical Technology Policy
CNAs	Copy number alterations
CNS	Central nervous system
CNV	Copy number variation
DDX41	*DEAD-box helicase 41*
DNMT3A	*DNA methyltransferase 3 alpha*
ECOG	Eastern Cooperative Oncology Group
EGFR	*Epidermal growth factor receptor*
EPCAM	Epithelial cell adhesion molecule
ESMO	European Society for Medical Oncology
ETV6	*ETS variant transcription factor 6*
EZH2	*Enhancer of zeste 2 polycomb repressive complex 2 subunit*
FANCC	*FA complementation group C*
FDA	Food and Drug Administration
FFPE	Formalin fixed paraffin embedded
FGFR1	*Fibroblast growth factor receptor 1*
FISH	Fluorescence *in situ* hybridization
FLT3	*Fms related receptor tyrosine kinase 3*
GATA2	*GATA binding protein 2*
GIS	Genomic instability score
HNPCC	Hereditary nonpolyposis colorectal cancer
HRD	Homologous recombination deficiency
IDH1	*Isocitrate dehydrogenase (NADP(+)) 1*
IDH2	*Isocitrate dehydrogenase (NADP(+)) 2*
INDELS	Insertion and deletion alterations
JAK2	*Janus kinase 2*
KRAS	*KRAS proto-oncogene, GTPase*
LDTs	Laboratory developed tests
LOH	Loss of heterozygosity
LST	Large scale state transitions
MLPA	Multiplex ligation dependent probe amplification
MPL	*MPL proto-oncogene,* thrombopoietin receptor
MMR	Mismatch repair
MNCs	Mononuclear cells
MRD	Minimum residual disease
MRE11A	*MRE11 homolog, double strand break repair nuclease*
mRNA	Messenger ribonucleic acid
MSI	Microsatellite instability
MSK-IMPACT	Memorial Sloan Kettering- integrated mutation profiling of actionable cancer targets
MUTYH	*MRE11 homolog, double strand break repair nuclease*

NBN	*Nibrin*
NCCN	National Comprehensive Cancer Network
NF1	*Neurofibromin 1*
NGS	Next generation sequencing
NRAS	*NRAS proto-oncogene, GTPase*
NPM1	*Nucleophosmin 1*
NSCLC	Non-small cell lung cancer
PALB2	*Partner and localizer of BRCA2*
PCR	Polymerase chain reaction
PDGFRA	*Platelet derived growth factor receptor alpha*
PDGFRB	*Platelet derived growth factor receptor beta*
PHF6	*PHD finger protein 6*
PPM1D	*Protein phosphatase, Mg2+/Mn2+ dependent 1D*
PTEN	*Phosphatase and tensin homolog*
RAD51C	*RAD51 paralog C*
RAD51D	*RAD51 paralog D*
RAS	Reticular activating system
RECQL4	*RecQ like helicase 4*
RINT1	*RAD50 interactor 1*
RNA	Ribonucleic acid
RTK	Receptor tyrosine kinase
RUNX1	*RUNX family transcription factor 1*
SCL	Small cell lung
SETBP1	*SET binding protein 1*
SF3B1	*Splicing factor 3b subunit 1*
SLX4	*SLX4 structure-specific endonuclease subunit*
SMARCA4	*SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4*
SNVs	RINT1Single nucleotide variants
SRSF2	*Serine and arginine rich splicing factor 2*
STAT3	*Signal transducer and activator of transcription 3*
STAG2	*Stromal antigen 2*
STK11	*Serine/threonine kinase 11*
TAI	Telomeric allelic imbalance
TET2	*Tet methylcytosine dioxygenase 2*
TF	Tumour fraction
TMB	Tumour mutational burden
TP53	*Tumor protein p53*
TW1	*Twist1*
U2AF1	*U2 small nuclear RNA auxiliary factor 1*
VUS	Variants of uncertain significance

WT1	*WT1 transcription factor*
XRCC2	X-ray repair cross complementing 2
ZRSR2	*Zinc finger CCCH-type, RNA binding motif and serine/arginine rich 2*

V. Scientific Background

NGS allows for the rapid sequencing of multiple strands of DNA. It is not limited to one specific type of test; rather, it encompasses numerous technologies that produce swift and high-volume sequencing. NGS can be used to sequence multiple genes, the exome, or even the entire genome. This is opposed to traditional Sanger sequencing, which is more useful for sequencing a specific gene (ACMG, 2012; Hulick, 2020).

NGS typically includes the following steps: the patient's DNA is prepared to serve as a template, followed by isolation of DNA fragments on solid surfaces such as small beads, generating sequence data. These results are then compared against a reference genome. Though any DNA sample may be used if the quality and quantity of that sample are sufficient, the methods of library generation and data analysis vary from panel to panel. Evaluating the results of a gene panel typically requires some expertise in bioinformatics. NGS reports data on all variants which are identified. As such, great care must be taken to evaluate these gene variants, especially variants of unknown significance (VUS) and secondary findings (Hulick, 2020; Rehm et al., 2013).

Panels that sequence a specific set of genes are referred to as "targeted panels" and may range from 5 to over 1000 genes. Targeted panels are generally more cost-effective than whole exome or whole genome sequencing and are useful for conditions where a disease has more than one causative gene. For example, nonsyndromic hearing loss may be caused by a variance in one of over 60 genes and sequencing each gene individually would not be cost effective. Many companies have developed a wide variety of gene panels. From the FDA-approved MSK-IMPACT all the way to well-validated proprietary panels, many different options of panel testing are available (Hulick, 2020).

While panel testing can be useful, there are still some instances where exome and genome sequencing may be necessary. While the exome is comprised of all of the protein-encoding genes and at least 85% of pathogenic mutations are found within the exome, it only represents ~1.5%-2% of the genome. This difference makes exome sequencing more cost effective than genome sequencing- the entire exome includes ~30 megabases as compared to the genome's 3.3 gigabases. However, sequencing an entire genome can still be useful, as pathogenic mutations can sometimes occur in a non-coding region of the genome. One example is mutations outside of the exome resulting in gene regulation dysfunction. Most clinical NGS testing use targeted panels or whole exome sequencing, with whole genome sequencing used only in select cases (Hulick, 2020).

Clinical genomics play a significant part in treatment, diagnosis, and understanding of cancer. Assessment of multiple pathogenic genes has become a widely used technique with the rise of NGS technologies, and the NCCN often recommends genetic panels in certain clinical situations. Some panels may also test for other genetic defects, such as microsatellite instabilities or

expression levels of specific proteins. Evaluation of genomic information (somatic changes, inherited germline changes, and so on) is widely prevalent in treatment and diagnosis of numerous types of cancer (Hulick, 2020). Genomic profiling of a tumor can help refine cancer subtype classification and help with identifying patients likely to benefit from systemic therapies, as well as help screen for germline variants influencing heritable cancer risk (Chakravarty & Solit, 2021).

With the declining costs associated with sequencing and the identification of new genomic biomarkers which are predictive of drug response, multigene NGS-based tumor genomic profiling panels are becoming more commonplace as a component in routine cancer care. Additionally, this genomic profiling can identify that refine or confirm a patient's cancer subtype diagnosis and provides clinicians with insight into both heritable cancer risk as well as the likelihood of cancer recurrence and death. Not all mutations within the same gene produce the same biological effect, nor do they have the same clinical significance. Thus, it is vital to improve the clinical reporting of these detected variant and to improve the overall knowledge surrounding different variants (Chakravarty & Solit, 2021).

Analytical Validity

Pathogenic variants and other NGS findings are traditionally confirmed by Sanger sequencing, the gold standard of gene sequencing (>99.99% accuracy). NGS has been shown to compare favorably to Sanger sequencing. In a study performed by Strom et al., 110 single-nucleotide variants (SNVs) were found by NGS. Of these SNVS, 103 met the minimum quality score threshold, set by the lab, of 500, with 7 falling below this threshold. However, 109 of the 110 total SNVs were validated by Sanger sequencing (Strom et al., 2014). Another study focused on the agreement between Sanger sequencing and NGS results, finding that out of 5,800 variants identified by NGS, only 2 did not have cross-method agreement. Overall, the agreement rate was 99.965%. The authors concluded that a single round of Sanger sequencing was "more likely to incorrectly refute a true-positive variant from NGS than to correctly identify a false-positive variant from NGS" (Beck et al., 2016).

D'Haene et al. designed and validated a custom NGS panel for routine diagnosis of gliomas, including 14 genes (*H3F3A, ACVR1, IDH1, PDGFRA, TERT, HIST1H3B, HIST1H3C, EGFR, BRAF, CDKN2A, PTEN, IDH2, TP54,* and *ATRX)* and one codeletion (1p/19). After validation to 52 known glioma samples, the panel was applied to 91 unknown brain lesions. For these brain lesions, a sensitivity of 99.4% and specificity of 100% was achieved. "Orthogonal" methods (such as in situ hybridization and immunohistochemistry) demonstrated high concordance with the panel (D'Haene et al., 2019).

Woodhouse et al. (2020) evaluated the analytical performance of FoundationOne Liquid CDx assay to detect genomic alterations in cancer patients. The assay was evaluated across more than 30 different cancer types in over 300 genes and greater than 30,000 gene variants. "Results demonstrated a 95% limit of detection of 0.40% variant allele fraction for select substitutions and insertions/deletions, 0.37% variant allele fraction for select rearrangements, 21.7% tumor fraction (TF) for copy number amplifications, and 30.4% TF for copy number losses. The false positive variant rate was 0.013% (approximately 1 in 8,000). Reproducibility of variant calling was 99.59% (Woodhouse et al., 2020)." In comparison to in situ hybridization and

immunohistochemistry, FoundationOne had an overall 96.3% positive percent agreement and > 99.9% negative percent agreement. "These study results demonstrate that FoundationOne Liquid CDx accurately and reproducibly detects the major types of genomic alterations in addition to complex biomarkers such as microsatellite instability, blood tumor mutational burden, and tumor fraction (Woodhouse et al., 2020)."

Clinical Utility and Validity

NGS has utility in numerous clinical scenarios and is especially useful in situations where multiple genes can cause the same phenotype, where other candidate genes were found to be normal, or where sequencing individual genes would not be timely or cost effective (Hulick, 2020).

Discussions of utility may also revolve around what is done with the findings of a gene panel. For instance, a study by Zehir et al. focused on the MSK-IMPACT gene panel. This panel of 410 cancer-related genes was used to sequence 10,945 tumors from 10,336 patients. Of these patients, 36.7% (3792/10336) were found to have a "clinically actionable" gene variant, such as *TP53* and *KRAS*. Of these, 527 patients were enrolled in clinical trials (Zehir et al., 2017). NGS has also helped provide diagnostic information to patients. A study focusing on 382 patients with a previously undiagnosed condition used NGS technology to diagnose 98 patients with exome or genome sequencing, allowing for changes in diagnostic testing, treatment, and genetic counseling. A total of 31 new syndromes were defined as well (Splinter et al., 2018).

Surrey et al. evaluated the clinical utility of a custom NGS panel for pediatric tumors. Sequencing was performed on 367 pediatric cancer samples. The authors found that results from the panel testing were "incorporated successfully into clinical care" for 88.7% of leukemias and lymphomas, 90.6% of central nervous system (CNS) cancers, and 62.6% of non-CNS solid tumors. A diagnosis change occurred in 3.3% of cases, and 19.4% of patients had variants requiring further germline testing (Surrey et al., 2019).

Tayshetye et al. (2020) analyzed the clinical utility of NGS in tumor testing using FoundationOne, a validated NGS genomic profiling test. 157 NGS results were collected of many different tumor types, with 63% being stage IV cancer at the time of testing. With NGS analysis, 185 genes with mutations were found in the RTK/RAS pathway, PI3K pathway, p53 pathway and cell cycle pathway. Overall, 82% of the patients had a mutation that could be treated with an FDA-approved treatment. NGS results were used in treatment decisions for 18% of these patients, and only 7% of the patients initiated therapy based on NGS results. The most common reason for not initiating NGS-based therapy was the lack of an FDA-approved medication used for that specific tumor type, as a major challenge is insurance approval for an off-label indication. The authors state that "while there are numerous potential benefits from the use of NGS, further studies are still needed to determine its full clinical utility (Tayshetye et al., 2020)."

Owattanapanich et al. (2021) analyzed the incidence and clinical impact of molecular genetic aberrations in Thai patients with AML and myelodysplastic syndrome- excess blasts (MDS-EB), as detected by NGS. The authors used a custom NGS panel targeting 42 genes recurrently mutated in myeloid neoplasms and found a median number of 3 mutations, with the most frequent alterations occurring in *FLT3* internal tandem duplications (ITD) (28.6%), *DNMT3A* (24.5%),

and *TW1* (22.4%). *FLT3*-ITD was more frequent in the de novo AML group than in the MDS/secondary AML group. In contrast, in the MDS/secondary AML group, *ASXL1*, *ETV6*, and *SRSF2* mutations were more frequent. Advanced age and *TP53* mutations were independent, poor prognostic factors for patients' survival and the authors note that "genetic landscape of AML patients for each disease type, each age group, and each nation differ." They note that "personalized treatment based on each molecular mutation in individual patients could improve their treatment responses and long-term survival outcomes" and conclude that a comprehensive genetic investigation should guide the most suitable treatment to improve an individual patient's outcome (Owattanapanich et al., 2021).

VI. Guidelines and Recommendations

National Comprehensive Cancer Network (NCCN)

Numerous gene panels have been recommended by the NCCN. Cancers, such as breast, ovarian, and leukemia, may be caused by many different gene variants, and the NCCN recommends panels in genetic testing for these conditions. These conditions are as follows:

Acute Lymphoblastic Leukemia (ALL):

The NCCN recommends comprehensive testing by NGS for gene fusions and pathogenic mutations, particularly if known to be *BCR-ABL1*/Ph-negative or Ph-like. For MRD assessment, the NCCN recommends NGS-based assays to detect clonal rearrangements in immunoglobin heavy chain gene and or T-cell receptor genes. They also note that PCR/NGS methods can detect leukemic cells at a sensitivity threshold of $<1 \times 10^{-6}$ (<0.0001%) bone marrow mononuclear cells (MNCs). Assays to detect alternative leukemia-specific fusion genes using NGS are in development but are not yet recommended for MRD quantification outside the context of a clinical trial (NCCN, 2021a).

Acute Myeloid Leukemia (AML):

The NCCN states that NGS analysis may be used "for the ongoing management of AML and various phases of treatment" of gene mutations involved with AML such as *TP53* (NCCN, 2022a).

Breast Cancer:

NCCN notes that *NTRK* mutations may be detected with NGS (NCCN, 2022b).

Central Nervous Cancers:

Evaluation of *IDH1* and *IDH2* mutations is highly recommended. The most common mutation of *IDH1* of R132H is reliably screened by immunohistochemistry, but sequencing (through Sanger or NGS-based assays) of *IDH1* and *IDH2* may also be highly recommended in the appropriate contexts. NGS is included as a "standard sequencing method" (NCCN, 2021c).

Chronic Lymphocytic Leukemia/ Small Lymphocytic Leukemia:

NCCN recommends assessing Minimal Residual Disease (MRD) using an assay with a sensitivity of 10^{-4} according to the standardized NGS method (NCCN, 2022c).

Colon and Rectal Cancer:

NCCN recommends that sequencing for *RAS* and *BRAF* genes and HER2 amplifications be performed if a patient is suspected or proven to have a metastatic synchronous adenocarcinoma. The NCCN does not recommend any sequencing method over another, but lists NGS and Sanger sequencing as possible methods (NCCN, 2021d, 2021h).

Esophageal and Esophagogastric Junction Cancers and Gastric Cancers:

NCCN recommends the use of NGS for assessing esophageal cancer and gastric cancer when there is limited diagnostic tissue available for testing and the patient is unable to undergo additional procedures. NGS can be considered instead of sequential testing for single biomarkers. NCCN notes that for esophageal and esophagogastric junction cancers and gastric cancer, NGS may be considered as part of the initial workup. For gastric cancer and esophageal squamous cell carcinoma and adenocarcinomas that are unresectable locally advanced, locally recurrent, or metastatic, with a Karnofsky performance score ≥60% or an ECOG performance score ≤2, NGS may be considered via validated assay. NCCN notes that NGS has certain limitations; therefore, gold-standard assays (immunohistochemistry/in-situ hybridization) should be considered first, followed by NGS testing as appropriate (NCCN, 2022d, 2022e).

Gastrointestinal Stromal Tumors:

NCCN recommends that all gastrointestinal stromal tumors lacking *KIT* or *PDFRA* should be tested for SDH deficiency and alternative driver mutations using NGS. In addition, NGS testing should be performed to identify alternative driver mutations such as *BRAF, NF1, NTRK*, and *FGFR* fusions, as these could provide insight for a targeted therapy (NCCN, 2022f).

Multiple Myeloma:

NCCN notes NGS as a valid method for informing treatment decisions. They comment that an NGS array on bone marrow may be useful in certain circumstances and that in certain circumstances, it may be useful to consider baseline clone identification and storage of aspirate sample for future MRD testing by NGS. NGS is recommended for follow-up/surveillance as needed in smoldering myeloma (asymptomatic). NGS is also listed as a way to assess minimum residual disease (MRD) and categorize responses to treatment (criterion is based on recommendations from the International Myeloma Working Group) (NCCN, 2022h).

Myelodysplastic Syndromes:

NCCN recommends that evaluation of mutations should include NGS panels incorporating the 21 most frequently mutated genes, which are as follows: *TET2, DNMT3A, ASXL1, EZH2, SF3B1, SRSF2, U2AF1, ZRSR2, RUNX1, TP53, STAG2, NRAS, CBL, NF1, JAK2, CALR, MPL, ETV6, GATA2, DDX41, IDH1, IDH2, SETBP1, PHF6, BCOR, FLT3, WT1, NPM1, STAT3*, and *PPM1D.*

NCCN added in version 3.2021 that NGS has low sensitivity for the *KIT D816V* mutation. In this case, allele-specific PCR is more sensitive and recommended in patients with high clinical suspicion of mast cell disease (NCCN, 2022i).

Myeloid/Lymphoid Neoplasms with Eosinophilia and Tyrosine Kinase Fusion Genes

NCCN recommends that "NGS may be used to identify novel fusion gene or cryptic rearrangements when clinical suspicion is high and fluorescence in situ hybridization (FISH) for *PDGFRA, PDGFRB, FGFR1, JAK2, ABL1, or FLT3* are negative... currently, the impact on outcomes of additional mutations detected by NGS is unclear and further studies are needed to determine the impact of mutations on disease course." In myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase fusion genes, an NGS myeloid mutation panel is recommended as a general diagnostic workup, though RT-PCR may be preferred over NGS for *FTL3*. NCCN also notes that "mutations detected y NGS may also provide a means to identify primary (clonal/neoplastic) eosinophilia from secondary (reactive) eosinophilia, including in cases where no rearrangements of *PDGFRA, PDGFRB, FGFR1, PCM1-JAK2, ETV6-JAK2,* or *BCR-JAK2* are detected. Mutations described include *TET2, ASXL1, EXH2,* or *SETBP1* and, recently, activating *STAT5* N642H mutations" (NCCN, 2021e).

Myeloproliferative Neoplasms:

NCCN states that NGS may be useful in establishing clonality in selected circumstances, such as the "triple negative" of non-mutated *JAK2, CALR,* and *MPL*. The NCCN also notes that workup may include a multi-gene NGS panel that includes all three of *JAK2, CALR,* and *MPL*. NCCN also notes that additional molecular testing using a multi-gene NGS panel should be considered to evaluate for higher-risk mutations associated with disease progression in patients with primary myelofibrosis (NCCN, 2021f).

Ovarian Cancer:

The NCCN recommends NGS for *BRCA1/2* somatic mutations, as clinically indicated (NCCN, 2022k).

Pancreatic Adenocarcinoma:

The NCCN states that NGS may be used to detect "actionable somatic findings", such as *ALK, NRG1, NTRK, ROS1, BRAF, BRCA1/2, HER2, KRAS, PALB2,* and MMR deficiency-related genes. Testing on tumor tissue is preferred but cell-free DNA testing can be considered if tumor tissue testing is not feasible (NCCN, 2021g).

B-Cell Lymphomas:

NCCN states that NGS may be used if a high suspicion of clonal process remains but other techniques have not clearly identified a clonal process. The NCCN states that an NGS panel including *TNFRSF14* and *STAT6* may be useful "under certain circumstances" for Follicular Lymphoma. NGS may also be useful for "treatment selection" (NCCN, 2021b).

T-Cell Lymphomas:

The NCCN also states that "genetic testing, including…NGS… to detect somatic mutations or genetic abnormalities are often informative and, in some cases, essential for an accurate and precise diagnostic and prognostic assessment of T-cell lymphomas". For hepatosplenic T-cell lymphoma, the NCCN finds NGS sequencing panels including *STAT3, STA5B, PIK3CD, SETD2, INO80, TET3,* and *SMARCA2* to be useful for diagnosis under certain circumstances (NCCN, 2022m).

Non-Small Cell Lung Cancer (NSCLC):

The NCCN recommends that testing be performed in a "panel-based approach, most typically performed by next-generation sequencing (NGS)" when feasible. NCCN notes that multiple studies suggest that NGS testing with broad gene coverage may allow for unambiguous determination of clonal relatedness among separate lung modules. RNA-based NGS should be considered in patients without identifiable driver oncogene mutations, especially in "never smokers", to maximize detection of fusion events. The NCCN mentions NGS as a commonly used method to detect sequence changes such as gene mutations in *EGFR, BRAF*, and *KRAS,* skipping events in *METex14,* insertion events in *EGFRex20,* and fusion events in *ALK* and *ROS1* (though DNA based NGS may underdetect *ROS1* fusions). For fusion detection, RNA-based NGS is preferable to DNA-based NGS. They note that studies exploring tumor relatedness by testing tissue from separately sampled lesions using broad gene coverage with an NGS approach suggests it may be superior to histopathologic assessment. However, the NCCN notes that NGS may be considered in biomarker analysis but cautions that not all types of alterations will be detected, that any method which investigates sequences other than a subset of highly specific alterations can identify variants of unknown significant (should not be considered as a basis for targeted therapy selection, even if other variants within that same gene are clinically actionable), and to be aware of the nuances of NGS (NCCN, 2022j).

Prostate Cancer:

The NCCN recommends NGS cancer predisposition screening for *BRCA1, BRCA2, ATM, PALB2, CHEK2, MLH1, MSH2, MSH6, and PMS2 (NCCN, 2022l).*

Small Bowel Adenocarcinoma:

Universal microsatellite instability (MSI) testing is recommended in all patients with a history of small bowel adenocarcinoma. NCCN recommends using NGS panels to test for MSI (NCCN, 2021i).

Soft Tissue Sarcoma:

NGS is mentioned among the techniques used to identify genetic aberrations in soft tissue sarcoma (NCCN, 2021j).

Systemic Mastocytosis:

NCCN recommends against NGS panels alone for detection of *KIT* D816V, citing their low sensitivity (approximately 5%). However, a myeloid mutation panel should be performed on bone marrow (although testing can be done on peripheral blood in the presence of an associated

hematologic neoplasm and/or circulating mast cells). Prognostically relevant mutations include *TET2, SRSF2, CBL, ASXL1, RUNX1, JAK2,* and *RAS (NCCN, 2021k).*

Genetic/Familial High-Risk Assessment for Colorectal Cancer:

NGS allows for the sequencing of multiple genes simultaneously (multi-gene testing) and the introduction of multi-gene testing for hereditary forms of cancer has rapidly altered the clinical approach to testing at-risk patients and their families. Multi-gene testing can simultaneously analyze sets of genes associated with a specific family cancer phenotype/phenotypes and may include syndrome-specific tests, cancer-specific tests, or comprehensive cancer panels. NCCN states that there are numerous scenarios in which multi-gene testing may be more effective. This includes greater efficiency in testing when more than one gene may explain presentation and family history, a higher chance of providing the patient with a possible explanation for their cause of cancer, competitive cost relative to sequentially testing single genes, and the chance of identifying pathogenic variants in multiple actionable genes that would me missed using cancer syndrome-specific panels, which could ultimately impact screening and management for the individual and their family members.

The NCCN notes certain cons associated with panel testing, such as slower turn around, the possibility of missing some mutations that would be detected with traditional single-gene analysis, and identification of mutations for more than one gene, which adds complexity that could lead to difficulty in making risk management recommendations. There is also a higher chance of identifying variants of unknown significance, unactionable variants, or variants that do not have a clear course of treatment. The NCCN also identifies two examples of clinical scenarios in which multi-gene testing should not be considered: "1) an individual from a family with a known mutation and there is no other reason for multi-gene testing; 2) the patient's family history is strongly suggestive of a known hereditary syndrome."

The NCCN panel recommends NGS as one of three options for patients or families where a colorectal or endometrial tumor is available- specifically, they note that in this situation, a comprehensive tumor NGS panel can be considered for workup and should include, at minimum, the 4 MMR genes and *EPCAM, BRAF*, MSI, and other known familial cancer genes

Overall, the NCCN acknowledges the significant benefits of panel testing, but states that choice of panel and testing is critical.

As a final aside, the NCCN is in agreement with the 2015 ASCO recommendations (NCCN, 2022g).

Genetic/Familial High-Risk Assessment for Breast, Ovarian, and Pancreatic Cancer:

In this guideline, the NCCN cites similar pros and cons to multi-gene testing as those covered in the previous guidelines for colorectal cancer. They also note that not all genes included on available multi-gene tests are necessarily clinically actionable and multi-gene panel testing increases the likelihood of finding pathogenic/likely pathogenic variants without clear clinical significance. The NCCN notes the following genes as pathogenic/likely pathogenic variants associated with breast/ovarian cancer: *BRCA1/2, ATM, BARD1, BRIP1, CDH1, CHEK2, NBN,*

PALB2, PTEN, RAD51C, RAD51D, STK11, and *TP53*. Lower penetrance genes that may be included as part of a multi-gene panel for breast and/or ovarian cancer include *FANCC, MRE11A, MUTYH* heterozygotes, *RECQL4, RINT1, SLX4, SMARCA4, and XRCC2* (NCCN, 2020).

American Society of Clinical Oncology (ASCO)

ASCO released guidelines discussing tumor testing for epithelial ovarian cancer. In it, they recommend germline sequencing of *BRCA1/2* "in the context of a multigene panel" that includes "at minimum" the following genes: *BRCA1, BRCA2, RAD51C, RAD51D, BRIP1, MLH1, MSH2, MSH6, PMS2,* and *PALB2* (Konstantinopoulos et al., 2020).

ASCO published guidelines regarding evaluating susceptibility to pancreatic cancer. In it, they recommend that germline genetic testing be performed using a multigene panel that includes the following genes: *APC, ATM, BRCA1/2, CDKN2A, MLH1, MSH2, MSH6, PMS2, EPCAM, PALB2, STK11, TP53*. An exception is if a genetic diagnosis has been previously confirmed in a family member; a panel should not be used in this case. Further, ASCO recommends that every patient diagnosed with pancreatic adenocarcinoma should undergo a risk assessment for hereditary syndromes associated with increased risk of pancreatic adenocarcinoma (Stoffel et al., 2018).

In 2015, ASCO published a policy statement update on genetic and genomic testing for cancer susceptibility that included recommendations for multi-gene panel testing for cancer susceptibility. ASCO recognizes that 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". ASCO notes that panel testing will identify variants of uncertain significance (VUSs) often, but that it is sufficient for cancer risk assessment to evaluate genes of established clinical utility (Robson et al., 2015).

ASCO states that there is little consensus as to which genes should be on gene panels and that clinical utility is "the fundamental issue with respect to testing for mutations in moderate-penetrance genes". At this time (2015) there is insufficient evidence to "conclusively demonstrate the clinical utility of testing for moderate-penetrance mutations" and that until these questions are answered, testing should be limited to mutations of established clinical utility (Robson et al., 2015).

American College of Medical Genetics (ACMG)

The ACMG published guidelines on inclusion criteria for genes with "various gene–disease evidence levels". For confirming a clinical diagnosis, the ACMG stated to include any gene associated (with a "moderate", "strong" or "definitive" association) with the disease, if the primary method of diagnosis was a "Disease-focused multigene panel or other non–sequencing-based ancillary assays". Genes with no emerging evidence or without evidence at all were to be excluded. Genes with emerging evidence should "typically" be excluded, although the ACMG notes some inclusions that may be "meaningful". The ACMG also states that genes with this level of evidence should be reported with a statement that disease association and inheritance has not been established.

For panels intended to "Establish genetic diagnosis for clinically complex cases" and that are used for conditions primarily diagnosed through exome/genome sequencing, genes that have evidence levels of "definitive", "strong" and "moderate" should be included. Genes of unknown significance should be qualified with a statement that disease association and inheritance have not been completely established (Bean et al., 2019).

The ACMG recommends that the selection of genes and transcripts in any given panel be limited to genes with "sufficient scientific evidence for a causative role in the disease". Genes without clear evidence of association with the disease should not be included.

ACMG recommends validating diagnostic testing through another method such as Sanger sequencing.

ACMG cannot recommend a minimum threshold for "coverage" as many factors of the platform and assay may influence minimum coverage. However, the ACMG recommends that each laboratory independently validate their panel tests (Rehm et al., 2013).

ACMG released a statement regarding some points to consider for germline findings using NGS in patients undergoing tumor testing. ACMG states that NGS has some limitations that make it harder to identify some types of germline variants, such as genomic rearrangements, large insertions/deletions, or expansion/contraction of repetitive sequences. In addition, the assay and analytical performance varies between laboratories. Therefore, confirmation with an orthogonal method such as PCR, microarray, or multiplex ligation-dependent probe amplification (MLPA) is recommended (Li et al., 2020).

The ACMG released a 2021 guideline on NGS for constitutional variants in the clinical laboratory, in which they note that diagnostic gene panels are optimal for well-defined clinical presentations that are genetically heterogeneous (have more than one pathogenic variant that can cause the diagnosed phenotype), when these pathogenic variants in the various disease-associated genes account for a significant fraction of cases. They address the fact that incidental findings should not be encountered, but also that broad panels may identify clinically significant findings unrelated to the initial test indication. Panels should be optimized by limiting the test to those genes relevant to a given disease.

In clinically relevant genomic regions that cannot be assayed reliably by NGS, ancillary assays such as Sanger sequencing of regions with low coverage by NGS, CNV detection, methylation, and repeat expansion. "Disease-targeted gene panels that include these areas should include appropriate additional methodologies to maximize clinical sensitivity" (Rehder et al., 2021).

Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists

The Joint Commission recommended that somatic variants be categorized by and reported based on their impact on clinical care. The Joint Commission notes that somatic variants include indels, SNVs, fusion genes from genomic rearrangements, and CNVs and should focus on their impact on clinical care. Any variant may be considered a biomarker if it predicts response to therapy,

influences prognosis, diagnosis, treatment decisions, or the gene function itself. The Joint Commission proposes four levels for these biomarkers which are as follows:

1. Level A, biomarkers that predict response or resistance to US FDA-approved therapies for a specific type of tumor or have been included in professional guidelines as therapeutic, diagnostic, and/or prognostic biomarkers for specific types of tumors;

2. Level B, biomarkers that predict response or resistance to a therapy based on well-powered studies with consensus from experts in the field, or have diagnostic and/or prognostic significance of certain diseases based on well-powered studies with expert consensus;

3. Level C, biomarkers that predict response or resistance to therapies approved by FDA or professional societies for a different tumor type (i.e., off-label use of a drug), serve as inclusion criteria for clinical trials, or have diagnostic and/or prognostic significance based on the results of multiple small studies;

4. Level D, biomarkers that show plausible therapeutic significance based on preclinical studies, or may assist disease diagnosis and/or prognosis themselves or along with other biomarkers based on small studies or multiple case reports with no consensus."

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

European Society for Medical Oncology (ESMO) Precision Medicine Working Group

ESMO released clinical practice guidelines on the use of NGS to diagnose tumors. Overall, ESMO suggests that NGS should be used routinely in patients with metastatic cancers including advanced lung adenocarcinoma, prostate cancer, ovarian cancer, and cholangiocarcinoma. For colon cancer, NGS can be an alternative option to PCR if it does not incur additional costs. Tumor mutational burden (TMB) should be tested in cervical cancer, salivary cancer, thyroid cancers, well-to-moderately differentiated neuroendocrine tumors, and vulvar cancer. Patients with other cancers may decide with their physician to order NGS on a large gene panel, if "pending no extra cost for the public health care system, and if the patient is informed about the low likelihood of benefit (Mosele et al., 2020)." ESMO states that more evidence is still needed to improve understanding on how to use NGS to treat patients based on precision biomarkers.

Recommendations according to cancer type are summarized below. Recommendations were provided based on the ESCAT scale ranking that calculates the number of patients that would need to be tested with NGS to identify one patient who could be matched to an effective drug. Level I means that the match between drug and genomic alterations has been validated in clinical

trials and should drive treatment decision in daily practice. Level II means that alteration has been associated with phase I/phase II trials. Level III means that genome alteration has been validated in another cancer, but not for that specific one. Level IV are hypothetically targetable alterations based on preclinical data (Mosele et al., 2020).

Cancer Type	Recommendation
Lung Adenocarcinoma	"Tumour multigene NGS to assess level I alterations. Larger panels can be used only on the basis of specific agreements with payers taking into account the overall cost of the strategy (drug included) and if they report accurate ranking of alterations. NGS can either be done on RNA or DNA, if it includes level I fusions in the panel.
Squamous cell lung cancer	No current indication for tumour multigene NGS
Breast cancer	No current indication for tumour multigene NGS
Colon cancer	Multigene tumour NGS can be an alternative option to PCR if it does not result in additional cost
Prostate cancer	Multigene tumour NGS to assess level I alterations. Larger panels can be used only on the basis of specific agreements with payers taking into account the overall cost of the strategy and if they report accurate ranking of alterations.
Gastric cancer	No current indication for tumour multigene NGS
Pancreatic cancer	No current indication for tumour multigene NGS
Hepatocellular carcinoma	No current indication for tumour multigene NGS
Cholangiocarcinoma	Multigene tumour NGS could be recommended to assess level I alterations. Larger panels can be used only on the basis of specific agreements with payers taking into account the overall cost of the strategy (drug included) and if they report accurate ranking of alterations. RNA-based NGS can be used.
Others	Tumour multigene NGS can be used in ovarian cancers to determine somatic BRCA1/2 mutations. In this latter case, larger panels can be used only on the basis of specific agreements with payers taking into account the overall cost of the strategy (drug included) and if they report accurate ranking of alterations. Large panel NGS can be used in carcinoma of unknown primary. It is recommended to determine TMB in cervical cancer, salivary cancer, thyroid cancers, well-to-moderately differentiated neuroendocrine

	tumours, vulvar cancer, pending drug access (and in TMB-high endometrial and SCL cancers if anti-PD1 antibody is not available otherwise) (Mosele et al., 2020)."

VII. Applicable State and Federal Regulations

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

A. Food and Drug Administration (FDA)

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

On September 30, 2021, the FDA approved ONCO/Reveal Dx Lung & Colon Cancer Assay (O/RDx-LCCA) by Pillar Biosciences, Inc. This device is a next generation sequencing based *in vitro* diagnostic test on DNA isolated from formalin-fixed paraffin-embedded (FFPE) NSCLC and colorectal cancer (CLC) tumors for detection of single nucleotide variants (SNVs) and deletions in 2 genes (*EGFR* and *KRAS*). This test is intended as a companion diagnostic to identify patients with NSCLC or CRC who may benefit from treatment with targeted therapies (FDA, 2021).

On November 6, 2020, the FDA approved FoundationOne Liquid CDx, by Foundation Medicine, Inc. This device is a next generation sequencing based *in vitro* diagnostic device for detection of substitutions, insertion and deletion alterations (indels), and copy number alterations (CNAs) in 311 genes, rearrangements in 3 genes, and copy number alterations in 3 genes. FoundationOne CDx also utilizes circulating cell-free DNA collected in FoundationOne® Liquid CDx Blood Sample Collection Kit to identify patients with non-small cell lung cancer, prostate cancer, ovarian cancer, or breast cancer who may benefit from treatment with the targeted therapies (FDA, 2020a).

On August 7, 2020, the FDA approved Guardant360 CDx, by Guardant Health, Inc. This device is a next generation sequencing based *in vitro* diagnostic device that uses targeted high throughput hybridization-based capture technology to detect SNVs, insertions, and deletions in 55 genes, copy number amplifications in 2 genes, and fusions in 4 genes. Guardant360 CDx also utilizes circulating cell-free DNA collected in Streck Cell-Free DNA Blood Collection Tubes to identify non-small cell lung cancer (NSCLC) patients who may benefit from treatment with the targeted therapy (FDA, 2020b).

On October 23, 2019, the FDA approved MyChoice HRD CDx, by Myriad Genetic Laboratories, Inc. This device is a next generation sequencing based *in vitro* diagnostic device for detection of single nucleotide variants, insertions, deletions, and large rearrangement variants of the BRCA1 and BRCA2 genes. This test also determines the Genomic Instability Score (GIS), a measurement of Loss of Heterozygosity (LOH), Telomeric Allelic Imbalance (TAI), and Large Scale State Transitions (LST), which is used to identify ovarian cancer patients with positive homologous recombination deficiency (HRD) status (FDA, 2019).

On November 30, 2017, the FDA approved FoundationOne CDx, by Foundation Medicine, Inc. This device is a next generation sequencing based *in vitro* diagnostic device for detection of substitutions, insertion and deletion alterations (indels), and copy number alterations (CNAs) in 324 genes and select gene rearrangements, as well as genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) using DNA isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens (FDA, 2017a).

On June 29, 2017, the FDA approved Praxis Extended RAS Panel, by Illumina, Inc. The Praxis™ Extended RAS Panel is a qualitative *in vitro* diagnostic test using targeted high throughput parallel sequencing for the detection of 56 specific mutations in RAS genes [KRAS (exons 2, 3, and 4) and NRAS (exons 2, 3, and 4)] in DNA extracted from formalin-fixed, paraffin-embedded (FFPE) colorectal cancer (CRC) tissue samples (FDA, 2017b).

On June 22, 2017, the FDA approved Oncomine Dx Target Test, by Life Technologies Corporation. The Oncomine Dx Target Test is a qualitative *in vitro* diagnostic test that uses targeted high throughput, parallel-sequencing technology to detect single nucleotide variants (SNVs) and deletions in 23 genes from DNA and fusions in ROS1 from RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples from patients with non-small cell lung cancer (NSCLC) using the Ion PGM Dx System (FDA, 2017c).

On December 19, 2016, the FDA approved FoundationFocus CDxBRCA, by Foundation Medicine, Inc. The FoundationFocus CDxBRCA is a next generation sequencing based *in vitro* diagnostic device for qualitative detection of BRCA1 and BRCA2 alterations in formalin-fixed paraffin-embedded (FFPE) ovarian tumor tissue. The FoundationFocus CDxBRCA assay detects sequence alterations in BRCA1 and BRCA2 (BRCA1/2) gene (FDA, 2016).

B. Centers for Medicare & Medicaid Services (CMS)

- N/A

VIII. Applicable CPT/HCPCS Procedure Codes

CPT	Code Description
81432	Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer); genomic sequence analysis panel, must include sequencing of at least 10 genes, always including BRCA1, BRCA2, CDH1, MLH1, MSH2, MSH6, PALB2, PTEN, STK11, and TP53

81433	Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer); duplication/deletion analysis panel, must include analyses for BRCA1, BRCA2, MLH1, MSH2, and STK11
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
81435	Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis); genomic sequence analysis panel, must include sequencing of at least 10 genes, including APC, BMPR1A, CDH1, MLH1, MSH2, MSH6, MUTYH, PTEN, SMAD4, and STK11
81436	Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis); duplication/deletion analysis panel, must include analysis of at least 5 genes, including MLH1, MSH2, EPCAM, SMAD4, and STK11
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
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
81455	Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm, DNA analysis, and RNA analysis when performed, 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, if performed
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/absence of variants and associated therapy(ies) to consider Proprietary test: Oncomine™ Dx Target Test Lab/Manufacturer: Thermo Fisher Scientific
0101U	Hereditary colon cancer disorders (eg, Lynch syndrome, PTEN hamartoma syndrome, Cowden syndrome, familial adenomatosis polyposis), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with MRNA analytics to resolve variants of unknown significance when

	indicated (15 genes [sequencing and deletion/duplication], EPCAM and GREM1 [deletion/duplication only]) Proprietary test: ColoNext® Lab/Manufacturer: Ambry Genetics®
0102U	Hereditary breast cancer-related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with MRNA analytics to resolve variants of unknown significance when indicated (17 genes [sequencing and deletion/duplication]) Proprietary test: BreastNext® Lab/Manufacturer: Ambry Genetics®
0103U	Hereditary ovarian cancer (eg, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis panel utilizing a combination of NGS, Sanger, MLPA, and array CGH, with MRNA analytics to resolve variants of unknown significance when indicated (24 genes [sequencing and deletion/duplication], EPCAM [deletion/duplication only]) Proprietary test: OvaNext® Lab/Manufacturer: Ambry Genetics®
0129U	Hereditary breast cancer–related disorders (eg, hereditary breast cancer, hereditary ovarian cancer, hereditary endometrial cancer), genomic sequence analysis and deletion/duplication analysis panel (ATM, BRCA1, BRCA2, CDH1, CHEK2, PALB2, PTEN, and TP53) Proprietary test: BRCAplus Lab/Manufacturer: Ambry Genetics
96040	Medical genetics and genetic counseling services, each 30 minutes face-to-face with patient/family
S0265	Genetic counseling, under physician supervision, each 15 minutes

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

IX. Evidence-based Scientific References

ACMG. (2012). Points to consider in the clinical application of genomic sequencing. *Genet Med*, *14*(8), 759-761. https://doi.org/10.1038/gim.2012.74

Bean, L. J. H., Funke, B., Carlston, C. M., Gannon, J. L., Kantarci, S., Krock, B. L., Zhang, S., Bayrak-Toydemir, P., & on behalf of the, A. L. Q. A. (2019). Diagnostic gene sequencing panels: from design to report—atechnical standard of the American College of Medical Genetics and Genomics(ACMG). *Genetics in Medicine*. https://doi.org/10.1038/s41436-019-0666-z

Beck, T. F., Mullikin, J. C., & Biesecker, L. G. (2016). Systematic Evaluation of Sanger Validation of Next-Generation Sequencing Variants. *Clin Chem*, *62*(4), 647-654. https://doi.org/10.1373/clinchem.2015.249623

Chakravarty, D., & Solit, D. B. (2021). Clinical cancer genomic profiling. *Nat Rev Genet*, *22*(8), 483-501. https://doi.org/10.1038/s41576-021-00338-8

D'Haene, N., Meléndez, B., Blanchard, O., De Nève, N., Lebrun, L., Van Campenhout, C., & Salmon, I. (2019). Design and Validation of a Gene-Targeted, Next-Generation Sequencing Panel for Routine Diagnosis in Gliomas. *Cancers (Basel), 11*(6). https://doi.org/10.3390/cancers11060773

FDA. (2016). *FoundationFocus CDxBRCA*. https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm?db=pma&id=389050

FDA. (2017a). *FoundationOne CDx*. https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm?db=pma&id=407172

FDA. (2017b). *Next generation sequencing oncology panel, somatic variant detection system*. https://www.accessdata.fda.gov/cdrh_docs/pdf16/P160038B.pdf

FDA. (2017c). *Oncomine Dx Target Test*. https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm?db=pma&id=393870

FDA. (2019). MyChoice HRD CDx. https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm?db=pma&id=440605

FDA. (2020a). FoundationOne Liquid CDx. https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm?db=pma&id=455653

FDA. (2020b). Guardant360 CDx. https://www.accessdata.fda.gov/scripts/cdrh/devicesatfda/index.cfm?db=pma&id=454228

FDA. (2021, July 30, 2021). *ONCO/Reveal Dx Lung & Colon Cancer Assay (O/RDx-LCCA)*. https://www.accessdata.fda.gov/cdrh_docs/pdf20/P200011B.pdf

Hulick, P. (2020). Next-generation DNA sequencing (NGS): Principles and clinical applications. https://www.uptodate.com/contents/next-generation-dna-sequencing-ngs-principles-and-clinical-applications?search=variant%20of%20unknown%20significance&source=search_result&selectedTitle=4~25&usage_type=default&display_rank=4

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

Li, M. M., Chao, E., Esplin, E. D., Miller, D. T., Nathanson, K. L., Plon, S. E., Scheuner, M. T., Stewart, D. R., Practice, A. P., & Guidelines, C. (2020). Points to consider for reporting of germline variation in patients undergoing tumor testing: a statement of the American College of Medical Genetics and Genomics (ACMG). *Genetics in Medicine*, *22*(7), 1142-1148. https://doi.org/10.1038/s41436-020-0783-8

Li, M. M., Datto, M., Duncavage, E. J., Kulkarni, S., Lindeman, N. I., Roy, S., Tsimberidou, A. M., Vnencak-Jones, C. L., Wolff, D. J., Younes, A., & Nikiforova, M. N. (2017). Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology,

American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn*, *19*(1), 4-23. https://doi.org/10.1016/j.jmoldx.2016.10.002

Mosele, F., Remon, J., Mateo, J., Westphalen, C. B., Barlesi, F., Lolkema, M. P., Normanno, N., Scarpa, A., Robson, M., Meric-Bernstam, F., Wagle, N., Stenzinger, A., Bonastre, J., Bayle, A., Michiels, S., Bièche, I., Rouleau, E., Jezdic, S., Douillard, J. Y., . . . André, F. (2020). Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. *Annals of Oncology*, *31*(11), 1491-1505. https://doi.org/10.1016/j.annonc.2020.07.014

NCCN. (2020). *Genetic/Familial High-Risk Assessment: Breast and Ovarian*. https://www.nccn.org/professionals/physician_gls/pdf/genetics_bop.pdf

NCCN. (2021a, January 7, 2022). *Acute Lymphoblastic Leukemia*. https://www.nccn.org/professionals/physician_gls/pdf/all.pdf

NCCN. (2021b, September 22, 2021). *B-Cell Lymphomas*. https://www.nccn.org/professionals/physician_gls/pdf/b-cell.pdf

NCCN. (2021c, September 8, 2021). *Central Nervous Cancers*. https://www.nccn.org/professionals/physician_gls/pdf/cns.pdf

NCCN. (2021d, September 10, 2021). *Colon Cancer*. https://www.nccn.org/professionals/physician_gls/pdf/colon.pdf

NCCN. (2021e, July 9, 2021). *Myeloid/Lymphoid Neoplasms with Eosinophilia and Tyrosine Kinase Fusion Genes*. https://www.nccn.org/professionals/physician_gls/pdf/mlne.pdf

NCCN. (2021f, August 18, 2021). *Myeloproliferative Neoplasms*. https://www.nccn.org/professionals/physician_gls/pdf/mpn.pdf

NCCN. (2021g, February 25, 2021). *Pancreatic Adenocarcinoma*. https://www.nccn.org/professionals/physician_gls/pdf/pancreatic.pdf

NCCN. (2021h, September 10, 2021). *Rectal Cancer, V.1.2020*. https://www.nccn.org/professionals/physician_gls/pdf/rectal.pdf

NCCN. (2021i, September 10, 2021). *Small Bowel Adenocarcinoma*. https://www.nccn.org/professionals/physician_gls/pdf/small_bowel.pdf

NCCN. (2021j, January 26, 2022). *Soft Tissue Sarcoma*. https://www.nccn.org/professionals/physician_gls/pdf/sarcoma.pdf

NCCN. (2021k, July 9, 2021). *Systemic Mastocytosis*. https://www.nccn.org/professionals/physician_gls/pdf/mastocytosis.pdf

NCCN. (2022a, December 2, 2021). *Acute Myeloid Leukemia*. https://www.nccn.org/professionals/physician_gls/pdf/aml.pdf

NCCN. (2022b, December 20, 2021). *Breast Cancer*. https://www.nccn.org/professionals/physician_gls/pdf/breast.pdf

NCCN. (2022c, January 18, 2022). *Chronic Lymphocytic Leukemia/ Small Lymphocytic Leukemia* https://www.nccn.org/professionals/physician_gls/pdf/cll.pdf

NCCN. (2022d, December 21, 2021). *Esophageal and Esophagogastric Junction Cancers*. https://www.nccn.org/professionals/physician_gls/pdf/esophageal.pdf

NCCN. (2022e, January 11, 2022). *Gastric Cancer*. https://www.nccn.org/professionals/physician_gls/pdf/gastric.pdf

NCCN. (2022f, January 21, 2022). *Gastrointestinal Stromal Tumors (GISTS)*. https://www.nccn.org/professionals/physician_gls/pdf/gist.pdf

NCCN. (2022g, August 11, 2021). *Genetic/Familial High-Risk Assessment: Colorectal*. https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf

NCCN. (2022h, December 14, 2021). *Multiple Myeloma*. https://www.nccn.org/professionals/physician_gls/pdf/myeloma.pdf

NCCN. (2022i, January 13, 2022). *Myelodysplastic Syndromes*. https://www.nccn.org/professionals/physician_gls/pdf/mds.pdf

NCCN. (2022j, December 7, 2021). *Non-Small Cell Lung Cancer*. https://www.nccn.org/professionals/physician_gls/pdf/nscl.pdf

NCCN. (2022k, January 18, 2022). *Ovarian Cancer Including Fallopian Tube Cancer and Primary Peritoneal Cancer*. https://www.nccn.org/professionals/physician_gls/pdf/ovarian.pdf

NCCN. (2022l, January 10, 2022). *Prostate Canceer*. https://www.nccn.org/professionals/physician_gls/pdf/prostate.pdf

NCCN. (2022m, December 22, 2021). *T-Cell Lymphomas*. https://www.nccn.org/professionals/physician_gls/pdf/t-cell.pdf

Owattanapanich, W., Herzig, J., Jahn, N., Panina, E., Ruchutrakool, T., Kungwankiattichai, S., Issaragrisil, S., Dohner, H., & Dohner, K. (2021). Genetic alterations in Thai adult patients with acute myeloid leukemia and myelodysplastic syndrome-excess blasts detected by next-generation sequencing technique. *Ann Hematol*, *100*(8), 1983-1993. https://doi.org/10.1007/s00277-021-04513-z

Rehder, C., Bean, L. J. H., Bick, D., Chao, E., Chung, W., Das, S., O'Daniel, J., Rehm, H., Shashi, V., Vincent, L. M., & Committee, A. L. Q. A. (2021). Next-generation sequencing for constitutional variants in the clinical laboratory, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med*, *23*(8), 1399-1415. https://doi.org/10.1038/s41436-021-01139-4

Rehm, H. L., Bale, S. J., Bayrak-Toydemir, P., Berg, J. S., Brown, K. K., Deignan, J. L., Friez, M. J., Funke, B. H., Hegde, M. R., & Lyon, E. (2013). ACMG clinical laboratory standards for next-generation sequencing. *Genet Med*, *15*(9), 733-747. https://doi.org/10.1038/gim.2013.92

Robson, M. E., Bradbury, A. R., Arun, B., Domchek, S. M., Ford, J. M., Hampel, H. L., Lipkin, S. M., Syngal, S., Wollins, D. S., & Lindor, N. M. (2015). American Society of Clinical Oncology Policy Statement Update: Genetic and Genomic Testing for Cancer Susceptibility. *Journal of Clinical Oncology*, *33*(31), 3660-3667. https://doi.org/10.1200/JCO.2015.63.0996

Splinter, K., Adams, D. R., Bacino, C. A., Bellen, H. J., Bernstein, J. A., Cheatle-Jarvela, A. M., Eng, C. M., Esteves, C., Gahl, W. A., Hamid, R., Jacob, H. J., Kikani, B., Koeller, D. M., Kohane, I. S., Lee, B. H., Loscalzo, J., Luo, X., McCray, A. T., Metz, T. O., . . . Ashley, E. A. (2018). Effect of Genetic Diagnosis on Patients with Previously Undiagnosed Disease. *N Engl J Med*, *379*(22), 2131-2139. https://doi.org/10.1056/NEJMoa1714458

Stoffel, E. M., McKernin, S. E., Brand, R., Canto, M., Goggins, M., Moravek, C., Nagarajan, A., Petersen, G. M., Simeone, D. M., Yurgelun, M., & Khorana, A. A. (2018). Evaluating Susceptibility to Pancreatic Cancer: ASCO Provisional Clinical Opinion. *Journal of Clinical Oncology*, *37*(2), 153-164. https://doi.org/10.1200/JCO.18.01489

Strom, S. P., Lee, H., Das, K., Vilain, E., Nelson, S. F., Grody, W. W., & Deignan, J. L. (2014). Assessing the necessity of confirmatory testing for exome-sequencing results in a clinical molecular diagnostic laboratory. *Genet Med*, *16*(7), 510-515. https://doi.org/10.1038/gim.2013.183

Surrey, L. F., MacFarland, S. P., Chang, F., Cao, K., Rathi, K. S., Akgumus, G. T., Gallo, D., Lin, F., Gleason, A., Raman, P., Aplenc, R., Bagatell, R., Minturn, J., Mosse, Y., Santi, M., Tasian, S. K., Waanders, A. J., Sarmady, M., Maris, J. M., . . . Li, M. M. (2019). Clinical utility of custom-designed NGS panel testing in pediatric tumors. *Genome Med*, *11*(1), 32. https://doi.org/10.1186/s13073-019-0644-8

Tayshetye, P., Miller, K., Monga, D., Brem, C., Silverman, J. F., & Finley, G. G. (2020). Molecular Profiling of Advanced Malignancies: A Community Oncology Network Experience and Review of Literature. *Front Med (Lausanne)*, *7*, 314. https://doi.org/10.3389/fmed.2020.00314

Woodhouse, R., Li, M., Hughes, J., Delfosse, D., Skoletsky, J., Ma, P., Meng, W., Dewal, N., Milbury, C., Clark, T., Donahue, A., Stover, D., Kennedy, M., Dacpano-Komansky, J., Burns, C., Vietz, C., Alexander, B., Hegde, P., & Dennis, L. (2020). Clinical and analytical validation of FoundationOne Liquid CDx, a novel 324-Gene cfDNA-based comprehensive genomic profiling assay for cancers of solid tumor origin. *PLOS ONE*, *15*(9), e0237802. https://doi.org/10.1371/journal.pone.0237802

Zehir, A., Benayed, R., Shah, R. H., Syed, A., Middha, S., Kim, H. R., Srinivasan, P., Gao, J., Chakravarty, D., Devlin, S. M., Hellmann, M. D., Barron, D. A., Schram, A. M., Hameed, M., Dogan, S., Ross, D. S., Hechtman, J. F., DeLair, D. F., Yao, J., . . . Berger, M. F. (2017). Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med*, *23*(6), 703-713. https://doi.org/10.1038/nm.4333