Molecular Oncology Testing for Solid Tumor Cancer Diagnosis, Prognosis, and Treatment Decisions (for Louisiana Only)

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Instructions for Use

Certain content mandated by Louisiana Department of Health

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Application

This Medical Policy only applies to the state of Louisiana. Portions of the coverage rationale contained in this policy represent Louisiana Medicaid coverage policy and are set forth below in accordance with state requirements.

Coverage Rationale

State-Specific Criteria

The coverage criteria for genetic counseling contained in this policy represents the Louisiana Medicaid Managed Care Organization Manual (LA MCO) coverage policy and is set forth below in accordance with State requirements.

Genetic Counseling

Genetic counseling before and after all genetic testing is required. Counseling must consist of at least all of the following and be documented in the medical record:

- Obtaining a structured family genetic history;
- Genetic risk assessment; and
- Counseling of the enrollee and family about diagnosis, prognosis, and treatment

(LA MCO Genetic Counseling and Testing)

Breast Cancer

Coverage of Oncotype DX Breast Cancer Assay for the Determination of Breast Cancer Prognosis

Coverage Criteria:

- Oncotype DX Breast Cancer Assay should be done within six months of the initial diagnosis of breast cancer

- Oncotype DX Breast Cancer Assay should be considered for individuals only after surgery and subsequent pathological examination of the tumor has been completed
- Histology indicates the cancer is ductal, lobular, mixed, or metaplastic
- Histology shows the cancer is not tubular or colloid
- Estrogen receptor is positive (ER+), or progesterone receptor is positive (PR+) or both
- HER2 receptor is negative
- Chemotherapy is a therapeutic option being considered for treatment and will be supervised by the practitioner ordering the gene expression profile
- Node negative or node positive (1-3 nodes only) on individuals who are post-menopausal

Gene expression profiling as a technique of managing the treatment of breast cancer is considered investigational and not medically necessary when a gene profiling test other than Oncotype DX Breast Cancer Assay is being used, including but not limited to:
- Breast Cancer Gene Expression Ratio (also known as Theros H/ISM)
- Breast Cancer IndexSM
- Insight® DX Breast Cancer Profile
- MammaPrint®
- Mammostrat
- Oncotype DX DCIS
- Pam50 Breast Cancer Intrinsic Classifier™
- The 41-gene signature assay
- The 76-gene "Rotterdam signature" assay
- THEROS Breast Cancer IndexSM

Gene expression profiling as a technique of managing the treatment of ductal carcinoma in situ (DCIS) is considered investigational and not medically necessary under all circumstances.

Repeat gene expression profiling with the Oncotype DX Breast Cancer Assay for the same tumor, such as a metastatic focus, or from more than one site when the primary tumor is multifocal is considered investigational and not medically necessary.
(Louisiana Department of Health and Hospitals, Health Plan Advisories 14-10, 2014)

Additional Non State-Specific Criteria

This policy applies to tests that have not been granted approval as an FDA cleared or approved Companion Diagnostic.

Lung Cancer

Molecular profiling of solid tumor tissue in metastatic non-small cell lung cancer is proven and medically necessary when the following criteria are met:
- No prior molecular profiling has been performed on the same tumor; and
- One of the following:
  - The multigene Next Generation Sequencing (NGS) panel selected has no more than 50 genes; or
  - Individual meets criteria for companion diagnostic testing*

Liquid Biopsy [cell-free DNA (cfDNA) or circulating tumor DNA (ctDNA)] molecular profiling tests of non-small cell lung cancer are proven and medically necessary when the following criteria are met:
- No prior molecular profiling has been performed on the same tumor; and
- The individual is not medically fit for invasive biopsy or tumor tissue testing is not feasible; and
- One of the following:
  - The multigene NGS panel selected has no more than 50 genes; or
  - Individual meets criteria for companion diagnostic testing*

*Refer to the Medical Policy titled Molecular Oncology Companion Diagnostic Testing (for Louisiana Only).

Prostate Cancer Gene Expression Profiling (GEP)

The use of the Oncotype DX® Genomic Prostate Score (GPS) is proven and medically necessary for individuals with biopsy-proven, untreated, localized adenocarcinoma of the prostate (no clinical evidence of metastasis or lymph node involvement) when:

- Test is ordered by a physician specializing in the treatment of organ confined prostate cancer including surgical oncology/urology, radiation oncology or medical oncology; and
- Results will be used to assist with treatment decision-making when the individual has not yet received treatment for prostate cancer and is a candidate for either active surveillance or definitive therapy and all of the following:
  - Life expectancy is greater than 10 years; and
  - Risk group is one of the following:
    - Very Low-Risk Prostate Cancer; or
    - Low-Risk Prostate Cancer; or
    - Favorable Intermediate-Risk Prostate Cancer

The use of the Prolaris® Biopsy prostate cancer prognostic test or Decipher® Prostate Biopsy genomic classifier is proven and medically necessary for individuals with biopsy-proven, untreated, localized adenocarcinoma of the prostate (no clinical evidence of metastasis or lymph node involvement) when:

- Test is ordered by a physician specializing in the treatment of organ confined prostate cancer including surgical oncology/urology, radiation oncology or medical oncology; and
- Results will be used to assist with treatment decision-making when the individual has not yet received treatment for prostate cancer and is a candidate for either active surveillance or definitive therapy and all of the following:
  - Life expectancy greater than 10 years; and
  - Risk group is one of the following:
    - Very Low-Risk Prostate Cancer; or
    - Low-Risk Prostate Cancer; or
    - Favorable Intermediate-Risk Prostate Cancer; or
    - Unfavorable Intermediate-Risk Prostate Cancer; or
    - High-Risk Prostate Cancer

The use of Decipher Prostate RP genomic classifier is proven and medically necessary to inform adjuvant treatment after radical prostatectomy for either of the following:

- Adverse features are found (e.g., high-grade disease, Gleason score 8 or higher, extracapsular extension, positive surgical margins, seminal vesicle invasion); or
- PSA is greater than zero at any point following prostatectomy

Molecular screening panel tests for prostate cancer are unproven and not medically necessary due to insufficient evidence of efficacy (e.g., ExoDx™ Prostate Test, My Prostate Score™, Confirm MDx™, Select MDx™).

Thyroid Cancer or Indeterminate Thyroid Nodule Testing

The use of GEP testing for thyroid nodules with indeterminate cytology [e.g., Afirma® Genomic Sequencing Classifier (GSC), ThyroSeq® V3, ThyGeNEXT®/ThyraMIR®] is proven and medically necessary when all of the following criteria are met:

- Follicular pathology on fine needle aspiration is indeterminate (Bethesda III/IV); and
- The results of the test will be used for making decisions about further surgery

Due to insufficient evidence of efficacy, molecular tests for indeterminate thyroid nodules other than those previously described as proven are unproven and not medically necessary, including but not limited to:

- Afirma® Xpression Atlas (XA)
- Comprehensive Genomic Profiling (CGP) (e.g., NeoTYPE® Thyroid Profile)

The use of more than one molecular profile test in an individual with an indeterminate thyroid nodule is unproven and not medically necessary due to insufficient evidence of efficacy.

CGP of confirmed anaplastic thyroid cancer is proven and medically necessary.

Uveal Melanoma Gene Expression Profiling (GEP)

GEP (e.g., DecisionDx®-UM) is considered proven and medically necessary when used to assist with predicting disease severity and making treatment decisions in the following situations:

- Individual has primary, localized uveal melanoma; and
- There is no evidence of metastatic disease; and
- Individual has not previously had DecisionDx-UM testing for current diagnosis

Due to insufficient evidence of efficacy, all other molecular testing of solid tumors with GEP, multigene NGS panels and/or CGP is unproven and not medically necessary, including but not limited to:

- NGS panels of > 50 genes unless otherwise specified
- Decipher® Bladder)
- ResponseDx Tissue of Origin™, CancerTYPE ID®, Rosetta Cancer Origin™, ProOnc
- PancraGEN®, PancreaSeq®
- Oncotype DX® colon cancer assay, Colorectal Cancer DSA™, GenefxH℠ Colon (also known as ColDx), OncoDefender™, CRC, ColoPrint®
- DecisionDx®-Melanoma, DermTech PLA™, myPath®-Melanoma
- MyPRS®/MyPRS Plus™
- Multi-cancer early detection/screening tests (e.g., Galleri®)
- TMPRSS2 fusion gene, ExoDX™ Prostate Test, MiPS (Mi Prostate Score Urine test), MyProstateScore (MPS, formerly MiPS), Confirm MDx™, Select MDx™
- Tumor-informed and tumor-naïve MRD assays (e.g., Invitae Personalized Cancer Monitoring, Signatera™, RaDaR®, Guardant Reveal™, Guardant Response™)
- Percepta® GSC for suspicious lung nodules
- Solid tumor profiling that includes Whole Exome, Whole Genome, or whole transcriptome Sequencing (e.g., Caris MI Tumor Seek™, Caris MI Profile™, Tempus xE)
- Whole genome methylation testing for tumors

Definitions

Comparative Genome Hybridization (CGH): CGH is a technology that can be used to detect genomic copy number variations (CNVs). Tests can use a variety of probes or single nucleotide polymorphisms (SNPS) to provide copy number and gene differentiating information. All platforms share that tumor (patient), and reference DNA are labeled with dyes or fluorescing probes and hybridized on the array, and a scanner measures differences in intensity between the probes, and the data is expressed as having greater or less intensity than the reference DNA (Cooley et al., 2013).

Comprehensive Genomic Profiling (CGP): A type of next-generation sequencing test that is able to detect all classes of genomic alterations, including cancer biomarkers, with a single sample (Singh et al., 2020).

Favorable Intermediate-Risk Prostate Cancer: Clinical/pathological features must include all of the following: No high- or very high-risk group features, Grade Group 1 or 2, less than 50% of biopsy cores are positive (e.g., < 6 of 12 cores) and has one or more intermediate risk factor (T2b-T2c, PSA less than 20) (NCCN Prostate Cancer, v2.2023).

Gene Expression Profiling (GEP): A laboratory test that analyzes mRNA patterns to determine gene activity (Kim et al., 2010). Also referred to as gene expression testing, gene expression classifier testing or gene expression assay.

High-Risk Prostate Cancer: Clinical/pathological features must include all of the following: No very high-risk features and exactly one of the following high-risk features: T3a OR Grade Group 4/5 OR PSA > 20 (NCCN Prostate Cancer, v2.2023).

Liquid Biopsy: Testing performed on a sample of bodily fluid to identify cancer cells from a tumor or pieces of DNA, RNA or other molecules that have been released from tumor cells and are circulating in an individual's body fluids. Liquid Biopsy may

be used for early detection of cancer, to help identify effective treatments or to monitor for return of cancer (NCI, Liquid Biopsy, 2023).

Low-Risk Prostate Cancer: Clinical/pathological features must include all of the following, but cancer does not qualify for very low-risk: PSA is less than 10, Grade Group 1, and T1-T2a disease (NCCN Prostate Cancer, v2.2023).

Next Generation Sequencing (NGS): New sequencing techniques that can quickly analyze multiple sections of DNA at the same time. Older forms of sequencing could only analyze one section of DNA at once (Kamps, et al. 2017).

Unfavorable Intermediate-Risk Prostate Cancer: Clinical/pathological features must include: No high- or very-high risk group features and one or more of the following: Grade Group 3, ≥ of 50% biopsy cores are positive (e.g., ≥ 6 of 12 cores), and either 2 or 3 intermediate risk factors (T2b-T2c disease, Grade Group 2 or 3, PSA 10-20) (NCCN Prostate Cancer, v2.2023).

Very High-Risk Prostate Cancer: Clinical/pathological features must include: 2 or 3 features of High-Risk Prostate Cancer, Primary Gleason pattern 5, T3b-T4 disease, and greater than 4 cores with Grade Group 4 or 5 (NCCN Prostate Cancer, v2.2023).

Very Low-Risk Prostate Cancer: Clinical/pathological features must include all of the following: PSA is less than 10, Grade Group 1, less than 3 biopsy cores positive with less than 50% cancer in each core and non-palpable disease (T1c) (NCCN Prostate Cancer, v2.2023).

Whole Exome Sequencing (WES): About 1% of a person's DNA makes protein. These protein making sections are called exons. All the exons together are called the exome. WES is a DNA analysis technique that looks at all the exons in a person, or a tissue type such as a tumor, at one time, rather than gene by gene (MedlinePlus, 2020).

Whole Genome Sequencing (WGS): WGS determines the sequence of the entire DNA in a person, or a tissue type, such as a tumor, which includes the protein making-(coding) as well as non-coding DNA elements (MedlinePlus, 2020).

Applicable Codes

The following list(s) of procedure and/or diagnosis codes is provided for reference purposes only and may not be all inclusive. Listing of a code in this policy does not imply that the service described by the code is a covered or non-covered health service. Benefit coverage for health services is determined by federal, state, or contractual requirements and applicable laws that may require coverage for a specific service. The inclusion of a code does not imply any right to reimbursement or guarantee claim payment. Other Policies and Guidelines may apply.

CPT Code	Description
*0005U	Oncology (prostate) gene expression profile by real-time RT-PCR of 3 genes (ERG, PCA3, and SPDEF), urine, algorithm reported as risk score
*0011M	Oncology, prostate cancer, mRNA expression assay of 12 genes (10 content and 2 housekeeping), RT-PCR test utilizing blood plasma and urine, algorithms to predict high-grade prostate cancer risk
*0012M	Oncology (urothelial), mRNA, gene expression profiling by real-time quantitative PCR of five genes (MDK, HOXA13, CDC2 [CDK1], IGFBP5, and CXCR2), utilizing urine, algorithm reported as a risk score for having urothelial carcinoma
*0013M	Oncology (urothelial), mRNA, gene expression profiling by real-time quantitative PCR of five genes (MDK, HOXA13, CDC2 [CDK1], IGFBP5, and CXCR2), utilizing urine, algorithm reported as a risk score for having recurrent urothelial carcinoma
*0016M	Oncology (bladder), mRNA, microarray gene expression profiling of 219 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as molecular subtype (luminal, luminal infiltrated, basal, basal claudin-low, neuroendocrine-like)

CPT Code	Description
*0017M	Oncology [diffuse large B-cell lymphoma (DLBCL)], mRNA, gene expression profiling by fluorescent probe hybridization of 20 genes, formalin-fixed paraffin-embedded tissue, algorithm reported as cell of origin
*0018U	Oncology (thyroid), microRNA profiling by RT-PCR of 10 microRNA sequences, utilizing fine needle aspirate, algorithm reported as a positive or negative result for moderate to high risk of malignancy
*0019U	Oncology, RNA, gene expression by whole transcriptome sequencing, formalin-fixed paraffin embedded tissue or fresh frozen tissue, predictive algorithm reported as potential targets for therapeutic agents
*0021U	Oncology (prostate), detection of 8 autoantibodies (ARF 6, NKX3-1, 5'-UTR-BMI1, CEP 164, 3'-UTR-Ropporin, Desmocollin, AURKAIP-1, CSNK2A2), multiplexed immunoassay and flow cytometry serum, algorithm reported as risk score
*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
*0026U	Oncology (thyroid), DNA and mRNA of 112 genes, next-generation sequencing, fine needle aspirate of thyroid nodule, algorithmic analysis reported as a categorical result ("Positive, high probability of malignancy" or "Negative, low probability of malignancy")
*0036U	Exome (i.e., somatic mutations), paired formalin-fixed paraffin-embedded tumor tissue and normal specimen, sequence analyses
*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
*0045U	Oncology (breast ductal carcinoma in situ), mRNA, gene expression profiling by real-time RT-PCR of 12 genes (7 content and 5 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as recurrence score
*0047U	Oncology (prostate), mRNA, gene expression profiling by real-time RT-PCR of 17 genes (12 content and 5 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a risk score
*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)
*0050U	Targeted genomic sequence analysis panel, acute myelogenous leukemia, DNA analysis, 194 genes, interrogation for sequence variants, copy number variants or rearrangements
*0069U	Oncology (colorectal), microRNA, RT-PCR expression profiling of miR-31-3p, formalin-fixed paraffin-embedded tissue, algorithm reported as an expression score
*0089U	Oncology (melanoma), gene expression profiling by RTqPCR PRAME and LINC00518, superficial collection using adhesive patch(es)
*0090U	Oncology (cutaneous melanoma), mRNA gene expression profiling by RT-PCR of 23 genes (14 content and 9 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a categorical result (i.e., benign, intermediate, malignant)
*0091U	Oncology (colorectal) screening, cell enumeration of circulating tumor cells, utilizing whole blood, algorithm, for the presence of adenoma or cancer, reported as a positive or negative result
*0113U	Oncology (prostate), measurement of PCA3 and TMPRSS2-ERG in urine and PSA in serum following prostatic massage, by RNA amplification and fluorescence-based detection, algorithm reported as risk score
*0118U	Transplantation medicine, quantification of donor-derived cell-free DNA using whole genome next-generation sequencing, plasma, reported as percentage of donor-derived cell-free DNA in the total cell-free DNA

CPT Code	Description
*0120U	Oncology (B-cell lymphoma classification), mRNA, gene expression profiling by fluorescent probe hybridization of 58 genes (45 content and 13 housekeeping genes), formalin-fixed paraffin-embedded tissue, algorithm reported as likelihood for primary mediastinal B-cell lymphoma (PMBCL) and diffuse large B-cell lymphoma (DLBCL) with cell of origin subtyping in the latter
*0153U	Oncology (breast), mRNA, gene expression profiling by next-generation sequencing of 101 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a triple negative breast cancer clinical subtype(s) with information on immune cell involvement
*0171U	Targeted genomic sequence analysis panel, acute myeloid leukemia, myelodysplastic syndrome, and myeloproliferative neoplasms, DNA analysis, 23 genes, interrogation for sequence variants, rearrangements and minimal residual disease, reported as presence/absence
*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)
*0204U	Oncology (thyroid), mRNA, gene expression analysis of 593 genes (including BRAF, RAS, RET, PAX8, and NTRK) for sequence variants and rearrangements, utilizing fine needle aspirate, reported as detected or not detected
*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
*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
*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
*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
*0245U	Oncology (thyroid), mutation analysis of 10 genes and 37 RNA fusions and expression of 4 mRNA markers using next-generation sequencing, fine needle aspirate, report includes associated risk of malignancy expressed as a percentage
*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
*0262U	Oncology (solid tumor), gene expression profiling by real-time RT-PCR of 7 gene pathways (ER, AR, PI3K, MAPK, HH, TGFB, Notch), formalin-fixed paraffin-embedded (FFPE), algorithm reported as gene pathway activity score
*0285U	Oncology, response to radiation, cell-free DNA, quantitative branched chain DNA amplification, plasma, reported as a radiation toxicity score
*0287U	Oncology (thyroid), DNA and mRNA, next-generation sequencing analysis of 112 genes, fine needle aspirate or formalin-fixed paraffin-embedded (FFPE) tissue, algorithmic prediction of cancer recurrence, reported as a categorical risk result (low, intermediate, high)
*0288U	Oncology (lung), mRNA, quantitative PCR analysis of 11 genes (BAG1, BRCA1, CDC6, CDK2AP1, ERBB3, FUT3, IL11, LCK, RND3, SH3BGR, WNT3A) and 3 reference genes (ESD, TBP, YAP1), formalin-fixed paraffin-embedded (FFPE) tumor tissue, algorithmic interpretation reported as a recurrence risk score
*0296U	Oncology (oral and/or oropharyngeal cancer), gene expression profiling by RNA sequencing at least 20 molecular features (e.g., human and/or microbial mRNA), saliva, algorithm reported as positive or negative for signature associated with malignancy

CPT Code	Description
*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
*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
*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
*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
*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
*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
*0313U	Oncology (pancreas), DNA and mRNA next-generation sequencing analysis of 74 genes and analysis of CEA (CEACAM5) gene expression, pancreatic cyst fluid, algorithm reported as a categorical result (i.e., negative, low probability of neoplasia or positive, high probability of neoplasia)
*0314U	Oncology (cutaneous melanoma), mRNA gene expression profiling by RT-PCR of 35 genes (32 content and 3 housekeeping), utilizing formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as a categorical result (i.e., benign, intermediate, malignant)
*0315U	Oncology (cutaneous squamous cell carcinoma), mRNA gene expression profiling by RT-PCR of 40 genes (34 content and 6 housekeeping), utilizing formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as a categorical risk result (i.e., Class 1, Class 2A, Class 2B)
*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
*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
*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 alterations
*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
*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
*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
*0339U	Oncology (prostate), mRNA expression profiling of HOXC6 and DLX1, reverse transcription polymerase chain reaction (RT-PCR), first-void urine following digital rectal examination, algorithm reported as probability of high-grade cancer

CPT Code	Description
*0340U	Oncology (pan-cancer), analysis of minimal residual disease (MRD) from plasma, with assays personalized to each patient based on prior next-generation sequencing of the patient's tumor and germline DNA, reported as absence or presence of MRD, with disease-burden correlation, if appropriate
*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
*0356U	Oncology (oropharyngeal or anal), evaluation of 17 DNA biomarkers using droplet digital PCR (ddPCR), cell-free DNA, algorithm reported as a prognostic risk score for cancer recurrence
*0362U	Oncology (papillary thyroid cancer), gene-expression profiling via targeted hybrid capture–enrichment RNA sequencing of 82 content genes and 10 housekeeping genes, fine needle aspirate or formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as one of three molecular subtypes
*0363U	Oncology (urothelial), mRNA, gene expression profiling by real-time quantitative PCR of 5 genes (MDK, HOXA13, CDC2 [CDK1], IGFBP5, and CXCR2), utilizing urine, algorithm incorporates age, sex, smoking history, and macrohematuria frequency, reported as a risk score for having urothelial carcinoma
*0364U	Oncology (hematolymphoid neoplasm), genomic sequence analysis using multiplex (PCR) and next-generation sequencing with algorithm, quantification of dominant clonal sequence(s), reported as presence or absence of minimal residual disease (MRD) with quantitation of disease burden, when appropriate
*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
0379U	Targeted genomic sequence analysis panel, solid organ neoplasm, DNA (523 genes) and RNA (55 genes) by next-generation sequencing, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability, and tumor mutational burden
*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
*0391U	Oncology (solid tumor), DNA and RNA by next-generation sequencing, utilizing formalin-fixed paraffin-embedded (FFPE) tissue, 437 genes, interpretive report for single nucleotide variants, splicesite variants, insertions/deletions, copy number alterations, gene fusions, tumor mutational burden, and microsatellite instability, with algorithm quantifying immunotherapy response score
*0409U	Oncology (solid tumor), DNA (80 genes) and RNA (36 genes), by next-generation sequencing from plasma, including single nucleotide variants, insertions/deletions, copy number alterations, microsatellite instability, and fusions, report showing identified mutations with clinical actionability
*0420U	Oncology (urothelial), mRNA expression profiling by real-time quantitative PCR of MDK, HOXA13, CDC2, IGFBP5, and CXCR2 in combination with droplet digital PCR (ddPCR) analysis of 6 single-nucleotide polymorphisms (SNPs) genes TERT and FGFR3, urine, algorithm reported as a risk score for urothelial carcinoma
*0421U	Oncology (colorectal) screening, quantitative real-time target and signal amplification of 8 RNA markers (GAPDH, SMAD4, ACY1, AREG, CDH1, KRAS, TNFRSF10B, EGLN2) and fecal hemoglobin, algorithm reported as a positive or negative for colorectal cancer risk
*0422U	Oncology (pan-solid tumor), analysis of DNA biomarker response to anti-cancer therapy using cell-free circulating DNA, biomarker comparison to a previous baseline pre-treatment cell-free circulating DNA analysis using next-generation sequencing, algorithm reported as a quantitative change from baseline, including specific alterations, if appropriate
*0424U	Oncology (prostate), exosome-based analysis of 53 small noncoding RNAs (sncRNAs) by quantitative reverse transcription polymerase chain reaction (RT-qPCR), urine, reported as no molecular evidence, low-, moderate- or elevated-risk of prostate cancer

CPT Code	Description
*0428U	Oncology (breast), targeted hybrid-capture genomic sequence analysis panel, circulating tumor DNA (ctDNA) analysis of 56 or more genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability, and tumor mutation burden
*0433U	Oncology (prostate), 5 DNA regulatory markers by quantitative PCR, whole blood, algorithm, including prostate-specific antigen, reported as likelihood of cancer
*81228	Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of genomic regions for copy number variants, comparative genomic hybridization [CGH] microarray analysis
81229	Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants, comparative genomic hybridization (CGH) microarray analysis
*81277	Cytogenomic neoplasia (genome-wide) microarray analysis, interrogation of genomic regions for copy number and loss-of-heterozygosity variants for chromosomal abnormalities
*81425	Genome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis
*81426	Genome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator genome (e.g., parents, siblings) (List separately in addition to code for primary procedure)
*81427	Genome (e.g., unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained genome sequence (e.g., updated knowledge or unrelated condition/syndrome)
*81445	Solid organ neoplasm, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants and copy number variants or rearrangements, if performed; DNA analysis or combined DNA and RNA analysis
*81449	Solid organ neoplasm, genomic sequence analysis panel, 5-50 genes, interrogation for sequence variants and copy number variants or rearrangements, if performed; RNA analysis
*81455	Solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes, genomic sequence analysis panel, interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis
*81456	Solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes, genomic sequence analysis panel, interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; RNA analysis
*81457	Solid organ neoplasm, genomic sequence analysis panel, interrogation for sequence variants; DNA analysis, microsatellite instability
*81458	Solid organ neoplasm, genomic sequence analysis panel, interrogation for sequence variants; DNA analysis, copy number variants and microsatellite instability
*81459	Solid organ neoplasm, genomic sequence analysis panel, interrogation for sequence variants; DNA analysis or combined DNA and RNA analysis, copy number variants, microsatellite instability, tumor mutation burden, and rearrangements
*81462	Solid organ neoplasm, genomic sequence analysis panel, cell-free nucleic acid (eg, plasma), interrogation for sequence variants; DNA analysis or combined DNA and RNA analysis, copy number variants and rearrangements
*81463	Solid organ neoplasm, genomic sequence analysis panel, cell-free nucleic acid (eg, plasma), interrogation for sequence variants; DNA analysis, copy number variants, and microsatellite instability
*81464	Solid organ neoplasm, genomic sequence analysis panel, cell-free nucleic acid (eg, plasma), interrogation for sequence variants; DNA analysis or combined DNA and RNA analysis, copy number variants, microsatellite instability, tumor mutation burden, and rearrangements
81479	Unlisted molecular pathology procedure
*81504	Oncology (tissue of origin), microarray gene expression profiling of > 2000 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as tissue similarity scores

CPT Code	Description
*81518	Oncology (breast), mRNA, gene expression profiling by real-time RT-PCR of 11 genes (7 content and 4 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithms reported as percentage risk for metastatic recurrence and likelihood of benefit from extended endocrine therapy
81519	Oncology (breast), mRNA, gene expression profiling by real-time RT-PCR of 21 genes, utilizing formalin-fixed paraffin embedded tissue, algorithm reported as recurrence score
*81520	Oncology (breast), mRNA gene expression profiling by hybrid capture of 58 genes (50 content and 8 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a recurrence risk score
*81521	Oncology (breast), mRNA, microarray gene expression profiling of 70 content genes and 465 housekeeping genes, utilizing fresh frozen or formalin-fixed paraffin-embedded tissue, algorithm reported as index related to risk of distant metastasis
*81522	Oncology (breast), mRNA, gene expression profiling by RT-PCR of 12 genes (8 content and 4 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as recurrence risk score
*81523	Oncology (breast), mRNA, next-generation sequencing gene expression profiling of 70 content genes and 31 housekeeping genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as index related to risk to distant metastasis
*81525	Oncology (colon), mRNA, gene expression profiling by real-time RT-PCR of 12 genes (7 content and 5 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a recurrence score
*81529	Oncology (cutaneous melanoma), mRNA, gene expression profiling by real-time RT-PCR of 31 genes (28 content and 3 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as recurrence risk, including likelihood of sentinel lymph node metastasis
*81540	Oncology (tumor of unknown origin), mRNA, gene expression profiling by real-time RT-PCR of 92 genes (87 content and 5 housekeeping) to classify tumor into main cancer type and subtype, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a probability of a predicted main cancer type and subtype
*81541	Oncology (prostate), mRNA gene expression profiling by real-time RT-PCR of 46 genes (31 content and 15 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a disease-specific mortality risk score
*81542	Oncology (prostate), mRNA, microarray gene expression profiling of 22 content genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as metastasis risk score
*81546	Oncology (thyroid), mRNA, gene expression analysis of 10,196 genes, utilizing fine needle aspirate, algorithm reported as a categorical result (e.g., benign or suspicious)
*81551	Oncology (prostate), promoter methylation profiling by real-time PCR of 3 genes (GSTP1, APC, RASSF1), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a likelihood of prostate cancer detection on repeat biopsy
*81552	Oncology (uveal melanoma), mRNA, gene expression profiling by real-time RT-PCR of 15 genes (12 content and 3 housekeeping), utilizing fine needle aspirate or formalin-fixed paraffin-embedded tissue, algorithm reported as risk of metastasis
*81599	Unlisted multianalyte assay with algorithmic analysis
*86152	Cell enumeration using immunologic selection and identification in fluid specimen (e.g., circulating tumor cells in blood)
*86153	Cell enumeration using immunologic selection and identification in fluid specimen (e.g., circulating tumor cells in blood); physician interpretation and report, when required

Codes labeled with an asterisk (*) are not on the State of Louisiana Medicaid Fee Schedule and therefore may not be covered by the State of Louisiana Medicaid Program.

HCPCS Code	Description
G0327	Colorectal cancer screening; blood-based biomarker

Diagnosis Code	Description
C90.10	Plasma cell leukemia not having achieved remission
C90.11	Plasma cell leukemia in remission
C90.12	Plasma cell leukemia in relapse
C91.00	Acute lymphoblastic leukemia not having achieved remission
C91.01	Acute lymphoblastic leukemia, in remission
C92.02	Acute myeloblastic leukemia, in relapse
C92.40	Acute promyelocytic leukemia, not having achieved remission
C92.41	Acute promyelocytic leukemia, in remission
C92.42	Acute promyelocytic leukemia, in relapse
C92.50	Acute myelomonocytic leukemia, not having achieved remission
C92.51	Acute myelomonocytic leukemia, in remission
C92.52	Acute myelomonocytic leukemia, in relapse
C92.60	Acute myeloid leukemia with 11q23-abnormality not having achieved remission
C92.61	Acute myeloid leukemia with 11q23-abnormality in remission
C92.62	Acute myeloid leukemia with 11q23-abnormality in relapse
C92.A0	Acute myeloid leukemia with multilineage dysplasia, not having achieved remission
C92.A1	Acute myeloid leukemia with multilineage dysplasia, in remission
C92.A2	Acute myeloid leukemia with multilineage dysplasia, in relapse
C95.90	Leukemia, unspecified not having achieved remission
C95.91	Leukemia, unspecified, in remission
C95.92	Leukemia, unspecified, in relapse

Description of Services

Technologies used for molecular profiling of solid tumor cancers vary, and can include, but are not limited to, tests that evaluate variations in the genes, such as Chromosome Microarray Analysis and Next Generation Sequencing, as well as others that assess the gene products, such as gene expression arrays and microRNA analysis. The amount of genetic material evaluated can range from a single gene to the whole exome or genome of a tumor. For the purposes of this policy, multi-gene analysis generally refers to a gene panel containing five or more genes, though some exceptions may apply as noted specifically in the policy. In some tests, expression patterns of defined genes are combined in a defined manner to provide an expression signature, a score, or a classifier for potential diagnosis and or prognosis of disease or to predict impact of intervention. Results of molecular profiling may assist individuals and healthcare providers with determining prognosis and selection of more effective and targeted cancer therapies (Chantrill et al., 2015).

Clinical Evidence

Thyroid Cancer/Indeterminate Thyroid Nodules

In 2022, Lee et al. conducted a systematic review and meta-analysis to appraise the diagnostic performance of the second-generation molecular tests in diagnosing thyroid nodules with indeterminate fine-needle aspiration biopsy results. Contained within the examination were 15 studies: 7 Afirma Genomic Sequencing Classifier (GSC), 6 ThyroSeq v3, and 2 ThyGeNext.

Studies on ThyGeNext were excluded from the meta-analysis due to their small sample sizes. Pooled data for GSC studies on 472 thyroid nodules displayed a sensitivity of 96.6 (95% confidence interval: 89.7–98.9%), specificity of 52.9% (23.4–80.5%), PPV of 63% (51–74%), and NPV of 96% (94–98%). Pooled data for ThyroSeq studies on 530 thyroid nodules presented a sensitivity of 95.1% (91.1–97.4%), specificity of 49.6% (29.3–70.1%), PPV of 70% (55–83%), and NPV of 92% (86–97%). There was not a statistically significant variance in the diagnostic performances of GSC and ThyroSeq (p-values for sensitivity = 0.89, specificity = 0.82, PPV = 0.43, NPV = 0.17). Limitations to the study include the small number of studies contained within the meta-analysis, no long-term analysis of the utility of the tests, and only two studies evaluated on ThyGeNext. The authors concluded from the review that high sensitivity and NPV in GSC and ThyroSeq V3 may help rule out malignancy amid thyroid nodules with indeterminate cytology results. There was no difference in diagnostic performances between the two molecular tests displaying that either test is suitable for the malignancy of thyroid nodules. Studies by Livhits et al. (2021) and Endo et al. (2019), previously discussed in this policy, were included in this systematic review by Lee et al.

Hu et al. (2021) investigated molecular findings across a large, real-world cohort of thyroid fine needle aspiration (FNA) samples through a retrospective analysis of RNA sequencing data files. Overall, there was a total of 50,644 consecutive Bethesda III-VI nodules included. The Afirma Genomic Sequencing Classifier (GSC), which uses whole transcriptome RNA sequencing to identify thyroid nodules as either benign or suspicious, confirmed that 66% of the 48,952 Bethesda III/IV FNA studied were benign. Among all Bethesda III/IV FNAs and 76% of Bethesda VI FNAs, the prevalence of *BRAF* V600E was 2%. Named were 130 different gene partners and fusions involving *NTRK, RET, BRAF*, and *ALK*, primarily prevalent in Bethesda V (10%). *BRAF* and *ALK* fusions were 81% and 67%, respectively; the positive predictive value of an *NTRK* or *RET* fusion for carcinoma or noninvasive follicular thyroid neoplasm with papillary-like nuclear features was > 95% among small consecutive Bethesda III/IV sample cohorts with one of these fusions' available surgical pathology excision data. The expanded Afirma Expression Atlas (XA) panel identified at least one genomic alteration in 70% of medullary thyroid carcinoma classifier positive FNAs, 44% of Bethesda III or IV Afirma GSC suspicious FNAs, 64% of Bethesda V FNAs, and 87% of Bethesda VI FNAs. Based on the results of this study, the authors felt the analytical and clinical validity of the Afirma GSC and XA assays were confirmed. However, the authors did not correlate the surgical pathology outcome with most of the FNA samples described or report surgical histology. There was no central blinded histopathologic review, and there is potential selection bias, especially among Bethesda V and VI samples.

In 2022, Babazadeh et al. reported on the clinical utility of Afirma XA testing during two years of clinical use. Afirma XA became available in 2018 and assesses 593 genes, including 905 potential variants and 235 fusions. Afirma XA was performed on 136 indeterminate nodules (103 of these met inclusion criteria). Forty-three of those had positive Afirma XA results, 83.7% of which were follicular cell-derived thyroid cancer on surgical histopathology. Overall PPV among Afirma GSC–suspicious indeterminate nodules during the same timeframe was 82.5%, similar to the Afirma XA results. Of the 60 nodules that tested negative with Afirma XA, 73.3% were follicular cell-derived thyroid cancer on surgical histopathology. The authors concluded that the Afirma XA positivity is predictive of follicular cell-derived thyroid cancer with PPV similar to that of GSC –suspicious results alone at the institution where the study took place. It is still uncertain whether Afirma XA results significantly increase the preoperative risk of malignancy for cytologically indeterminate nodules. More extensive studies on variants and fusions associated with varied risks of malignancy are needed. Longer-term data collection of Afirma XA results and related clinical variables is principal in standardizing how thyroid cancer specialists should use this molecular test.

A Hayes Molecular Test Assessment found limited but positive evidence supporting the Afirma GSC assay for identification of benign thyroid nodules in results deemed indeterminate by cytopathology so that individuals may avoid unnecessary surgical intervention. The evidence showed the GSC test has a high sensitivity and NPV, and the specificity and PPV varied between studies due to the lack of Afirma benign nodules resected to assess test performance. The evidence acclaims the GSC test had better specificity and PPV when equated to the previous version of the test, the Genomic Expression Classifier, however studies could not confirm statistically significant differences in the values due to the limited number of resected nodules. Additional studies are required to report the follow up of individuals with Afirma benign outcomes, specifically around missed malignancies, to support the test performance [Hayes, Afirma Genomic Sequencing Classifier (Veracyte Inc.), 2021, updated 2022].

Hayes assessed the use of the ThyGeNEXT and ThyraMIR tests in a Molecular Test Assessment. The assessment uncovered inadequate evidence supporting the use of the ThyGeNEXT and ThyraMIR tests to assist with reclassifying thyroid nodules with indeterminate cytology [Hayes, ThyGeNEXT and ThyraMIR (Interpace Diagnostics Group Inc.) 2021, updated 2022].

A Hayes Molecular Test Assessment addressing the ThyroSeq v3 test uncovered two studies and concluded there is inadequate evidence to support the use of the ThyroSeq test in the preoperative evaluation of thyroid nodules with indeterminate cytology to evaluate the possibility of cancer in a specified nodule and to offer prognostic information for treatment management [Hayes, ThyroSeq v3 Genomic Classifier (GC) (University of Pittsburgh Medical Center, CBLPath Inc.), 2019, updated 2021].

In a prospective blinded, multicenter study by Steward et al. (2019, included in the Lee et al. 2022 systematic review and the Hayes ThyroSeq v3 Genomic Classifier Molecular Test Assessment above), authors sought to find the diagnostic exactness of a multigene classifier test (ThyroSeq v3) for cytologically indeterminate thyroid nodules. The study enrolled 782 individuals with a total of 1,013 nodules. Of those, 286 FNA samples from 256 individuals met inclusion criteria and underwent molecular analysis with the multigene GC (ThyroSeq v3). The primary outcome of this study was the correct separation of benign histopathological nodules from cancer and noninvasive follicular thyroid neoplasms with papillary-like nuclei (NIFTP) in samples with Bethesda III and IV cytology. Of the 286 cytologically indeterminate nodules, 206 (72%) were benign, 69 (24%) were malignant, and 11 (4%) were noninvasive follicular thyroid neoplasms with papillary-like nuclei (NIFTP). Overall, 257 (90%) nodules (154 Bethesda III, 93 Bethesda IV, and 10 Bethesda V) had informative GC analysis, with 61% classified as negative and 39% as positive. The test collectively established a 94% (95% CI, 86%-98%) sensitivity and 82% (95% CI, 75%-87%) specificity in Bethesda III and IV nodules. With a cancer/NIFTP incidence of 28%, the negative predictive value (NPV) was 97% (95% CI, 93%-99%), and the PPV was 66% (95% CI, 56%-75%). The detected 3% false-negative rate was comparable to benign cytology; the missed cancers were all low-risk tumors. Between nodules testing positive, precise groups of genetic variations had cancer likelihoods fluctuating from 59% to 100%. Limitations to the study include a small sample size and no long-term clinical impact outcomes established. The authors concluded the multigene genomic classifier test (ThyroSeq v3) showed high sensitivity/NPV and relatively high specificity/PPV, which could eliminate the need for diagnostic surgical procedures in up to 82% of all benign thyroid nodules with indeterminate cytology and 61% of individuals with Bethesda III to IV indeterminate nodules.

Angell et al. (2019) reported on their clinical and analytical validation of the Afirma® XA, which uses whole transcriptome RNA-sequencing to detect gene variations and fusions from a panel of over 500 genes in thyroid fine needle aspiration (FNA) samples. From the same sample, DNA and RNA were purified using 943 blinded FNAs and multiple methodologies were used for comparison, including whole-transcriptome RNA-seq, targeted RNA-seq, and targeted DNA-seq. To define performance for fusions between whole transcriptome RNA-seq and targeted RNA-seq, 695 additional blinded FNAs were used. Of variants detected in DNA at 5 or 20% variant allele frequency, 74 and 88% were also detected by XA, respectively, and XA variant detection was 89% compared to another RNA-based detection method. Analytical validation studies showed high intra-plate reproducibility (89%-94%), inter-plate reproducibility (86–91%), and inter-lab accuracy (90%). Multiple variants and fusions formerly described across the spectrum of thyroid cancers were identified by XA, some of which have approved or investigational targeted therapies. The sensitivity of XA as a standalone test was 49% in 190 Bethesda III/IV nodules. Limitations of measuring variants in expressed RNA were identified, including the fact that some variants and fusions that were identified by an alternative method were not identified by XA; the researchers were not able to determine the reason for the difference, nor which tests was “correct”. The authors concluded that the data from this study supports the clinical and analytical validity of XA for GSC suspicious or for Bethesda V/VI nodules. The asserted that XA may also enhance genomic insight when the Afirma GSC is used first for Bethesda III/IV nodules as a rule-out test and results are GSC suspicious and may ultimately help to inform personalized clinical decision-making in individuals with thyroid nodules and thyroid cancer. Further studies addressing the clinical utility of this test are needed.

Deaver et al. (2018) conducted a retrospective analysis of 2019 thyroid FNA from 2011 to 2015. The samples were categorized using the Bethesda System for reporting thyroid cytology into B3 and B4 nodules. GEC results from Afirma were available for 54% of B3 cases, with about half having a benign classification. In the B4 group, 52% had GEC, with 28.6% classified as benign. The authors followed 73 benign GEC cases. Five underwent surgery and no malignancy was found. The remainder continued to have a stable size, and in those that had repeat FNA, about 72%, no malignancy was noted. The authors concluded that GEC results accurately predicted benign thyroid nodules.

In a meta-analysis of the gene expression classifier (GEC) for the diagnosis of indeterminate thyroid nodules, Santhanam et al. (2016) evaluated 7 out of 58 potential studies. The reference standard for determination of benign or malignant nodules was the histopathology of the thyroidectomy specimen. A QUADAS-2 report for all studies included in the final analysis was tabulated for risk of bias and applicability. The pooled sensitivity of the GEC for malignant histology was 95.7% (95% CI 92.2-97.9, I (2) value 45.4%, p = 0.09), and the pooled specificity was 30.5% (95% CI 26.0-35.3, I (2) value 92.1%, p < 0.01). Overall, the

diagnostic odds ratio was 7.9 (95% CI 4.1-15.1). Although the meta-analysis revealed a high pooled sensitivity and low specificity for the Afirma GEC, individuals with a benign GEC were not followed long enough to ascertain the actual false-negative rates of the index test.

The Afirma gene classifier, a gene expression analysis of 167 genes, has a sensitivity of 92% with a negative predictive value (NPV) of 93% in the largest prospective study of indeterminate nodules to date (Alexander et al., 2012). However, a study performed in a community hospital–based thyroid surgery practice (Harell and Bimston, 2014) showed a lower NPV (89.6%) than other studies in the literature, leading some to conclude (Zhang and Lin, 2016, Marti et al., 2015) that the Afirma test will only provide the most useful information in a practice setting with a prevalence of malignancy in indeterminate thyroid lesions of 15% to 21% where a NPV > 95% and PPV > 25% would be expected. Outside this range it is unlikely the test can provide information that would alter management. Marti et al. (2015) conducted a retrospective review of the Afirma gene classifier at two institutions from February 2013 to December 2014 and found that there were wide variations in the Afirma GEC-benign call rate, PPV, and NPV between the two institutions: one a comprehensive health system with a TMC prevalence of 30–38% and the second a tertiary referral cancer center with a prevalence 10-19%. Each had differing rates of malignancy in indeterminate thyroid nodules and Afirma did not routinely alter management in both institution and the NPV ranged from 86-98%. In addition, the Afirma 167 gene classifier appears to be less accurate in nodules with that contain benign Hurthle cells. In several studies that examined the cytology population percentage of Hurthle cells, the test was more likely to report a suspicious for malignancy result for which the patient was sent for surgery, and therefore limited the clinical utility of the test (Harrell and Bimston, 2014, Brauner et al., 2015, Lastra et al., 2014).

In a retrospective analysis of 189 thyroid FNAs with indeterminate cytology, Yang et al. (2016) examined the refining role of the Afirma GEC test in a 20-month period after implementation. Correlation with surgical follow-up, when available, was performed. The excisional rate of atypia of undetermined significance-follicular lesion of undetermined significance in the pre-GEC category was 63%, which decreased to 35% in the post-GEC category, whereas the malignancy rate in the excised thyroids increased from 35% in the pre-GEC category to 47% in the post-GEC category. Similar findings also were obtained for suspicious for follicular neoplasm-follicular neoplasm lesions. The authors concluded that the strength of the GEC test appears to lie in its ability to reclassify 42% of indeterminate cytology cases as benign, thereby decreasing the number of unnecessary surgical procedures.

Pagan et al. (2016) investigated the prevalence of genetic alterations in diverse subtypes of thyroid nodules beyond papillary thyroid carcinomas (PTC) in 851 variants and 133 fusions in 524 genes. After adding a cohort of tissue samples, the authors found 38/76 (50%) of histopathology malignant samples and 15/75 (20%) of benign samples to harbor a genetic alteration. In a direct comparison of the same FNA also tested by an RNA-based gene expression classifier (GEC), the sensitivity of genetic alterations alone was 42%, compared to the 91% sensitivity achieved by the GEC. The specificity based only on genetic alterations was 84%, compared to 77% specificity with the GEC. Due to the finding that variants are also found in benign nodules, the authors conclude that testing only GEC suspicious nodules may be helpful in avoiding false positives and altering the extent of treatment when selected mutations are found. Sipos et al. (2016) retrospectively evaluated the long-term follow-up of patients with a 'benign' Afirma GEC to determine impact on management compared to published data. During 36 months of follow-up, 17 of 98 patients (17.3%) had thyroid surgery; the majority (88%) being performed within 2 years. According to the authors, this represents a reduction in thyroid surgeries compared to patients that did not have a GEC performed on suspicious lesions. Limitations of this study are small patient population and non-randomization of patients.

MicroRNAs (miRNA) are small noncoding RNAs that regulate gene expression. Research has demonstrated that a number of miRNAs are differentially expressed between benign and malignant thyroid nodules which have led to the development of miRNA based diagnostic lab tests, and in some cases, labs may offer miRNA testing in conjunction with gene variant and expression analysis. Wylie et al. (2016) conducted a study examining genetic variant and miRNA analysis on archived pathology samples from the University of Michigan. The samples consisted of an initial set of 235 aspirates representing 118 nodules with benign cytology, including 13 with surgical outcome (12 benign, 1 malignant), 73 with malignant cytology, including 51 with surgical outcome (1 benign, 50 malignant), and 44 with indeterminate cytology, all with available surgical outcome. The second set of aspirates consisted of 42 distinct nodules with indeterminate cytology and surgical outcome. Thirty-one miRNAs were analyzed as well as 17 genetic alterations in the BRAF, RAS, RET and PAX8 genes, considered standard mutation testing. Furthermore, 54 samples that were negative by the 17-mutation panel were interrogated using a miRNA classification algorithm, commercially available as the ThyraMIR Thyroid miRNA Classifier, which analyzes in parallel 20 genes through next generation sequencing and 46 mRNA transcripts. The authors found that standard mutation testing alone had a sensitivity of

61%, consistent with the literature. Machine learning was utilized to group miRNA analysis into two groups of miRNAs, classifier A and classifier B. When miRNA classifier A was included in the analysis, the sensitivity rose to 78%, and 94% with classifier B. The authors calculated that this leads to a low residual risk of cancer (8%) among specimens negative by mutation and miRNA testing and corresponds to a calculated improvement from 78–90% NPV to 94–98% NPV at 20–40% cancer prevalence. These results contributed to the development of ThyraMIR. In the small cohort that underwent evaluation by ThyraMIR, the authors report a diagnostic sensitivity of 85% and specificity of 95%.

Clinical Practice Guidelines

American Thyroid Association (ATA)

In this guideline on the clinical management of thyroid nodules, Haugen et al. (2016) provide the following recommendations regarding the use of molecular profiling:

- Nondiagnostic cytology-some studies suggests that use of a thyroid core needle biopsy with BRAF testing, a gene panel, or a gene expression analysis may provide clinical guidance in these cases, but the full clinical impact of these approaches for nodules with nondiagnostic cytology remains unknown. If molecular testing is being considered, patients should be counseled regarding the potential benefits and limitations of testing and about the possible uncertainties in the therapeutic and long-term clinical implications of results
- Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance (AUS/FLUS) - investigations such as repeat FNA or molecular testing may be used to supplement malignancy risk assessment in lieu of proceeding directly with a strategy of either surveillance or diagnostic surgery. Informed patient preference and feasibility should be considered in clinical decision-making. The authors reviewed available data for multi-gene panels of BRAF, NRAS, HRAS, and KRAS point mutations, as well as RET/PTC1 and RET/PTC3, with or without PAX8/PPAR$_{\gamma}$ rearrangements, and a mRNA expression profile of 167 genes, and concluded that more data was needed to fully understand how such tests can impact clinical management. They conclude that there is currently no single optimal molecular test that can definitively rule in or rule out malignancy in all cases of indeterminate cytology
- Follicular Neoplasm/Suspicious for Follicular Neoplasm Cytology-after consideration of clinical and sonographic features, molecular testing may be used to supplement malignancy risk assessment data in lieu of proceeding directly with surgery
- Suspicious for Malignant Cytology-After consideration of clinical and sonographic features, mutational testing for BRAF or the seven-gene mutation marker panel (BRAF, RAS, RET/PTC, PAX8/PPAR$_{\gamma}$) may be considered in nodules with SUSP cytology if such data would be expected to alter surgical decision-making. Molecular testing using the 167 GEC has a PPV that is similar to cytology alone (76%) and a NPV of 85% and it is therefore not indicated in patients with this cytological diagnosis
- Malignant cytology-while studies have been presented in the literature that suggest that BRAF and other multi-gene panels may be useful in prognosis and treatment decisions, more studies are needed to establish the impact of molecular profiling involving multiple mutations or other genetic alterations on clinical management of individuals with primary thyroid medullary cancer
- Post-operative radioiodine (RAI) therapy. Molecular testing to guide postoperative RAI use is not recommended at this time

American Association of Endocrine Surgeons (AAES)

The AAES (Patel et al., 2020) developed evidence-based recommendations to aid clinicians in the optimal surgical management of thyroid disease, including the following statements which address molecular testing:

- If thyroidectomy is preferred for clinical reasons, then molecular testing is unnecessary (strong recommendation, moderate-quality evidence)
- When the need for thyroidectomy is unclear after consideration of clinical, imaging, and cytologic features molecular testing may be considered as a diagnostic adjunct for cytologically indeterminate nodules (strong recommendation, moderate-quality evidence)
- Accuracy of molecular testing relies on institutional malignancy rates and should be locally examined for optimal extrapolation of results to thyroid cancer risk (strong recommendation, moderate-quality evidence)
- For nodules that are cytologically categorized as Bethesda III, clinical factors, radiological features, and patient preference should inform decision-making regarding whether or not to proceed with repeat biopsy, molecular testing, diagnostic thyroidectomy, or observation (strong recommendation, moderate-quality evidence)
- Diagnostic thyroidectomy and/or molecular testing are accepted options for individuals with nodules cytologically categorized as Bethesda IV (strong recommendation, moderate-quality evidence)

American Association of Clinical Endocrinologists, American College of Endocrinology, Associazione Medici Endocrinologi (AACE/ACE/AME)

The AACE/ACE/AME updated their guidelines on the management of thyroid nodules in 2016 (Gharib et al., 2016). They state that molecular profiling should be considered in nodules with indeterminate cytology, and not in those who are found to be clearly benign or malignant. They favor profiles that include BRAF, RET/PTC, PAX8/PPARG and RAS mutations. They find that there is insufficient evidence either for, or against, gene expression classifiers. There is insufficient evidence to use molecular profiling to determine the extent of surgical interventions, or for use with low-risk indeterminate cytology cases.

National Comprehensive Cancer Network (NCCN)

The 2022 NCCN guidelines for thyroid carcinoma indicate that molecular diagnostics may be helpful to reclassify follicular lesions, based on genetic profile, as more /less likely to be benign or malignant. In addition, molecular testing specific to medullary thyroid cancer in Bethesda lll-Vl nodules may identify these unique carcinoma types, as it is challenging to explicitly identify them via cytology (category 2B evidence). Although past studies have shown that molecular diagnostics do not perform well for Hürthle cell neoplasms, modern genomic classifiers are promising with regard to Hürthle cell specimens. A requirement for the diagnosis of Hürthle cell and follicular carcinomas is evidence of either vascular or capsular invasion, which fine needle aspiration cannot determine; use of molecular diagnostics may be considered in these situations. Molecular markers, however, should be interpreted with caution and used in conjunction with individualized clinical, radiographic and cytologic features. The NCCN panel notes that molecular testing has been shown to have benefit for making targeted treatment decisions, especially those related to use of drug therapy or clinical trial participation. Some mutations may also have prognostic importance. Molecular testing of single genes or a gene expression classifier panel test may be considered and should be selected by the clinician based on the specific clinical question being asked. (NCCN Thyroid Carcinoma, v3.2022).

Lung Cancer

Sakata et al. (2022) conducted a multicenter retrospective study to evaluate the success rate of genetic alteration testing in four driver genes [epidermal growth factor (EGFR), anaplastic lymphoma kinase (ALK), c-ros oncogene 1 (ROS1), and *v-raf* murine sarcoma viral oncogene homolog B1 (BRAF)] using the Oncomine Dx Target Test Multi-CDx System in patients with non-small-cell lung cancer (NSCLC). A total of 533 patients with NSCLC whose diagnoses were confirmed using histological or cytological methods, and who had undergone testing for 46 genes using the Oncomine Dx Target Test Multi-CDx System between June 2019 and January 2020, were enrolled in the study. The median age was 72 years (range 25-94 years) and 345 patients (64.7%) were male. The percentages of patients with adenocarcinoma detected histologically or those with stage IV disease were 73.2% and 46.0%, respectively. PD-L1 status was evaluated in 497 patients; among these, 133 (25.0%) showed more than 50% PD-L1 expression. Evaluation of patient smoking history showed that 138 (25.9%) had never smoked, whereas 394 patients (74.1%) had a history of smoking. The success rate of genetic alteration testing for all four genes was 80.1% (95% CI 76.5%-83.4%). Surgical resection was associated with the highest success rate (88.0%), which was significantly higher than that for bronchoscopic biopsy (76.8%, p = .005). Multivariate analysis revealed a difference for surgical resection alone (p = .006, 95% CI 1.36-6.18, odds ratio 2.90). The authors concluded that optimizing specimen quantity and quality may improve the use of driver gene testing in clinical settings. Limitations include the absence of data on the exact number of submitted slides and the amount of DNA or RNA input in the submitted samples for Oncomine Dx Target Test Multi-CDx System testing. In addition, the study is limited by its retrospective observations conducted immediately after approval of the Oncomine Dx Target Test Multi-CDx System. Subsequently, several modifications were made for conducting NGS tests, including those using the Oncomine Dx Target Test Multi-CDx System at each hospital.

A comparison study by Yao et al. (2021) was performed to develop a quick gene testing procedure using fresh core needle biopsy samples from NSCLC patients. Thirty patients with NSCLC confirmed by frozen section examination were enrolled to compare the results of multi-gene mutation testing using fresh frozen (FF) tissues and paired formalin-fixed paraffin-embedded (FFPE) tissues. A total of 77 fresh NSCLC tissue samples obtained from core needle biopsy were evaluated by frozen section examination. The 77 patients consisted of 39 males (50.6%) and 38 females (49.4%) with a median age of 65 years (range, 42–85 years) of which 32 were smokers (41.6%) vs. 45 nonsmokers (58.4%). Frozen section examination revealed 70 (90.9%) AC, 6 (7.8%) SCC, and 1 (1.3%) adenosquamous carcinoma (ASC), which is consistent with the final pathological diagnosis using FFPE tissues. If the NSCLC diagnosis and adequate tumor cell counts were confirmed by histopathology, the fresh tissues were used to extract DNA and subsequent gene testing by ARMS-PCR. The paired FFPE core needle biopsy samples were from 30 NSCLC patients in stage IV, randomly selected for this study, who also underwent gene testing. The 77 fresh samples showed an EGFR mutation rate of 61.0%. The clinical treatment strategy for patients was optimized based on gene test results. Using

this procedure of gene mutation testing, the time interval between physicians requesting and obtaining a test result has been shortened to fewer than 2 days. Following a comparison of gene testing results with fresh tissues and paired FFPE tissues from the 30 patients, no difference in the DNA concentration extracted from fresh tissues and FFPE tissues was found. DNA purity, however, was higher in fresh tissues than that in FFPE tissues. Gene testing detected the same gene mutations in 93.3% of cases in fresh tissues and paired FFPE tissues. The authors concluded that gene testing procedure using fresh biopsy samples greatly shortens the waiting time of patients. The multi-gene mutation testing using fresh core needle biopsy samples from NSCLC patients is a reasonable, achievable, and quick approach. The authors stated that fresh tissues may serve as a potential alternative to FFPE tissues for gene testing in NSCLC patients. Limitations to this study include a risk of misdiagnosis during frozen section examination and uncertain diagnosis of fresh tissues related to lack of pathologist experience. Additionally, the sensitivity and specificity of gene testing using FF tissues are 96 and 75% when compared with FFPE tissues. The high sensitivity and low specificity may be attributed to the selection of cases through frozen section examination. The sample size is too small to prove the usefulness of this test as a diagnostic tool. Further research with randomized controlled trials is needed to validate these findings.

Wang et al. (2020) conducted a cohort study using a multiplexed PCR-based panel developed to simultaneously test 118 hotspot mutations and fusions in nine driver genes capable of comprehensively determining patient genotypes as tumor predictive biomarkers. Surgically resected samples from 214 NSCLC patients (168 patients with adenocarcinomas and 46 with squamous cell cancers) were included in this cohort study. A multiplexed PCR-based assay was developed to simultaneously test 118 hotspot mutations and fusions in nine driver genes. The sensitivity of the kit was 1% for gene mutation and 450 copies for gene fusion. Genetic alterations were detected in 143 (66.8%) patients by the assay. The three most common alterations identified were EGFR mutations (50.9%), KRAS mutations (8.4%) and ALK fusions (4.7%). Eight (3.7%) patients harbored concurrent mutations, and the most common partners were EGFR mutations which were observed in the eight patients. No associations between survival and EGFR, KRAS, and ALK status were observed. Patients with two or more alterations exhibited shorter DFS compared to those with single mutations ($p = 0.032$), whilst had no difference in overall survival (OS) ($p = 0.245$). However, only TNM stage was an independent predictor of OS (HR = 2.905, $p < 0.001$) as well as DFS (HR = 2.114, $p < 0.001$) in this cohort in multivariate analysis. Patients with the L858R mutation had longer DFS ($p = 0.014$) compared to other sensitizing mutations and tended to have better OS ($p = 0.06$). The authors concluded that the mutational profile of oncogenic driver genes plays an important role in NSCLC as several core oncogenic driver genes have been considered to be tumor predictive biomarkers. Furthermore, the authors stated that this study suggested a multiplex gene panel testing technique may be used to detect nine driver genes in a limited number of specimens. In addition, this methodology would have the potential to save both specimens and time compared to the combination of all assays by other methods. A small sample size which may have reduced statistical power makes it difficult to decide whether these conclusions can be generalized to a larger population. The findings of this study need to be validated by well-designed studies.

Drilon et al. (2015) identified 31 patients with lung adenocarcinoma with a ≤ 15 pack-year smoking history whose tumors previously tested "negative" for alterations in 11 genes (mutations in EGFR, ERBB2, KRAS, NRAS, BRAF, MAP2K1, PIK3CA, and AKT1 and fusions involving ALK, ROS1, and RET) via multiple non-NGS methods. A broad, hybrid capture–based NGS assay (Foundation One) was performed (4,557 exons of 287 cancer-related genes and 47 introns of 19 genes frequently rearranged in solid tumors). A genomic alteration with a corresponding targeted therapeutic based on the National Comprehensive Cancer Network (NCCN) guidelines for non–small cell lung cancer (NSCLC) was found in 26% (n = 8 of 31) of patients. The drivers identified in tumors from these 8 patients were EGFR G719A, BRAF V600E, SOCS5-ALK, HIP1-ALK, CD74-ROS1, KIF5B-RET (n = 2), and CCDC6-RET. Six of these patients went on to receive targeted therapy. The authors noted that the reasons for non-detection of these genomic alterations via non-NGS testing can be varied such as lower sensitivity, complex rearrangements undetectable by standard FISH, and, possibly, heterogeneity between different tumor biopsies or sites. They concluded that broad, hybrid capture–based NGS assays have the potential to uncover clinically actionable genomic alterations in never smokers or ≤ 15 pack-year smokers whose lung adenocarcinomas do not harbor a potential driver via non-NGS testing. (Oxnard et al., 2016, Riediger et al., 2016).

Kris et al. (2014) reported on the Lung Cancer Mutation Consortium's study of the frequency of oncogenic drivers in patients with lung adenocarcinoma. These oncogenic drivers are then analyzed to determine if there is a way to guide treatment. Fourteen study sites from 2009 to 2012, enrolled patients with metastatic lung adenocarcinoma and used a multiplex assay to test for drivers in 10 genes (full genotyping). Tumors from 1,007 patients were tested for at least 1 gene and 733 for 10 genes. Of the 733 patients, an oncogenic driver was found in 466 (64%) with 182 tumors (25%) had the KRAS driver; sensitizing EGFR, 122 (17%); ALK rearrangements, 57 (8%); other EGFR, 29 (4%); 2 or more genes, 24 (3%); ERBB2 (formerly HER2), 19 (3%);

BRAF, 16 (2%); PIK3CA, 6 (< 1%); MET amplification, 5 (< 1%); NRAS, 5 (< 1%); MEK1, 1 (< 1%); AKT1, 0. Twenty-four of the 733 patient had two oncogenic drivers identified. Of the total 1007 patients, the results were used to select a targeted therapy or trial in 28%. Among the 1,007 patients tested for at least 1 driver, 93% had sufficient information to be included in the survival analysis (456 were alive and 482 had died); among this group, median follow-up was 1.67 years (IQR, 0.9-2.69); range, 0-18.56. For the patients with an oncogenic driver and genotype directed therapy (n = 260), the median survival was 3.5 years [interquartile range (IQR), 1.96-7.70] compared with 2.4 years (IQR, 0.88-6.20) for the 318 patients with any oncogenic driver(s) who did not receive genotype-directed therapy [propensity score–adjusted hazard ratio, 0.69 (95% CI, 0.53-0.9), p = .006].

Clinical Practice Guidelines

American College of Chest Physicians (ACCP)

In an evidence-based clinical practice guideline for the diagnosis and management of lung cancer, the ACCP states that the epidemiology of lung cancer is an active field. According to the ACCP, researchers in molecular epidemiology are making advances in the identification of biomarkers of risk and for early detection, although these are not yet mature enough for clinical application (Detterbeck et al., 2013).

American Society of Clinical Oncology (ASCO)

ASCO endorsed the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology Clinical Practice Guideline Update with minor modifications (Kalemkerian et al., 2018). The guidelines, supported by ASCO, include the following relevant points, considered to be 'expert' consensus opinion.

- Physicians may use molecular biomarker testing in tumors with:
  - An adenocarcinoma component
  - Nonsquamous, non–small-cell histology
  - Any non–small-cell histology when clinical features indicate a higher probability of an oncogenic driver [e.g., young age (< 50 years); light or absent tobacco exposure]
- BRAF testing should be performed on all patients with advanced lung adenocarcinoma, irrespective of clinical characteristics. RET, or KRAS, or MET molecular testing are not recommended as single gene routine stand-alone assays outside the context of a clinical trial. It is appropriate to include these as part of larger testing panels performed either initially or when routine EGFR, ALK, BRAF, and ROS1 testing is negative
- Multiplexed genetic sequencing panels are preferred where available over multiple single-gene tests to identify other treatment options beyond EGFR, ALK, BRAF, and ROS1
- Circulating tumor cell free DNA testing, also called a liquid biopsy, should not be routinely considered due to lack of evidence of efficacy. However, the expert consensus opinion provided is that cfDNA may be used in some clinical settings in which tissue is limited and/or insufficient for molecular testing to identify EGFR mutations

National Comprehensive Cancer Network (NCCN)

NCCN guidelines for NSCLC indicate that numerous gene alterations impacting treatment selection have been identified. Thus, testing for these alterations is necessary to identify the most effective targeted therapies and avoid treatment unlikely to provide clinical benefit. NCCN recommends that when feasible, testing be performed via a broad, panel-based approach, most often performed by next generation sequencing (NGS). In addition, the guidelines include a discussion of the role of plasma cell-free/circulating tumor DNA testing, stating that cell-free/circulating tumor DNA testing should not be used in lieu of a tissue diagnosis. However, NCCN also suggests that the use of cell-free/circulating tumor DNA testing can be considered in specific clinical circumstances, including the following:

- If a patient is medically unfit for invasive tissue sampling
- In the initial diagnostic setting, if following pathologic confirmation of a NSCLC diagnosis there is not sufficient material for molecular analysis, cell-free/circulating tumor DNA should be used only if follow up tissue based analysis is planned for all patients in which an oncogenic driver is not identified
- In the setting of initial diagnosis, if tissue-based testing doesn't fully assess all recommended biomarkers due to tissue quantity or testing methods available, repeat biopsy or cell-free/circulating tumor DNA testing may be considered (NCCN Non-Small Cell Lung Cancer, v5.2022)

Melanoma

Cutaneous Melanoma

In their Molecular Test Assessment on the DecisionDXMelanoma gene expression test, Hayes identified ten studies (including the Zager, 2018 study below) that met the defined criteria for their review. One study reported the reproducibility and technical reliability of the test and another reported failure rates for samples submitted from a single center. Seven of the studies focused the clinical validity of the test to inform risk of recurrence or metastasis and the last study assessed the clinical validity of the test to predict the likelihood of sentinel lymph nodes. They did not identify any studies in peer-reviewed literature that met criteria and addressed the clinical utility of the test to improve clinical decision making and patient outcomes. Hayes concluded that there was a low-quality body of evidence for the analytical and clinical validity of this test to identify the risk of recurrence or metastasis or to predict sentinel lymph node positivity for patients with American Joint Committee on Cancer (AJCC) stage I, II, or III cutaneous melanoma (Hayes, DecisionDx-Melanoma, 2022).

An Ontario Health Technology Assessment (2021) that evaluated the diagnostic accuracy, clinical utility and budget impact of pigmented lesion assays (PLA) for people with suspected melanoma skin lesions. The systematic review included seven studies consisting of six cohort studies [including three Ferris studies (2017, 2018 and 2019) that were previously discussed in this policy] and one survey that were conducted in dermatology offices, examining adults (> 18 years old) with suspected melanoma lesions using the DermTech pigmented lesion assay. The authors stated that the risk of bias in the included studies was generally moderate to high, and the quality of evidence was very low. Limitations noted in the review included the potential bias from the industry sponsored studies, overestimation of the diagnostic accuracy of PLA, the diagnostic accuracy of visual assessment may have been underestimated when compared to published literature, and many parameters and assumptions used by the economic analysis were not reported in the study, which they stated had potentially serious limitations. They concluded that there was no evidence demonstrating the impact of PLA on patient outcomes and that the low-quality evidence for the diagnostic accuracy of PLA remains uncertain when compared to visual inspection alone. They also stated that the evidence is uncertain about whether PLA has an impact on clinical decision making and that the cost-effectiveness of this test compared with the standard care pathway is also uncertain.

Marchetti et al. (2020) completed a systematic review and meta-analysis to assess the performance of prognostic gene expression profile (GEP) tests in patients with American Joint Committee on Cancer (AJCC) stage I or stage II cutaneous melanoma. The review included seven studies with a total of 1,450 participants. One study was determined to have a moderate risk of bias and the other six studies were determined to have a high risk of bias. There were 623 participants with stage I disease and 212 with stage II disease that were tested with DecisionDx-Melanoma. The authors found that DecisionDx-Melanoma correctly classified recurrence in 29% of the participants with stage I disease and 82% of those with stage II disease. It also found that the test correctly classified 90% with stage I disease and 44% with stage II disease among participants without recurrence. When they reviewed the data for MelaGenix, which had 88 participants with stage I disease and 245 with stage II disease, they found that the test correctly classified 32% with Stage I disease and 76% with stage II disease among those with recurrence. Among those participants tested with MelaGenix, the test correctly classified 77% with stage I disease and 43% with stage II disease. Limitations noted by the authors include the heterogeneity in study designs and data reporting, the lack of availability of individual participant data, short follow-up and significant censoring, the variability in the definitions used for melanoma recurrence, and the risk of bias and quality of the evidence. The authors concluded that the prognostic ability of DecisionDx-Melanoma and MelaGenix to predict recurrence among patients with localized melanoma varied by AJCC stage and appeared to be poor for patients with stage I disease. They recommend more rigorously structured studies be performed to better quantify the association of GEP tests with melanoma outcomes and to demonstrate clinical utility.

A recent meta-analysis (Greenhaw et al., 2020) reported on the strength of the prognostic value of the 31-gene expression profile for cutaneous melanoma. To perform the assessment, meta-analysis was performed on 3 studies that met inclusion criteria. Clinical outcome for the 31 gene expression test were compared with the American Joint Committee on Cancer Staging. The 31-gene expression profile was able to identify the American Joint Committee on Cancer stage 1 to 3 patient categories with a high likelihood for distant metastases and recurrence. When the gene expression profile and sentinel lymph node biopsy were evaluated in conjunction, sensitivity and negative predictive value related to distant metastasis-free survival both improved. The authors concluded that the 31-gene test accurately and consistently identified melanoma patients who were at increased risk of metastasis, functioned independently of other clinicopathologic factors, and improved accuracy of current risk stratification. Several limitations were noted, however. There is a possibility that unpublished negative-result studies exist that were not considered in this analysis. The studies included had different designs, which could impact the strength of

the effect of gene expression profiling due to evolving treatments and population differences. Follow up time also varied across the studies, which is a consideration when interpreting overall survival estimates. Further studies are needed to evaluate most appropriate follow up and treatment of individuals identified as high-risk via the 31-gene expression in conjunction with other clinicopathologic factors.

Hayes published a Molecular Test Assessment on the myPath Melanoma gene expression test. The test is intended to be used as an adjunct diagnostic tool to distinguish between benign nevi and malignant melanoma when histopathologic results of a patient are not clear. Their assessment included seven studies that consisted of one study looking at analytical validity, four studies on clinical validity, and two clinical utility studies. All seven studies were assessed to be of very low quality due to small sample sizes, study design, lack of test accuracy measurements, questionable study comparators and/or removal of challenging cases for clinical validity. Based on their review, Hayes concluded that there was limited evidence that supports the myPath Melanoma test as a diagnostic adjunct tool and that the evidence was insufficient to support the use of the test as a guide to manage treatment decisions. They also stated that the studies were limited in showing that test results have a positive impact on health outcomes. Hayes recommended more studies to evaluate the impact of myPath Melanoma for rare or challenging types of melanoma and on clinical practice along with studies that show how the test results are used in conjunction with other clinical information to develop a treatment plan [Hayes, myPath Melanoma (Myriad Genetics) 2018, updated 2022].

Zager et al. (2018, included in Hayes DecisionDx-Melanoma Molecular Test Assessment, above) conducted a multicenter trial of archived primary melanoma tumors from 523 patients, using a 31 gene expression classifier to classify patients as Class 1 (low risk) and Class 2 (high risk). The 5-year recurrence free survival (RFS) rates for Class 1 and Class 2 were 88% and 52%, respectively. Distant metastasis-free survival rates (DMFS) were 93% for Class 1 versus 60% for Class 2. The gene expression classifier was a significant predictor of RFS and DMFS in univariate analysis in addition to with Breslow thickness, ulceration, mitotic rate, and sentinel lymph node (SLN) status. GEP, tumor thickness and SLN status were significant predictors of RFS and DMFS in a multivariate model that also included ulceration and mitotic rate. The authors concluded that the 31 gene expression classifier provided value to prognostication, and more prospective studies are needed.

Ardakani et al. (2017) assessed the ability of CGH to differentiate between melanocytic naevi and melanoma in cases where the two-show overlapping histological features. Melanomas are characterized by CNVs, while naevi are normal. The team used 19 formalin fixed, paraffin embedded (FFPE) unambiguous naevi and 19 melanomas and tested them using a SurePrint G3 Human CGH 8 x 60K array. CGH was able to differentiate between the naevi and the melanoma in 95% of cases. One naevus showed two large CNV. The authors concluded that CGH may be a good adjunctive test to resolve histologically equivocal melanocytic samples.

Berger et al. (2016) conducted a retrospective analysis to ascertain clinical management changes to 156 patients with cutaneous melanoma, based on the outcome of DecisionDx-Melanoma. Molecular risk classification by gene expression profiling has clinical impact and influences physicians to direct clinical management of CM patients. The vast majority of the changes implemented after the receipt of test results were reflective of the low or high recurrence risk associated with the patient's molecular classification. Because follow-up data was not collected for this patient cohort, the study is limited for the assessment of the impact of gene expression profile-based management changes on healthcare resource utilization and patient outcome.

Uveal Melanoma

Singh et al. (2022) conducted a retrospective 10-year cohort study to assess the accuracy of the predicted metastasis-free survival (MFS) rate by a gene expression profiling (GEP) test in patients with uveal melanoma (UM) by comparing the patients' GEP test results to what they found in their clinics. The authors reported that the test predicted worse outcomes for patients with UM than what occurred. The study included a retrospective record review of 352 consecutive patients from two clinics with a mean age at diagnosis of 59.4 years (+ 13.0 years) who were followed for a median interval of 38.0 months (19.0 - 57.0 months). All patients had undergone a fine-needle aspiration biopsy GEP test of which, 43% showed class 1A (low risk) UM, 22% showed class 1B (intermediate risk) UM, and 35% showed class 2 (high risk) UM. The MFS was specified as time-to-metastasis for those who developed metastases, or the last follow-up date was used for those who did not develop metastatic disease. There were 48 patients who developed metastasis with 40 who had class 2 tumors, 5 with class 1A tumors and 3 with class 1B tumors. The authors found that the observed 3-year MFS was 93% for all class 1 tumors and 67% for class 2 tumors while the 5-year MFS was 87% for patients with class 1 tumors and 47% for those with class 2 tumors. Limitations of this cohort

study included its retrospective design, small population size and small number of included study sites. The authors concluded that, in general, the MFS was better for smaller than larger tumors and that the predicted MFS for class 2 UM tumors appears to be worse than what they found to have actually occurred in the patient population. They recommended that future studies include the tumor size in the prediction model to enhance the accuracy of the GEP test.

Hayes completed a Molecular Test Assessment of the DecisionDx-UM test, a quantitative reverse transcriptase PCR-based profiling test intended to identify the likelihood of metastasis within 5 years in patients with UM. The evidence base examined in the assessment included one study each on analytical validity, clinical validity, and clinical utility, which was the Plasseraud (2016) study reviewed below. When reviewed together, the overall quality of the body of evidence was assessed to be very low due to small sample sizes, short follow-up periods, the sensitivity and linearity of the test, and the ambiguity of the role of DecisionDx-UM in physician decisions. Hayes concluded that the evidence was insufficient to support the use of the DecisionDx-UM test to identify the likelihood of metastasis within 5 years in patients with UM because the validity of the test and the impact on patient management was unclear. The assessment stated that additional studies are needed to support the use of this test [Hayes, DecisionDx-UM (Castle Biosciences Inc.), 2020, updated 2022].

In a 5-year clinical outcome report from a prospective registry of individuals tested with a prognostic 15-gene expression profile (15-GEP) test for UM and a meta-analysis with published cohorts, Aaberg et al. (2020) found that testing with the 15-GEP test guided management of individuals with UM. UM, a rare intraocular cancer, has a 30-50% risk of metastasis within 5 years of diagnosis. The prognostic 15-GEP was designed to predict 5-year metastatic risk using three risk categories indicating low, intermediate, and high-risk groups. In this study, 89 patients who had undergone 15-GEP testing were prospectively enrolled at four separate locations. Clinical outcomes and management plans were tracked every six months. Eighty percent of class 1 (low-risk) participants received low-intensity management and all class 2 (high-risk) patients received high-intensity management ($p < 0.0001$). Five-year melanoma survival rates were 94% for class 1 and 63% for class 2. Five-year metastasis-free survival rates were 90% for class 1 and 41% for class 2. By meta-analysis performed on several prior studies to evaluate clinical outcomes of patients tested with15-GEP, class 2 was associated with an increased risk for both metastasis and mortality and was also the only independent predictor of metastasis.

Klufas et al. (2017) retrospectively reviewed the role of gene expression profile analysis (GEP) vs. chromosome 3 specific analysis. Records of consecutive patients diagnosed with posterior UM who underwent intraoperative fine needle aspiration biopsy prior to placement of an iodine-125 radioactive plaque between 2012 and 2014 were reviewed. Two cohorts of patients were identified. Cohort 1 had 44 patients, and tumors had both GEP and FISH analysis. Cohort 2 had 43 patients, and those tumors had GEP, and multiplex ligation-dependent probe amplification (MLPA) results were obtained. Discordance between GEP and chromosome 3 status by FISH and MLPA occurred in the series at a rate of 15.9 and 16.3%, respectively. The authors concluded that caution must be advised when counseling a patient with a good-prognosis GEP "Class 1" result that the uveal tumor may actually harbor monosomy 3, which is associated with a poor prognosis for metastasis in nearly 20% of the patients.

Plasseraud et al. (2016, included in the Hayes DecisionDx-UM 2020 Molecular Test Assessment above) evaluated the clinical validity and utility of DecisionDx-UM in a prospective, multicenter, study (supported by Castle Biosciences, Inc.). Seventy patients were enrolled to document patient management differences and clinical outcomes associated with low-risk Class 1 and high-risk Class 2 results indicated by DecisionDx-UM testing. Thirty-seven patients in the prospective study were Class 1 and 33 were Class 2. Class 1 patients had 100% 3-year metastasis-free survival compared to 63% for Class 2 (log rank test $p = 0.003$) with 27.3 median follow-up months in this interim analysis. Class 2 patients received significantly higher-intensity monitoring and more oncology/clinical trial referrals compared to Class 1 patients (Fisher's exact test $p = 2.1 \times 10(-13)$ and $p = 0.04$, resp.). In the authors' opinion, the results of this study provide additional, prospective evidence in an independent cohort of patients for which Class 1 and Class 2 patients are managed according to the differential metastatic risk indicated by DecisionDx-UM. A study limitation is financial sponsorship/support by the manufacturer which increases the risk of bias.

Clinical Practice Guidelines

American Academy of Dermatology (AAD)

Guidelines from the American Academy of Dermatology (AAD), updated in 2019, included recommendations for diagnostic, prognostic, and therapeutic molecular testing (Swetter et al., 2019).

- Ancillary diagnostic molecular techniques [e.g., comparative genomic hybridization; fluorescence in situ hybridization, gene expression profiling (GEP)] may be used for equivocal melanocytic neoplasms

- Routine molecular testing, including GEP, for prognostication is discouraged until better use criteria are defined. The application of molecular information for clinical management (e.g., sentinel lymph node eligibility, follow-up, and/or therapeutic choice) is not recommended outside of a clinical study or trial
- Testing of the primary cutaneous melanoma for oncogenic mutations (e.g., BRAF, NRAS) is not recommended in the absence of metastatic disease

National Comprehensive Cancer Network (NCCN)

NCCN Cutaneous Melanoma guidelines (v3.2022) indicate that for diagnostic testing, prognostic testing, and somatic testing, there is agreement that any ancillary testing should be used as an adjunct to clinical and expert dermatopathological examination and that it should be interpreted within the context of their findings.. For prognostic testing, the guidelines state that it is “unclear whether these tests provide clinically actionable prognostic information” and that “the impact of these tests on treatment outcomes or follow up schedules has not been established”.
The guideline further states the following:

- Prognostic GEP to differentiate melanomas at low versus high risk for metastasis should not replace pathologic staging procedures, and the use of GEP testing according to specific AJCC-8 melanoma stage requires further prospective investigation in large, contemporary data sets of unselected patients
- It remains unclear whether available GEP tests are reliably predictive of outcome across the risk spectrum as these tests have not been prospectively validated with clinical studies to accurately define the clinical utility of the tests
- Pre-diagnostic noninvasive patch testing may be helpful to guide biopsy decisions
- Mutational analysis for BRAF or multigene testing of the primary lesion is not recommended for patients with cutaneous melanoma, unless required to guide adjuvant or other systemic therapy or consideration of clinical trials
- BRAF mutation testing is recommended for patients with stage III melanoma for whom future BRAF-directed therapy may be an option

NCCN Uveal Melanoma guidelines address the staging and management of uveal melanoma, stating that biopsy is not usually necessary for the initial diagnosis of uveal melanoma and selection of first line treatment, but it may be helpful when there is uncertainty regarding diagnosis and may also provide prognostic information that can help guide follow up. Risks/benefits of biopsy for prognostic purposes should be carefully considered and discussed at length. Molecular/chromosomal testing for prognostic purposes is preferred over cytology alone if biopsy is performed. NCCN outlines tumor markers that have been shown to be associated with increased risk or shorter time to development of distant metastases and notes the development of gene expression profiling for prognostic purposes, which is recommended for stratification if biopsy is performed (NCCN Uveal Melanoma, v2.2022).

Cancers of Unknown Primary (CUP)

Ding et al. (2022) conducted a systematic review and meta-analysis to identify studies investigating the efficacy of site-specific therapy on patients with cancer of unknown primary (CUP). A systematic search in PubMed, Web of Science, Embase, Cochrane Library, and ClinicalTrials.gov, and of conference abstracts from January 1976 to January 2021 was performed to identify studies investigating the efficacy of site-specific therapy on patients with CUP. The quality of included studies was evaluated using the Cochrane risk of bias tool and Newcastle-Ottawa scale. Eligible studies were weighted and pooled for meta-analysis. Hazard ratios (HRs) for overall survival (OS) and progression-free survival (PFS) were assessed to compare the efficacy of site-specific therapy with empiric therapy in patients with CUP. In addition, subgroup analyses were conducted. Five studies comprising 1,114 patients were identified, of which 454 patients received site-specific therapy, and 660 patients received empiric therapy. Our meta-analysis revealed that site-specific therapy was not significantly associated with improved PFS [HR 0.93, 95% confidence interval (CI) 0.74-1.17, p = 0.534] and OS (HR 0.75, 95% CI 0.55-1.03, p = 0.069), compared with empiric therapy. However, during subgroup analysis significantly improved OS was associated with site-specific therapy in the high-accuracy predictive assay subgroup (HR 0.46, 95% CI 0.26-0.81, p = 0.008) compared with the low accuracy predictive assay subgroup (HR 0.93, 95% CI 0.75-1.15, p = 0.509). Furthermore, compared with patients with less responsive tumor types, more survival benefit from site-specific therapy was found in patients with more responsive tumors (HR 0.67, 95% CI 0.46-0.97, p = 0.037). The authors concluded that their results suggest that site-specific therapy is not significantly associated with improved survival outcomes; however, it might benefit patients with CUP with responsive tumor types. This is a non-randomized study and is limited due to a heterogeneous patient population. Further investigation is needed before clinical usefulness of this procedure is proven.

Ross et al. (2021) performed a retrospective analysis of cancer of unknown primary (CUP) origin cases referred for comprehensive genomic profiling (CGP) to determine how many were potentially eligible for enrollment into an experimental CUPISCO arm, an ongoing randomized trial using CGP to assign patients with CUP to targeted or immunotherapy treatment arms based on genomic profiling (NCT03498521). Centrally reviewed adenocarcinoma and undifferentiated CUP specimens in the FoundationCore database were analyzed using the hybrid capture based FoundationOne CDx assay (mean coverage, > 600×). Presence of genomic alterations, microsatellite instability (MSI), tumor mutational burden (TMB), genomic loss of heterozygosity (gLOH), and programmed death-ligand 1 (PD-L1) positivity were determined. A total of 96 of 303 patients (31.7%) could be matched to an experimental CUPISCO arm. Key genomic alterations included ERBB2 (7.3%), PIK3CA (6.3%), NF1 (5.6%), NF2 (4.6%), BRAF (4.3%), IDH1 (3.3%), PTEN, FGFR2, EGFR (3.6% each), MET (4.3%), CDK6 (3.0%), FBXW7, CDK4 (2.3% each), IDH2, RET, ROS1, NTRK (1.0% each), and ALK (0.7%). Median TMB was 3.75 mutations per megabase of DNA; 34 patients (11.6%) had a TMB ≥ 16 mutations per megabase. Three patients (1%) had high MSI, and 42 (14%) displayed high PD-L1 expression (tumor proportion score ≥ 50%). gLOH could be assessed in 199 of 303 specimens; 19.6% had a score of > 16%. The authors concluded that 32 percent of patients would have been eligible for targeted therapy in CUPISCO. Future studies, including additional biomarkers such as PD-L1 positivity and gLOH, may identify a greater proportion potentially benefiting from CGP-informed treatment. Clinical trial identification number: NCT03498521. The findings of this retrospective analysis of carcinoma of unknown primary origin (CUP) cases validate the experimental treatment arms being used in the CUPISCO study (NCT03498521) using comprehensive genomic profiling to assign patients with CUP to targeted or immunotherapy treatment arms based on the presence of pathogenic genomic alterations. The authors also concluded the findings suggest that future studies including additional biomarkers and treatment arms, such as programmed death-ligand 1 positivity and genomic loss of heterozygosity, may identify a greater proportion of patients with CUP potentially benefiting from comprehensive genomic profiling-informed treatment. A limitation is that this study lacks detailed clinical data for each specimen, including whether any patients received specialized therapy and subsequently demonstrated therapeutic benefit. Further research is needed to validate these findings.

Lombardo et al. (2020) conducted a systematic review to describe genes and molecular pathways involved in cancer of unknown primary (CUP) pathogenesis and focus on available data of targeted genotype-directed treatment. This systematic review consisted of studies of patients with CUP, whose tumor specimen was evaluated through a next-generation sequencing (NGS) panel, performed on June 10, 2019, according to PRISMA criteria from PubMed, ASCO meeting library and Clinicaltrial.gov identifying potentially targetable alterations for which approved/off-label/in clinical trials drugs are available. Case reports about CUP patients treated with targeted therapies driven by NGS results in order to explore the clinical role of NGS in this setting were identified. Fifteen publications of which eleven studies (9 full-text articles and 2 abstracts) have analyzed the genomic profiling of CUPs through NGS technology, with different platforms and with different patient's cohorts, ranging from 16 to 1,806 patients were included. Among these studies, 85% of patients demonstrated at least one molecular alteration, the most frequent involving TP53 (41.88%), KRAS (18.81%), CDKN2A (8.8%), and PIK3CA (9.3%). A mean of 47.3% of patients harbored a potentially targetable alteration for which approved/off-label/in clinical trials drugs were available. Four case reports were identified in order to evaluate the clinical relevance of a specific targeted therapy identified through NGS. The authors concluded NGS may represent a tool to improve diagnosis and treatment of CUP by identifying therapeutically actionable alterations and providing insights into tumor biology. Potential limitations of a tissue-agnostic therapeutic approach include that extrapolating therapeutic actionability from one cancer histology to another might provide uncertain. Therefore, for CUP patients it would be still important to consider putative primary sites even when candidate actionable driver mutations are found. Therefore, for CUP patients it would be important to consider putative primary sites even when candidate actionable driver mutations are found. In addition, redundancy in activation of pathways of resistance does often take place as a mechanism of primary as well as secondary resistance. Further research is needed to determine the clinical relevance of these findings.

A Hayes molecular test assessment report concluded that there is insufficient evidence to draw conclusions regarding the effectiveness of the CancerTYPE ID gene expression test to aid in identifying the site of origin for cancers in patients with indeterminate, uncertain, or differential diagnoses. Peer-reviewed literature supporting the entire assay process as well as publications demonstrating that CancerTYPE ID provides accurate, clinically actionable information resulting in improved outcomes [Hayes, CancerTYPE ID (bioTheranostics Inc.), 2018, updated 2022].

A systematic review conducted by Binder et al. (2018) to determine incidence and survival trends and to discuss the value of comprehensive genomic profiling (CGP) in cancer of unknown primary (CUP) patients. Age-standardized incidence rates (ASR) per 100,000 were calculated for 2,935 CUP patients from 1981 to 2014 using cancer registry data of the canton of Zurich,

Switzerland. Kaplan-Meier survival curves were estimated for sex, age, and histological groups. Cox proportional hazards regression models were used to estimate adjusted hazard ratios (HR). A literature review was conducted to assess the current use of CGP in CUP patients. ASR of CUP increased from 10.3 to 17.6 between 1981 and 1997 and decreased to 5.8/100,000 in 2014. Mean overall survival remained stable. Mortality was lower for patients with squamous cell carcinoma [HR 0.48 (95% CI, 0.41-0.57)], neuroendocrine carcinoma [0.75 (0.63-0.88)], and higher for unclassified neoplasms [1.25 (1.13-1.66)] compared to adenocarcinomas. The literature review identified 10 studies using CGP of CUP tissue. Clinically relevant mutations were identified in up to 85% of CUP patients, of which 13%-64% may benefit from currently available drugs. The authors concluded that CUP incidence decreased most likely due to improved diagnostics, however, mortality did not improve over the last 34 years. CGP testing may help to identify molecular signatures in CUP patients and enable targeted treatment. Given poor prognosis and limited treatment options for patients with CUP, genomic profiling using NGS technologies may meet a clinical need. The findings of this study need to be validated by well-designed studies. Further investigation is needed before clinical usefulness of this procedure is proven.

Varadhachary and Raber (2014) reviewed the research, diagnosis, and treatment of CUP, noting that the performance of tissue-of-origin molecular-profiling assays in known cancers has been validated with the use of independent, blinded evaluation of sets of tumor samples, with an accuracy of approximately 90%. Based on these findings, the authors comment that the feasibility of using formalin-fixed samples obtained from small, core-needle biopsy or using samples obtained by means of fine-needle aspiration makes this method practical for use in the clinic setting. However, without randomized, controlled trials it is difficult to gauge the therapeutic effect of tissue-of-origin molecular-profiling assays. Further, they suggest that creative trial designs are urgently needed to study subsets of unknown primary cancers and the effect of these assays on survival and quality of life of patients.

Meleth et al. (2013) conducted a technology assessment on genetic testing or molecular pathology testing for cancer of unknown primary cancers with CancerTypeID, miRview, or PathworkDx to determine analytical validity, clinical validity, and clinical utility. The results showed that the clinical accuracy of all the three tests is similar, ranging from 85 percent to 88 percent. The evidence that the tests contribute to identifying a TOO is moderate; however, the researchers noted that they did not have sufficient evidence to assess the effect of the tests on treatment decision and outcomes.

Clinical Practice Guidelines

European Society for Medical Oncology (ESMO)

In a clinical practice guideline for the diagnosis, treatment, and follow-up on CUP, ESMO (Fizazi et al., 2015) did not identify any significant differences in the tumor microRNA expression profile when CUP metastases biologically assigned to a primary tissue of origin were compared with metastases from typical solid tumors of known origin. Although they noted that these tests may aid in the diagnosis of the putative primary tumor site in some patients, their impact on patient outcome via administration of primary site-specific therapy remains questionable and unproven in randomized trials.

National Institute for Health and Care Excellence (NICE)

In a guideline on the diagnosis and management of metastatic malignant disease of unknown primary origin in adults, the National Institute of Health, and Care Excellence (NICE, 2010, updated 2014) does not recommend the use of gene-expression-based profiling to identify primary tumors in patients with provisional CUP. They also do not recommend the use of gene expression-based profiling when deciding which treatment to offer patients with confirmed CUP.

National Comprehensive Cancer Network (NCCN)

National Comprehensive Cancer Network (NCCN) clinical practice guidelines for occult primary state that while there may be a diagnostic benefit of gene expression profiling (GEP) assays, it is similar to immunohistochemical staining in terms of accuracy of tumor classification and a clinical benefit for GEP has not been demonstrated. The panel does not recommend gene sequencing for the identification of tissue of origin as standard management in the diagnostic workup of patients with occult primary tumors. Molecular profiling of tumor tissue using NGS or other techniques which identify gene fusions may be considered after initial determination of histology has been made. Testing on tumor tissue is preferred, but cell-free DNA can be considered if tumor tissue testing is not feasible. NCCN suggests that pathologists and oncologists collaborate on the judicious use of modalities including immunohistochemistry, GEP and NGS on a case-by-case basis, with the best individualized patient outcome in mind [NCCN Occult primary (Cancer of Unknown Primary [CUP]), v2.2023].

Colorectal Cancer (CRC)

Azeez et al. (2022) conducted a prospective transcriptome profiling study, using an RNA sequencing (RNA-Seq) approach, to uncover the possible novel targets of gemini curcumin (Gemini-Cur) on colorectal cancer (CRC) and related cellular pathways. After confirming the cytotoxic effect of Gemini-Cur by tetrazolium salt (MTT) and apoptotic assays, RNA sequencing was used to identify differentially expressed genes (DEGs) in HCT-116 cells. On a total of 3,892 Differentially Expressed Genes (DEGs) (padj < 0.01), 442 genes showed a log2 FC < |2| (including 244 upregulated and 198 downregulated). Gene ontology (GO) enrichment analysis was performed. Protein-protein interaction (PPI) and gene-pathway networks were constructed by using STRING and Cytoscape. The pathway analysis showed that Gemini-Cur predominantly modulates pathways related to the cell cycle. The gene network analysis revealed five central genes, namely GADD45G, ATF3, BUB1B, CCNA2 and CDK1. Real-time PCR and Western blotting analysis confirmed the significant modulation of these genes in Gemini-Cur-treated compared to non-treated cells. Exploration of the genes with abnormal expression during the treatment of colon cancer with Gemini-Cur is essential to provide a deeper understanding of the mechanisms involved. The authors stated that the data of this study helps to determine top DEGs as possible cellular targets and figure out potential biological pathways in colon cancer that are modulated by curcumin. The authors concluded that RNA sequencing revealed novel potential targets of curcumin on cancer cells. Further studies are required to elucidate the molecular mechanism of action of Gemini-Cur regarding the modulation of the expression of hub genes in different cancer cell lines and non-cancerous controls which will facilitate the findings of curcumin targets in colon cancer.

Yothers et al. (2022) conducted a patient-specific meta-analysis of 12-gene colon cancer recurrence score validation studies for recurrence risk assessment after surgery with or without fluorouracil (5FU) and oxaliplatin. Three validation studies of the 12-gene colon recurrence score assay were used with pre-specified patient-specific meta-analysis (PSMA) methods to integrate the 12-gene Oncotype DX Colon Recurrence Score result (RS) with the clinical and pathology risk factors stage, T-stage, mis-match repair (MMR) status, and number of nodes examined to calculate individualized recurrence risk estimates. Baseline risk estimation used the most recent studies, so the risk estimates reflect current medical practice. The effect of 5FU was estimated with a meta-analysis of two studies. The effect of oxaliplatin was estimated using one of the RS assay validation studies, in which patients were randomized to 5FU with or without oxaliplatin. The RS result and each of the clinical-pathologic factors provided independent prognostic information for recurrence. Among stage II, T3, MMR-proficient patients with ≥ 12 nodes examined (the most common scenario), patients with RS ≤ 30 (approximately 48%) have estimated 5-year recurrence risk ≤ 10% with surgery alone. Among stage IIIA/B, T3, MMR-deficient patients with ≥ 12 nodes examined, patients with RS ≤ 19 (approximately 14%) have an estimated 5-year recurrence risk ≤ 10% with surgery alone. Among stage IIIA/B, T3, MMR-proficient patients with ≥ 12 nodes examined, those with RS ≤ 14 (approximately 6%) have estimated 5-year recurrence risk ≤ 10% with 5FU alone. The authors concluded that the PSMA integrates the 12-gene colon RS result with clinical and pathology factors to provide individualized recurrence risk estimates that reflect current medical practice. The risk estimates are in a range that may help inform treatment decisions for a substantial number of stage II and stage III patients. Limitations include that the estimated effect of 5FU is from a meta-analysis of a randomized study and a non-randomized treatment comparison with covariate adjustment to reduce bias. The SUNRISE study was a retrospective analysis that selected patients who had not received adjuvant chemotherapy after resection for stage II or III colon cancer and this may have led to selection of patients whom clinicians had considered to be at lower risk of recurrence. Also, the PSMA risk assessment used a baseline risk assessment from the last two enrolling studies (NSABP C-07, enrolling from 2000–2002 and SUNRISE, enrolling from 2000–2005). If further improvements in patient outcomes have occurred since this time, they are not reflected in the present recurrence risk estimates. Finally, the RS result is not predictive, that is, it is not associated with the relative treatment effect of chemotherapy with 5FU or oxaliplatin. Further research with randomized controlled trials is needed to validate these findings.

Daemen et al. (2021) conducted a retrospective study and review of randomized, open-label, prospective, parallel three-arm, phase 3 trial, sponsored by F. Hoffmann-La Roche, to improve high-risk classification by identifying biological pathways associated with outcome in adjuvant stage II/III colorectal cancer (CRC). A total of 1,062 patients with stage III or high-risk stage II colon carcinoma from the three-arm randomized phase 3 AVANT trial were included in this retrospective study. The authors performed expression profiling to identify a prognostic signature. Data from validation cohort GSE39582, The Cancer Genome Atlas, and cell lines were used to further validate the prognostic biology. Retrospective analysis of the adjuvant AVANT trial uncovered a prognostic signature capturing three biological functions-stromal, proliferative and immune-that outperformed the Consensus Molecular Subtypes (CMS) and recurrence prediction signatures like Oncotype Dx in an independent cohort. Importantly, within the immune component, high granzyme B (GZMB) expression had a significant prognostic impact while other individual T-effector genes were less or not prognostic. In addition, the authors found GZMB to be endogenously expressed in CMS2 tumor cells and to be prognostic in a T cell independent fashion. The authors concluded that this study

furthers their understanding of the underlying biology that propagates stage II/III CRC disease progression and provides scientific rationale for future high-risk stratification and targeted treatment evaluation in biomarker defined subpopulations of resectable high-risk CRC. The results also shed light on an alternative GZMB source with context-specific implications on the disease's unique biology. A limitation to this study is that these results need to be clinically validated in a prospective study.

He et al. (2018) examined the clinicopathological features that could impact the sensitivity and specificity of SEPT9 analysis. A total of 1,160 patients were included in the study from hospitals in China, which included 300 patients with colorectal cancer, 122 patients with adenoma, 103 patients with hyperplastic polyps, 568 normal participants (no evidence of disease), and 67 patients with other gastrointestinal diseases. Overall, the sensitivity and specificity of SEPT9 was impacted by cancer stage, size, invasion depth, classification, differentiation, and metastasis. It was also noted that SEPT9 detected adenomas, hyperplastic polyps, and other gastrointestinal diseases such as inflammatory bowel disease. When screening an average risk population, these non-colorectal cancer disorders are much more common and could lead to false positives and unnecessary intervention.

Molecular technologies are also under investigation to screen for colon cancer, such as the Epi proColon 2.0 assay that measures the methylated Septin9 (SEPT9), a circulating tumor cell marker. The premise of this test is that during colorectal cancer development, the tumor will release cell free DNA (cfDNA) into the bloodstream, and the ratio of SEPT9 DNA be detected through specialized techniques and can predict the presence of early colorectal cancer. A meta-analysis of one cohort study and thirteen case-controlled studies representing 9,870 cases demonstrated a pooled sensitivity of 0.66 and specificity of 0.91. The authors compared this to data available for the gold standard test, fecal occult blood testing (FOBT) of a sensitivity of 0.60 and specificity of 0.91, equal to SEPT9. The authors combined the results of FOBT and SEPT9 and achieved a detection rate of colorectal cancer of 88.7% with a specificity of 78.8%. They concluded that FOBT and SEPT9 complement each other, but further studies are needed to determine the best screening tests and approaches (Yan et al., 2016).

Zhang et al. (2016) retrospectively reviewed the prognostic role of CDX2 expression in patients with stage 1 and stage III metastatic colorectal cancer (CRC) after complete surgical resection. The patient cohort (n = 145) included 66 patients with CDX2-negative metastatic CRC and a comparison cohort of 79 patients with CDX2-positive metastatic CRC. The prevalence of absent CDX2 expression in this cohort was 5.6%. After adjusting for covariates in a multivariate model, the association of a lack of CDX2 expression and OS remained statistically significant (HR, 4.52; 95% CI, 2.50-8.17; PÂ < .0001). In addition, the median PFS (3 vs. 10 months; HR, 2.23; 95% CI, 1.52-3.27; PÂ < .0001) for first-line chemotherapy was significantly decreased in patients with CDX2-negative metastatic CRC. The authors concluded that the results showed that a lack of CDX2 expression in metastatic CRC is an adverse prognostic feature and a potential negative predictor of the response to chemotherapy. Further research with randomized controlled trials is needed to validate these findings.

To evaluate whether patients with CDX2-negative tumors might benefit from adjuvant chemotherapy, Dalerba et al. (2016) investigated the association between CDX2 status, and assessed at either the mRNA or protein level, the disease-free survival among patients who either did or did not receive adjuvant chemotherapy. Reviewing a database of 669 patients with stage II colon cancer and 1,228 patients with stage III colon cancer, the authors reported that their results confirmed that treatment with CDX2 as a biomarker in colon cancer adjuvant chemotherapy was associated with a higher rate of disease-free survival in both the stage II subgroup (91% with chemotherapy vs. 56% with no chemotherapy, p = 0.006) and the stage III subgroup (74% with chemotherapy vs. 37% with no chemotherapy, p < 0.001) of the CDX2-negative patient population (Fig. 5). A test for the interaction between the biomarker and the treatment revealed that the benefit observed in CDX2-negative cohorts was superior to that observed in CDX2-positive cohorts in both the stage II subgroup (p = 0.02 for the interaction) and the stage III subgroup (p = 0.005 for the interaction). In the authors' opinion, their results indicate that patients with stage II or stage III CDX2-negative colon cancer might benefit from adjuvant chemotherapy and that adjuvant chemotherapy might be a treatment option for patients with stage II CDX2-negative disease, who are commonly treated with surgery alone. Given the exploratory and retrospective design of this study, these results will need to be further validated through randomized, clinical trials, in conjunction with genomic DNA sequencing studies.

Yamanaka et al. (2016) evaluated the 12-gene Recurrence Score assay for stage II and III colon cancer without chemotherapy to reveal the natural course of recurrence risk in stage III disease (the Sunrise Study). A cohort-sampling design was used. From 1,487 consecutive patients with stage II to III disease who had surgery alone, 630 patients were sampled for inclusion with a 1:2 ratio of recurrence and nonrecurrence. Sampling was stratified by stage (II v III). The assay was performed on formalin-fixed, paraffin-embedded primary cancer tissue. Association of the Recurrence Score result with recurrence-free interval (RFI) was

assessed by using weighted Cox proportional hazards regression. With respect to prespecified subgroups, as defined by low (< 30), intermediate (30 to 40), and high (≥ 41) Recurrence Score risk groups, patients with stage II disease in the high-risk group had a 5-year risk of recurrence similar to patients with stage IIIA to IIIB disease in the low-risk group (19% v 20%), whereas patients with stage IIIA to IIIB disease in the high-risk group had a recurrence risk similar to that of patients with stage IIIC disease in the low-risk group (approximately 38%). The authors conclude that this validation study of the 12-gene Recurrence Score assay in stage III colon cancer without chemotherapy showed the heterogeneity of recurrence risks in stage III as well as in stage II colon cancer.

ColonSentry is a blood-based gene expression test that assesses the expression of ANXA3, CLEC4D, LMNB1, PRRG4, TNFAIP6, VNN1, and IL2RB genes using real time PCR, and reports results as a cumulative relative risk score (CURR). In a 2014 evaluation of available data, Heichman reviewed the work of Han et al. (2008) and Marshall et al. (2010) that explored the clinical utility of the test and reported that in a case-controlled study of 202 colorectal cancer patients and 208 matched healthy controls, a specificity of 70% for distinguishing cancer from healthy controls, and a sensitivity of 72% for identifying colorectal cancer was found. Larger, prospective studies are needed to further confirm the performance of this test.

Clinical Practice Guidelines

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

Together, the ASP, CAP, AMP and ASCO convened an expert panel to create evidence-based guidelines for standard molecular biomarker testing in individuals diagnosed with CRC, which included a comprehensive search of the published literature including over 4,000 articles. Twenty-one recommendations were made, which include specifics regarding individual gene testing and requirements for laboratories. The guideline asserts that evidence supports testing for variations in specific genes in the EGFR signaling pathway because they may provide information that is clinically relevant for targeted therapy of CRC with anti-EGFR monoclonal antibodies. Some biomarkers, such as *BRAF* and DNA mismatch repair (MMR) have been shown to have clear value for prognostication and others (*KRAS* and *NRAS*) are evidence-backed for negative predictive value for benefit to anti-EGFR therapies. (Sepulveda et al., 2017)

National Comprehensive Cancer Network (NCCN)

NCCN Clinical Practice Guidelines for colon cancer indicate that the role of targeted therapy for treatment of advanced or metastatic CRC has become more common and as such, biomarker testing for tumor gene status of *KRAS/NRAS* and *BRAF* mutations, as well as HER2 amplifications and MSI/MMR status (if not previously done), are recommended for patients with metastatic CRC, either via individual gene testing or as part of an NGS panel (no specific methodology is recommended). In a footnote for pedunculated or sessile polyp (adenoma) with invasive cancer, NCCN notes that “It has not been established if molecular markers are useful in treatment determination (predictive markers) and prognosis.” With regard to multigene assays, Immunoscore and ctDNA, the guidelines assert that while these tests can further inform risk of recurrence, the added value is questioned and the evidence of predictive value related to benefit of chemotherapy is lacking, thus, the NCCN panel believes there is insufficient evidence to recommend the use of multigene assays, Immunoscore or post-surgical ctDNA to estimate risk recurrence or to assist with selection of adjuvant therapy in colon cancer. The panel encourages clinical trial enrollment to generate further data on these tests. (NCCN Colon cancer, v2.2022)

Prostate Cancer

Decipher, Oncotype DX Prostate, Prolaris, and Promark

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

In a 2021 systematic review, Jairath et al. evaluated the available evidence supporting clinical utility of the Decipher genomic classifier (GC.) A total of 144 studies were identified and of those, 42 studies including 30,407 individuals met inclusion criteria for this review with GC performance data available for localized, post-prostatectomy, nonmetastatic castration-resistant and metastatic hormone-sensitive prostate cancer (PCa). Participants were part of retrospective studies (n = 12,141), prospective registries (17,053) and prospective and post hoc randomized trial analyses (n = 1,213). On multivariate analysis, 32 studies showed that GC was independently prognostic for study endpoints including biochemical failure, metastasis, adverse pathology, and both cancer-specific and overall survival. In 24 studies, GC improve discrimination over standard of care and in 5 studies, GC changed clinical management in the settings of active surveillance and post-prostatectomy. The strength of the evidence was found to be levels 1 and 2 as per Simon criteria for all disease states except high-risk PCa and was found to be grade A and B by American Urological Association (AUA) criteria, depending on state of disease. Based on this review, the authors assert that consistent data has emerged from diverse levels of evidence and when evaluated overall, clinical utility of the Decipher GC has been demonstrated. Utility is strongest for intermediate-risk PCa and postprostatectomy use in clinical decision-making. Authors Marascio (2020), Berlin (2019), Kim (2019), Klein (2016), Glass (2016), and Marrone (2015), previously cited in this policy, were included in this systematic review.

Feng et al. (2021) performed an ancillary study to validate the Decipher GC in men who received salvage radiation for elevated prostate-specific antigen (PSA) after surgery in the context of a phase 3 randomized trial. They used specimens from the placebo-controlled, phase 3 NRG/RTOG 9601 clinical trial and extracted RNA from the highest-grade tumor tissue available in 2019 (NRG/RTOG 9601 was conducted 1998-2003). Median follow up time was 13 years. GC scores were assigned (0-1) to whole transcriptomes and the predictive ability of GC for distant metastasis was evaluated. Additional outcomes including prostate cancer-specific mortality (PCSM) and overall survival (OS) were also measured. The authors analyzed GC scores from 352 randomized participants who met quality-controlled inclusion criteria. The GC was found to have an association with distant metastasis [hazard ratio (HR), 1.17; 95% CI, 1.05-1.32; p = .006], PCSM (HR, 1.39; 95% CI, 1.20-1.63; p < .001) and OS (HR, 1.17; 95% CI, 1.06-1.29; p = .002) after adjusting for Gleason score, T stage, margin status, age, race/ethnicity, entry PSA and treatment arm, suggesting that not all men with biochemically recurrent cancer after surgical intervention will benefit equally from addition of hormone therapy to salvage radiotherapy. The researchers propose that the Decipher GC may hold promise for risk stratification and treatment decisions involving hormone therapy for prostate cancer recurrence after surgery. Noted study challenges include the limited availability of samples from NRG/RTOG 9601 and ability of available samples to meet quality control requirements (22.4% of total trial samples did not pass quality control), as the median age of tissue samples was older than 20 years.

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

Decipher Biopsy testing was used in a multi-institutional study of 855 men newly diagnosed with prostate cancer between February 2015 and October 2019. Vince et al. (2021) sought to assess the clinical utility of this test in localized prostate cancer patients. Participating patients were tracked through the prospective Michigan Urological Surgery Improvement Collaborative and were linked to the Decipher Genomics Resource Information Database. An independent third party performed patient matching using two or more unique identifiers. Of the 855 men in the study, 264 participated in active surveillance and 454 went on to radical therapy. In the men that elected active surveillance, after adjustment for NCCN risk group, PSA, prostate volume, body mass index, percent positive cores and age, a high risk Decipher score was independently associated with shorter time to treatment. This was true for patients who underwent radical therapy as well; high risk Decipher score was independently associated with a shorter time to failure of treatment. The authors concluded that in this prospective statewide registry, there was a strong association with a high-risk Decipher Biopsy score and conversion from active surveillance to definitive treatment and treatment failure. The authors mention phase 3 randomized trial NCT04396808 which is estimated to conclude in 2023, and which will, in their opinion, provide level 1 evidence of the clinical impact of Decipher biopsy testing.

In a retrospective, observational study, Morris et al. (2021) compared the predictive ability and clinical utility of the cell cycle progression (CCP) gene expression classifier test (Prolaris), multiparametric magnetic resonance imaging (mpMRI) with Prostate Imaging Reporting and Data Systems (PI-RADS) scoring and clinical/pathological data in individuals with localized prostate cancer, a CCP score and an mpMRI PI-RADS v2 score. The study was made up of two cohorts; the first included 156 individuals with newly diagnosed prostate cancer (with or without previous negative biopsy) and the second included 66 individuals who had initiated active surveillance without CCP testing, but then received the test during their active surveillance. Each individual was given a combined score using CCP results and UCSF Cancer of the Prostate Risk Assessment (CAPRA) score; this was the clinical cell-cycle risk score (CCR). The researchers found small but significant correlations between PI-RADS score and CCP (rs = 0.22, p = 8.1 x 10-4), CAPRA (rs = 0.36, p = 4.8 x 10-8), or CCR (rs = 0.37, p = 2.0 £ 10-8. This may indicate that a large part of the prognostic information identified in the testing performed is independent. PI-RADS score did not prove to be a significant factor for prediction of post-radical prostatectomy Gleason score. However, both CCP and CCR were shown to be significant and independent, in their predictions regarding active surveillance versus curative treatment in cohort 1 per multivariate analysis. CCR at or below the threshold for active surveillance reduced the likelihood of choosing curative treatment over active surveillance, which the authors assert validates the clinical utility of the active surveillance threshold. Overall, the authors state that their results support CCP as a better tool to predict both tumor grade and management of individuals with prostate cancer than PI-RADS. They stress the importance of obtaining molecular information from men with newly diagnosed prostate cancer to assess risk and determine the best clinical management for the individual. Notably, the majority of the authors associated with this study are either employed by or associated with the manufacturer of the test under study. Additional limitations include the retrospective nature of the study, cohort sizes, dependence on quality and accuracy of biopsy and the lack of long-term outcomes.

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

In a multicenter, retrospective, observational study, Kaul et al. (2019, included in Hayes, Prolaris Biopsy Test, 2019) aimed to evaluate the selection of active surveillance along with the safety and durability of the clinical cell cycle risk (CCR) score, which is a combination score of clinical data and molecular data (Prolaris). Individuals with low-risk prostate cancer (according to both CCR score (DSM ≤ 3.2%) and NCCN guidelines) who had previously undergone CCP testing during the course of their care were tracked. Initial treatment selection (active surveillance vs. treatment) and duration of active surveillance were evaluated. Adverse events measured were biochemical recurrence and metastasis of disease. Of 664 individuals with low-risk disease (per CCR score and NCCN guideline), 82.4% (547) chose active surveillance and 17.6% underwent definitive treatment ≤ 6 months after diagnosis. The median follow-up period from biopsy was 2.2 years. Only 0.4% of the 547 individuals who chose active surveillance experienced an adverse event and two-thirds of the individuals remained on active surveillance for more than 3 years. Only markers of tumor aggressiveness showed a significant difference between the two groups; individuals who underwent definitive treatment within 6 months of diagnosis had more aggressive pathological features than those who chose active surveillance. The authors determined that based on the collective data from the study, the use of the CCR score in evaluating prostate cancer risk can safely increase selection of active surveillance when compared with the use of only clinical/pathological criteria and potentially allow more individuals to avoid unnecessary treatment of prostate cancer and treatment-related side effects. Limitations included the lack of a control group to assess active surveillance selection and durability in men who did not receive a CCR score, a relatively short median follow-up time and cohort of individuals with low-risk prostate cancer only. In addition, several study authors are employed by or have associations with the manufacturer of the test being evaluated in this study, creating the potential for bias.

The Prolaris test for use with biopsy and post-prostatectomy underwent assessment by Hayes in 2019. For the Prolaris Biopsy test, Hayes found insufficient evidence to support the analytical and clinical validity of this test to aid in prediction of prostate cancer specific mortality and metastasis, and studies supporting clinical utility were limited as well (Hayes, Prolaris Biopsy Test [Myriad Genetic Laboratories Inc.], 2019, updated 2022). Regarding the use of Prolaris post-prostatectomy for determination of biochemical recurrence risk within ten years of prostatectomy, Hayes found minimal evidence of analytical validity and preliminary evidence for clinical validity, but no studies that provided evidence for clinical utility of Prolaris for post-prostatectomy use (Hayes, Prolaris Post-Prostatectomy [Myriad Genetic Laboratories Inc.], 2019, updated 2022).

Kornberg et al. (2019) evaluated the Oncotype DX Prostate test to determine if the assay results are associated with an increased risk of adverse pathology. The patient cohort was men who were enrolled in active surveillance and underwent radical prostatectomy. A total of 215 men were included and 179 (83%) were determined to be at low risk and 36 (17%) were at intermediate risk. Analysis showed that a higher GPS was associated with an increased risk of adverse pathology at delayed radical prostatectomy (HR/5 units 1.16, 95% CI 1.06-1.26, $p < 0.01$). A higher GPS was also associated with an increased risk of biochemical recurrence (HR/5 units 1.10, 95% CI 1.00-1.21, $p = 0.04$). The researchers concluded that in patients who undergo radical prostatectomy after a period on active surveillance, a higher GPS by Oncotype DX Prostate is associated with an increased risk of adverse pathology. In addition, the higher GPS is associated with biochemical recurrence following radical prostatectomy.

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

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

In a meta-analysis of the Decipher GC performance, five studies including 975 individuals (855 of whom had individual, patient-level data) were examined for assess ability of Decipher to predict metastasis of prostate cancer in individuals who had undergone prostatectomy (Spratt et al., 2017, included in the 2021 Jairath systematic review.) Meta-analyses were performed by pooling HRs for each study using random-effects modeling. Overall, patients were stratified by Decipher as either low (60.9%), intermediate (22.6%) or high (16.5%) risk; ten year cumulative metastases rates were 5.5%, 15% and 26.7% (p,.001) respectively. Pooled Decipher HRs reveal an HR of 1.52 (95% CI, 1.39 to 1.67; I2 = 0%) per 0.1 unit. Using only a clinical model, the C-index for 10 year distant metastases was 0.76, increasing to 0.81 with addition of Decipher results. The researchers concluded that Decipher GC has the ability to improve prognostication for individuals with prostate cancer post-prostatectomy and recommend ongoing study of the best methods of incorporating this type of testing into clinical practice.

Den et al. (2016) conducted a retrospective review of 2,341 consecutive radical prostatectomy patients to understand the relationship between the Decipher classifier test and patient tumor characteristics. Decipher score had a positive correlation with pathologic Gleason score [PGS; r = 0.37, 95% confidence interval (CI) 0.34 − 0.41], pathologic T-stage (r = 0.31, 95% CI 0.28 − 0.35), CAPRA-S (r = 0.32, 95% CI 0.28 − 0.37) and patient age (r = 0.09, 95% CI 0.05-0.13). Decipher reclassified 52%, 76% and 40% of patients in CAPRA-S low-, intermediate- and high-risk groups, respectively. The authors detected a 28% incidence of high-risk disease through the Decipher score in pT2 patients and 7% low risk in pT3b/pT4, PGS 8 − 10 patients. There was no significant difference in the Decipher score between patients from community centers and those from academic centers (p = 0.82). The authors concluded that although Decipher correlated with baseline tumor characteristics for over 2,000 patients, there was significant reclassification of tumor aggressiveness as compared to clinical parameters alone. In their opinion, utilization of the Decipher genomic classifier can have major implications in assessment of postoperative risk that may impact physician-patient decision making and ultimately patient management.

Brand et al. (2016) performed a meta-analysis of two independent clinical validation studies of a 17-gene biopsy-based genomic assay (Oncotype Dx Prostate Cancer Assay) as a predictor of favorable pathology at radical prostatectomy. Patient-specific meta-analysis was performed on data from 2 studies (732 patients) using the Genomic Prostate Score (GPS; scale 0-100) together with Cancer of the Prostate Risk Assessment (CAPRA) score or NCCN risk group as predictors of the likelihood of favorable pathology (LFP). Risk profile curves associating GPS with LFP by CAPRA score and NCCN risk group were generated. Patient-specific meta-analysis generated risk profiles ensure more precise estimates of LFP with narrower confidence intervals either study alone. GPS added significant predictive value to each clinical classifier. The authors concluded that a model utilizing GPS and CAPRA provided the most risk discrimination, and in a decision curve analysis, greater net benefit was shown when combining GPS with each clinical classifier compared with the classifier alone. Although the clinical characteristics of the 2 patient cohorts were similar, there were nonetheless some key differences in the representation of different racial groups and higher risk patients. The risk estimates were numerically different in the 2 studies, although the confidence levels overlapped.

In a 2015 retrospective study, Cuzick et al. (included in Hayes, Prolaris Biopsy Test, 2019) sought to validate a predefined prognostic score from a test using CCP to assist providers in choosing the most appropriate management for individuals with newly diagnosed, localized prostate cancer. Study participants included individuals with localized prostate cancer diagnosed using needle biopsy; all individuals were being managed conservatively. The primary endpoint of the study was death due to prostate cancer. Validation was done using CCP score independently and in a prespecified linear combination with standardly used clinical information (CCR scores). Clinical information included baseline PSA, Gleason score, clinical stage, extent of disease and age, which were then combined into a sole risk assessment score (CAPRA). An independent validation cohort of 585 individuals, all of whom had full data available, made up the study. CCP score hazard ratio was s 2.08 (95% CI [1.76, 2.46], p < 10-13) per one unit change of the score in the independent validation. In the multivariate analysis which included CAPRA, CCP score hazard ratio was 1.76 (95% CI [1.44, 2.14], p < 10-6). In addition, the predefined CCR score was high predictive with a hazard ratio of 2.17 (95% CI [1.83, 2.57], x2 89.0, p < 0-20), thoroughly encompassing all prognostic information. The authors indicate that the prognostic value of the CCP score from needle biopsies was confirmed by this study; for individuals being managed conservatively, CCP scores were highly prognostic for death from prostate cancer and provided data that was not available based on clinical information alone. They indicate that the CCP score can provide useful information for ascertaining which individuals with prostate cancer can be safely treated with conservative methods and avoid radical treatment. A limitation of this study was that a large number of initial participants were excluded due to quality issues, inadequate tumor available or missing clinical data. In addition, all study participants were symptomatic with worse prognoses than contemporary cohorts of screen-detected cancers. Thus, the study population is not necessarily representative of current populations of individuals with prostate cancer. In addition, for the majority of cases, changes in treatment greater than or equal to six months after diagnosis were not recorded. Lastly, several of the authors are employees of or otherwise associated with the test manufacturer, which could present risk of bias.

Other Prostate Cancer Assays

Although many additional genomic panel tests related to screening and stratifying risk in individuals with prostate cancer are commercially available, the evidence to support the clinical validity and utility of these tests is currently lacking.

In a 2023 Molecular Test Assessment, Hayes found a low-quality body of evidence addressing the clinical benefit of the ExoDx Prostate Test, which is proposed for use in individuals ≥ 50 years of age with PSA levels 2 to 10 ng/mL to aid in decision-making related to initial or repeat prostate biopsy. Although four studies addressing the clinical validity of test were reviewed,

the evidence indicates low to acceptable ability to detect clinically significant prostate cancer. No studies were found that compared ExoDx Prostate's clinical performance with other PSA derivatives, MRI, or other commercially available similar tests. Evidence for clinical utility was insufficient (Hayes, ExoDx Prostate Test [Exosome Diagnostics Inc.], 2023).

Tosoian et al. (2021) sought to validate an optimal threshold for the use of the MyProstateScore test in ruling out grade group ≥ 2 cancer in individuals referred for prostate biopsy. In this study, men who had not yet received prostate biopsy provided urine samples prior to biopsy and a MyProstateScore was generated using a model which leverages serum prostate specific antigen (PSA), urinary prostate cancer antigen 3 and urinary TMPRSS2:ERG. The study enrolled individuals from academic and community settings for an overall population of 1,525 individuals. The researchers found that at a threshold of 10, MyProstateScore had 97% sensitivity and 98% negative predictive value for grade group ≥ 2 cancer. The authors concluded that MyProstateScore provided exceptional sensitivity and negative predictive value for ruling out grade group ≥ 2 in a large and pertinent population of individuals referred for prostate biopsy. Study limitations included the use of systematic biopsy as a reference standard, as biopsy appears to miss approximately 15-20% of cancers, which would include a proportion of grade group ≥ 2 cancers. In addition, not all grade group ≥ 2 cancers will ultimately be clinically significant. The authors encourage additional validation studies with longer term outcomes for this group. Furthermore, there were no individuals with a history of negative biopsy included in this study and the study was performed without use of multiparametric MRI, which is commonly used during diagnosis. Further data is needed to confirm the findings of this study and further assess clinical utility.

A prospective, randomized, blinded two-armed clinical utility study was conducted by Tutrone et al. (2020) to evaluate the impact of the ExoDx Prostate (IntelliScore) test (EPI) on the decision whether to perform a biopsy in a real-world clinical setting. EPI is designed to assess risk for high grade prostate cancer. The study enrolled 1,094 patients from 24 urology practices and a total of 72 urologists. All patients underwent EPI testing but were randomized into EPI vs. Control. Only the EPI arm received results for the biopsy. In the EPI group (458) of the participants received negative EPI scores. Of these, 63% were recommended to defer biopsy and 74% of those did indeed defer the biopsy. Of those with positive EPI scores, 87% were recommended by urologist to proceed with biopsy and 72% of participants complied with that recommendation. Ultimately, this led to detection of 305 more high grade prostate cancer in comparison with control group and the researchers estimated that 49% fewer high-grade cancers were missed due to deferred biopsy compared to standard of care. Sixty-eight percent of participating urologists indicated that the EPI influenced their decision regarding biopsy recommendation. The authors stated that this was the first report on a prostate cancer biomarker utility study with a blinded control group and felt that the study showed that the EPI test influenced decision making regarding prostate biopsy and patient stratification. Despite these positive outcomes, there were limitations. In the EPI group, there was a 5.7% assay failure, and in the entire group of participants, there was a failure rate of 7.1%. Data is lacking regarding long-term outcomes of the participants who deferred biopsy after using EPI, and the large number of testing sites and urologists involved required the use of streamlined questionnaires, limiting feedback. Lastly, a small number of participants (< 5%) had undergone pre-biopsy MRI, which can help refine biopsy accuracy and provide additional information related to EPI test performance. The researchers suggest that future studies could include a larger percentage of patients with MRI data available.

Another molecular test used to assess risk for prostate cancer is ConfirmMDx. This test uses tissue from a negative prostate biopsy to identify genetic biomarkers which can then be used to help determine if an individual may be ruled out for repeat biopsy or to predict likelihood of Gleason score ≤ 6 or ≥ 7 prostate cancer on repeat biopsy when individuals have high-risk clinical pathological features associated with prostate cancer. In a Molecular Test Assessment [ConfirmMDx for Prostate Cancer (MDxHealth Inc.), 2019, updated 2022], Hayes found positive but insufficient evidence to support use of ConfirmMDx for ruling out prostate cancer in repeat biopsy and insufficient evidence for prediction of Gleason score ≤ 6 or ≥ 7 prostate cancer on repeat biopsy. Additional studies are required to evaluate whether ConfirmMDx results in improved patient outcomes in individuals with high-risk clinical features of prostate cancer.

McKiernan et al. (2018) assessed the performance and utility of ExoDx Prostate IntelliScore (EPI) urine exosome gene expression assay versus SOC parameters for discriminating grades of prostate cancer from benign disease. This study compared EPI results with biopsy outcomes in men with age ≥ 50 yr. and prostate-specific antigen (PSA) 2–10 ng/ml, scheduled for initial prostate biopsy. The results were that in a total of 503 patients, with median age of 64 yr., median PSA 5.4 ng/ml, 14% African American, 70% Caucasian, 53% positive biopsy rate (22% GG1, 17% GG2, and 15% ≥ GG3), EPI was superior to SOC with an area under the curve (AUC) 0.70 versus 0.62, respectively, comparable with previously published results (n = 519 patients, EPI AUC 0.71). Using a validated cut-point 15.6 would have avoided 26% of unnecessary prostate biopsies and 20% of total biopsies, with negative predictive value (NPV) 89% and missing 7% of ≥ GG2 PCa. Setting a different

cut-point 20 would avoid 40% of unnecessary biopsies and 31% of total biopsies, with NPV 89% and missing 11% of ≥ GG2 PCa. This study concluded that EPI has been validated in over 1,000 patients across two prospective validation trials for risk stratification of high-grade and low-grade from benign disease. The use of test may improve identification of patients with higher grade disease and could reduce unnecessary biopsies, although 10% of prostate cancer cases would be missed based on the test characteristics.

A study from McKiernan et al. (2016) evaluated the performance of the EPI urine exosome assay. The study compared those patients who received standard of care (SOC) alone to those who received SOC plus this novel exosome assay. SOC was defined as PSA levels, age, race, and family history. EPI urine exosome assay is a noninvasive, urinary 3-gene expression assay that is designed to discriminate high-grade (> Gleason Score 7) from low-grade (Gleason Score 6) and benign disease. The researchers compared the urine exosome gene expression assay with biopsy outcomes in 499 patients with PSA levels of 2 to 20 ng/mL. After this first phase, the derived prognostic score was validated in 1064 patients that included PCA-free men, 50 years or older, scheduled for an initial or repeated prostate needle biopsy due to suspicious digital rectal examination (DRE) findings and/or PSA levels (limit range, 2.0-20.0 ng/mL). This study found that in 255 men in the training target population (median age 62 years and median PSA level 5.0 ng/mL, and initial biopsy), the urine exosome gene expression assay plus SOC was associated with enhanced discrimination between GS7 or greater and GS6 and benign disease [AUC 0.77 (95% CI, 0.71-0.83) vs. SOC AUC 0.66 (95% CI, 0.58-0.72) ($p < .001$)]. The validation study found that in 519 patients, urine exosome gene expression assay plus SOC AUC 0.73 (95% CI, 0.68-0.77) was superior to SOC AUC 0.63 (95% CI, 0.58-0.68) ($p < .001$). Using a predefined cut point, 138 of 519 (27%) biopsies would have been avoided, missing only 5% of patients with dominant pattern 4 high-risk GS7 disease. This study concluded that the urine exosome gene expression assay was associated with improved identification of patients with higher-grade prostate cancer among men with elevated PSA levels and could reduce the total number of unnecessary biopsies.

In a review of tissue-based genomic biomarkers for prostate cancer, Moschini et al. (2016), report that available genomic assays have improved the prognostic ability over clinicopathologic parameters of localized PCa. However, these assays should be prospectively applied, or even retrospectively applied to prospective studies, to validate their clinical utility in prognostication and even prediction in terms of what treatment should be applied either at a new diagnosis or post-RP.

Clinical Practice Guidelines

American Association of Clinical Urologists

In a 2018 position statement endorsed by the Large Urology Group Practice Association (LUGPA), the AACU states that they "support the use of tissue-based molecular testing as a component of risk stratification in prostate cancer treatment decision making. We also support ongoing research to further refine the prognostic power of these tests."

American Society of Clinical Oncology (ASCO)

Eggener et al. (2020) published the recent ASCO guideline on molecular biomarkers in localized prostate cancer and summarized the evidence as follows:
"Few biomarkers had rigorous testing involving multiple cohorts and only 5 of these tests are commercially available currently: Oncotype Dx Prostate, Prolaris, Decipher, Decipher PORTOS, and ProMark. With various degrees of value and validation, multiple biomarkers have been shown to refine risk stratification and can be considered for select men to improve management decisions. There is a paucity of prospective studies assessing short- and long-term outcomes of patients when these markers are integrated into clinical decision making."

ASCO made four specific recommendations:

- Commercially available molecular biomarker tests (i.e., Oncotype Dx Prostate, Prolaris, Decipher, and ProMark) may be offered in situations in which the assay result, when considered as a whole with routine clinical factors, is likely to affect management. Routine ordering of molecular biomarkers is not recommended (Type: Evidence based; Evidence quality: Intermediate; Strength of recommendation: Moderate)
- Any additional molecular biomarkers evaluated do not have sufficient data to be clinically actionable or are not commercially available and thus should not be offered (Type: Evidence based; Evidence quality: Insufficient; Strength of recommendation: Moderate)
- Consideration of a commercially available molecular biomarker test (e.g., Decipher Genomic Classifier) is recommended in situations in which the assay result, when considered as a whole with routine clinical factors, is likely to affect management.

In the absence of prospective clinical trial data, routine use of genomic biomarkers in the postprostatectomy setting to determine adjuvant versus salvage radiation or to initiate systemic therapies should not be offered (Type: Evidence based; Evidence quality: Intermediate; Strength of recommendation: Moderate)

- In men with newly diagnosed prostate cancer eligible for active surveillance, both magnetic resonance imaging and genomics intend to identify clinically significant cancers. The Expert Panel endorses their use only in situations in which the result, when considered with routine clinical factors, is likely to affect management. This may include, for instance, the initial management of men who are potentially eligible for active surveillance, where each of these approaches may provide clinically relevant and actionable information. These tests may provide information independent of routine clinical parameters and independent of one another (Type: Informal consensus; benefits/harms ratio unknown; Evidence quality: Low; Strength of recommendation: Weak)

American Urological Association (AUA)/American Society for Radiation Oncology (ASTRO)

The AUA and ASTRO published a three part updated guideline addressing clinically localized prostate cancer in 2022. This guideline was endorsed by the Society for Urologic Oncology (SUO) and provides the following recommendations regarding use of genomic testing:

- Clinicians may use tissue-based genomic biomarkers selectively when added risk stratification has the potential to impact clinical decision-making. (Expert Opinion)
- Clinicians should not use tissue-based genomic biomarkers routinely for risk stratification or to assist with clinical decision-making. (Moderate Recommendation; Evidence Level: Grade B)
- Patient and tumor risk factors should be fully assessed to guide decision regarding offering germline testing which would include mutations that are known to be associated with aggressive prostate cancer types or are known to have implications for treatment. (Expert Opinion)

The guideline further states the use of genomic classifiers (GCs) to improve outcomes in individuals with clinically localized prostate cancer has not been validated in high quality, prospective clinical trials to date. This is the reason the AUA/ASTRO guideline does not recommend routine use at this time. Existing published data supporting predictive ability of genomic classifiers have mostly been based on tissue analysis of radical prostatectomy samples; thus the impact of heterogeneity of tissue and under-sampling on the ability to prognosticate with GCs is still uncertain. Accumulating evidence has, shown that GC scores based on biopsy specimens (specifically Decipher), do correlate with clinical outcomes. (Eastham et al., 2022)

American Urological Association (AUA)/Society for Urologic Oncology (SUO)

In a 2023 guideline addressing the early detection of prostate cancer, the AUA and SUO (Wei et al.) include the following recommendation:
“Clinicians may use adjunctive urine or serum markers when further risk stratification would influence the decision regarding whether to proceed with biopsy (Conditional Recommendation; Evidence Level: Grade C).”

National Comprehensive Cancer Network (NCCN)

NCCN clinical practice guidelines for prostate cancer (NCCN Prostate Cancer, v2.2023) state that Decipher, Oncotype DX Prostate and Prolaris molecular assays may be considered in men with low or favorable intermediate risk prostate cancer and a life expectancy greater than or equal to ten years during initial risk stratification to help guide decision-making regarding management. Individuals with unfavorable intermediate and high-risk disease may consider the use of Decipher and Prolaris molecular assays. Further, the Decipher test should be considered if not previously performed to inform adjuvant therapy when adverse features are found post prostatectomy and can be part of the discussion of risk stratification in patients with prostate specific antigen persistence/recurrence after radical prostatectomy (category 2B evidence).

The discussion section of the NCCN guideline states “These molecular biomarker tests have been developed with extensive industry support, guidance, and involvement, and have been marketed under the less rigorous FDA regulatory pathway for biomarkers. Although full assessment of their clinical utility requires prospective randomized clinical trials, which are unlikely to be done, the panel believes that men with low or favorable intermediate disease may consider the use of Decipher, Oncotype DX Prostate or Prolaris during initial risk stratification. In addition, Decipher may be considered during work up for radical prostatectomy PSA persistence or recurrence (category 2B for the latter setting). Future comparative effectiveness research may allow these tests and others like them to gain additional evidence regarding their utility for better risk stratification of men with prostate cancer.”

NCCN categorizes prostate cancer risk groups as follows:

Risk Group	Clinical/Pathological Features		
Very low	Has all of the following: • cT1c • Grade Group 1 • PSA < 10 ng/mL • Fewer than 3 prostate biopsy fragments/cores positive, ≤ 50% cancer in each fragment/core • PSA density < 0.15 ng/mL/g		
Low	Has all of the following, but does not qualify for very low-risk: • cT1–cT2a • Grade Group 1 • PSA < 10 ng/mL		
Intermediate	Has all of the following: • No high-risk group features • No very high-risk group features • Has one or more intermediate risk factors (IRFs): ○ cT2b–cT2c ○ Grade Group 2 or 3 ○ PSA 10–20 ng/mL	Favorable intermediate	Has all of the following: • 1 IRF • Grade Group 1 or 2 • < 50% biopsy cores positive (e.g., < 6 of 12 cores)
		Unfavorable intermediate	Has one or more of the following: • 2 or 3 IRFs • Grade Group 3 • ≥ 50% biopsy cores positive (e.g., ≥ 6 of 12 cores)
High	Has no very high-risk features and has exactly one high-risk feature: • cT3a; or • Grade Group 4 or Grade Group 5; or • PSA > 20 ng/mL		
Very high	Has at least one of the following: • cT3b–cT4 • Primary Gleason pattern 5 • 2 or 3 high-risk features • > 4 cores with Grade Group 4 or 5		

In its 2023 version 2 guideline addressing prostate cancer early detection, the NCCN panel recommends "consideration of biomarker tests that have been validated in peer-reviewed, multi-site studies using an independent cohort of pts. These tests include Select MDx and ExoDx Prostate tests (in addition to other tests such as % PSA, Prostate Health Index and 4Kscore®), which may further define the probability of grade group ≥ prostate cancer in pts w/ PSA levels > 3 ng/mL who have not yet had a biopsy". In addition, the panel indicates that ExoDx, SelectMDx and Confirm MDx could be considered for individuals with at least one previous negative biopsy who are suspected to be at higher risk. Validation of these tests across diverse populations, however, has been variable, and the results of such assays can be complicated and require a degree of caution in their interpretation. The panel goes on to state that no biomarker test can be recommended over any other for early prostate cancer detection due to the quality and quantity of evidence available at this time.

Pancreatic Cancer and Ampullary Adenocarcinoma

A Hayes Precision Medicine Research Brief was published regarding PancreaSeq, a next generation sequencing-based test that analyzes 74 genes isolated from pancreatic cyst fluid to evaluate the risk of malignancy. Hayes concluded that there is currently not enough published peer-reviewed literature to evaluate the evidence related to PancreaSeq Genomic Classifier for characterization of pancreatic cysts in full assessment [Hayes, PancreaSeq Genomic Classifier (University of Pittsburgh Medical Center MGP Laboratory), 2022].

A Hayes Molecular Test Assessment concluded that there is insufficient evidence to support the use of the PancraGEN test to assess the risk of cancer in pancreatic cysts to help physicians choose appropriate surveillance strategies or surgical options for patients with pancreatic cysts. No peer-reviewed articles were identified that assesses the analytical validity, clinical validity, or clinical utility of the current version of the PancraGEN test [Hayes, PancraGEN (Interpace Diagnostics), 2022].

Although current guidelines recommend somatic genomic sequencing for advanced pancreatic cancer patients, the benefit of this testing remains unclear. A 2021 systematic review and meta-analysis (Meti et al.) found that genomic sequencing can frequently identify targetable alterations in pancreatic cancer. In this review, 19 prospective studies of pancreatic cancer patients were analyzed. Each study conducted genomic sequencing to assist with clinical treatment selection. Methodologies for sequencing, definitions of targetable alterations and treatment selection approaches varied across studies and were unfortunately not completely reported. Of 1,382 sequenced patients, 590 had a targetable alteration. Twelve percent received matched therapy based on the results of the testing. Only one observational study reported an improvement in outcomes. The authors note that continued efforts to study targetable alterations for pancreatic cancer should focus on their clinical benefit. They recommend large collaborative studies to move forward with precision oncology for pancreatic cancer in the future.

A retrospective study was performed by Kandimalla et al. (2021) using a genome-wide DNA methylation analysis of multiple GI cancers to develop a pan-GI diagnostic assay and validate the tissue-specific differentially methylated regions (DMRs) in 300 cell-free DNA specimens for early detection and/or population screening of all GI cancers. The study design involved tissue discovery followed by plasma cell-free DNA (cfDNA) validation. Methylation data from 1,781 tumor and adjacent normal tissues and DMRs between individual GI cancers and adjacent normal were studied including colorectal cancer (CRC), hepatocellular carcinoma (HCC), esophageal squamous cell carcinoma (ESCC), gastric cancer (GC), esophageal adenocarcinoma (EAC), and pancreatic ductal adenocarcinoma (PDAC). By comparing data from tumor versus normal tissues within each GI cancer, as well as across all GI cancers, a total of 67,832 regions of interest (ROI) were identified based on differentially methylated probes with a $p < 0.001$ and an absolute delta beta of 0.20 across all the comparisons. Three distinct categories of DMR panels were developed to include (i) cancer-specific biomarker panels with AUC values of 0.98 (CRC), 0.98 (HCC), 0.94 (ESCC), 0.90 (GC), 0.90 (EAC), and 0.85 (PDAC); (ii) a pan-GI panel that detected all GI cancers with an AUC of 0.88; and (iii) a multi-cancer (tissue of origin) prediction panel, EpiPanGI Dx, with a prediction accuracy of 0.85-0.95 for most GI cancers. The authors concluded that by using a novel biomarker discovery approach, they were able to provide the first evidence for a cfDNA methylation assay that offers strong diagnostic accuracy for multi-detection GI cancers in a non-invasive and cost-effective manner. This study is limited by its retrospective observations, limited sample size used to represent each stage, and lack of mutation profiles of cfDNA samples to be able to directly compare or combine the diagnostic performance of the methylation assay relative to genomic mutations. Further investigation with prospective evaluation is needed to determine the clinical relevance of these findings.

O'Kane et al. (2019) reported on the COMPASS trial for pancreatic ductal adenocarcinoma (*PDAC*). Patients were recruited before chemotherapy for whole genome sequencing (WGS) and RNA sequencing (RNASeq). The tumor tissue was analyzed, and tumor responses and clinical outcomes were correlated. Of the 157 patients that had a tumor biopsy, 141 genomes were reported. Twenty-five (21%) had a Moffitt basal-like RNA signature which is usually associated with chemotherapy resistance. GATA6 expression was able to separate the Moffitt subgroup from those with classical tumors. Also, 30% of patients had potentially actionable genetic alterations including BRAF variants (n = 4) and a NTRK3-EML4 fusion in KRAS WT tumors (8%). The researchers concluded that there are subsets of patients with advanced PDAC that have actionable variants.

Singhi et al. (2018) studied the clinical validity of using pre-operative pancreatic cyst fluid (PCF) for next generation sequencing (NGS) of KRAS, GNAS, TP53, PIK3CA and PTEN genes to predict benign vs. malignant lesions. PCF samples from 595 patients (626 samples) were obtained through fine needle aspiration and subjected to NGS for the 5 genes. A different cohort of 159 PCF specimens was also evaluated for KRAS/GNAS mutations by Sanger sequencing. Of the 595 patients, 308 (49%) had KRAS or GNAS mutations and 35 had a mutation in TP53, PIK3CA, or PTEN. Follow up diagnostic pathology was available in 102 patients. For these 102 patients, NGS testing of PCF for KRAS/GNAS had a 100% sensitivity (n = 56) and 96% specificity for an intraductal papillary mucinous neoplasm. In the separate cohort of Sanger sequencing patients, KRAS/GNAS mutations detection had a 65% sensitivity and 100% specificity. By NGS, the combination of KRAS/GNAS mutations and alterations in TP53/PIK3CA/PTEN had an 89% sensitivity and 100% specificity for advanced cancer. The study concluded that in comparison to Sanger sequencing, preoperative NGS of PCF for KRAS/GNAS mutations is highly sensitive for IPMNs and specific for mucinous PCs. In addition, the combination of TP53/PIK3CA/PTEN alterations is a useful preoperative marker for advanced cancer.

Lowery et al. (2018) performed comprehensive germline testing (GT) in a cohort of patients with exocrine pancreatic neoplasms. The genotype and phenotype associations were used to identify biomarkers for therapy response. Six hundred fifteen patients were prospectively tested for somatic tumor and matched sample profiling for 410-468 genes. PGAs were present in 122 (19.8%) of 615 patients involving 24 different genes, including BRCA1/2, ATM, PALB2, and multiple additional genes associated with the DNA damage response pathway. Of these patients with germline alterations, 41.8% did not meet current guidelines for GT. The study concluded that the data supported routinely offering GT in all pancreatic ductal adenocarcinoma patients with a broad panel of known hereditary cancer predisposition genes.

Wong et al. (2019) reported on ampullary cancer (AC) and germline alterations in BRCA2, ERBB2, and ELF3. Forty-five patients with pathologically confirmed AC were tested with the Memorial Sloan Kettering Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) test (410-468 genes). Twenty-three patients were also tested with GT with MSK-IMPACT (76-88 genes). Eight of 44 patients (18%) were identified as harboring pathogenic mutations in BRCA2, ATM, RAD50, and MUTYH. Additionally, they found a wide spectrum of SAs in genes such as KRAS, MDM2, ERBB2, ELF3, and PIK3CA. Two patients in the cohort underwent SA-targeted therapy, and 1 had a partial radiographic response.

Clinical Practice Guidelines

American Society of Clinical Oncology (ASCO)

Sohal et al. published an update to the ASCO Metastatic Pancreatic Cancer Guideline in 2020, noting that a complete discussion of molecular biomarker testing is outside the scope of the guideline, but a modification to the recommendations around molecular testing was made. This includes recommendation that all patients with pancreatic cancer should be offered information about biomarker testing and biomarker testing (specifically NTRK fusion testing) should be used in patient selection for targeted therapies.

In a guideline from ASCO in 2016, clinical decision support was outlined for metastatic pancreatic cancer. Sohal et al. (2018) published an update to this guideline that incorporated new evidence. The researchers conducted a literature review and found two new studies to include. The recommendations included that select patients should be tested for mismatch repair deficiency or microsatellite instability, and pembrolizumab is recommended for patients with mismatch repair deficiency or high microsatellite instability tumors.

National Comprehensive Cancer Network (NCCN)

NCCN Pancreatic Adenocarcinoma guidelines include a footnote recommending tumor/somatic molecular profiling in cases of metastatic or locally advanced disease when an individual is a candidate for anti-cancer therapy to identify potential uncommon mutations. Recommendations further include specific testing for fusions (ALK, NRG1, NTRK, ROS1 FGFR2, RET), mutations (BRAF, BRCA1/2, KRAS, PALB2) amplifications (HER2) and microsatellite instability (MSI), and/or mismatch repair (MMR) deficiency. It is preferred that testing is done on tumor tissue; however, cell-free testing can be considered if tumor tissue testing is not feasible. (NCCN Pancreatic Adenocarcinoma, v1.2022)

Multi-Cancer Early Detection Tests

Galleri

The Galleri (GRAIL, Menlo Park, CA) multi-cancer early detection test is a qualitative, next-generation sequencing (NGS), in vitro test that was designed to detect DNA methylation patterns using cell-free DNA (cfDNA) that has been isolated from human peripheral whole blood. Specific DNA methylation patterns can serve as a signal of cancer and may be able to provide more information regarding the origin of the cancer signal.

Klein et al. (2021) documented the results of an observational study to validate a multi-cancer early detection test designed to complement existing screening methods and potentially increase the number of cancers found through population screening, potentially impacting, and improving clinical outcomes. Including 4,077 participants in an independent validation set (cancer n = 2,823, non-cancer n = 1,254), sensitivity, specificity, and cancer signal origin (CSO) were measured. This population was a pre-specified sub-study of the Circulating Cell-free Genome Atlas Study, a prospective, multi-center, observational study designed to collect biological samples (blood and tumor tissue) both from participants with newly diagnosed cancer and from participants without a diagnosis of cancer to characterize population heterogenicity in cancer and cancer-free participants so that models for distinguishing between cancer and non-cancer could be developed. According to the authors, the Atlas study

demonstrated that MCED testing using cfDNA in combination with machine learning could detect cancer signals across various cancer types and predict cancer signal origin with high accuracy. The objective of the current study is to further validate an MCED test that has been refined for use as a screening tool. Overall sensitivity for cancer signal detection was 51.5% and showed increasing sensitivity with stage of cancer. Cancer signal detection specificity was 99.5% (95% confidence interval). Cancer signals were detected across more than 50 cancer types. CSO prediction in true positives was 88.7% overall. The researchers concluded that the MCED test showed high specificity and accuracy in prediction of CSO and detected signals across multiple cancer types. A noted limitation is that blood sample collection from participants with cancer done after biopsies had been performed could increase the possibility that tumor cfDNA fraction could also increase relative to pre-biopsy. In addition, CCGA is a case-control study, so would not reflect performance in a screening population. Further studies evaluating test performance and clinical utility in target-use population are needed.

In a prospective case-control sub-study of the Atlas and STRIVE studies (NCT02889978 and NCT03085888), the performance of targeted methylation analysis of cfDNA in detecting and localizing multiple cancer types across all stages, with high specificity, was assessed. A total of 6,689 participants [2,482 with cancer (over 50 types), 4,207 without cancer] were grouped into training or validation sets. Cell-free DNA was sequenced, targeting a panel of over 100,000 informative methylation areas. From this, a classifier was developed and validated for detection of cancer and localization of tissue of origin. The publication (Liu et al., 2020) documented consistent performance in both the training and validation sets. In the validation set, specificity was 99.3%. Stage I-III sensitivity was 67.3% in a pre-selected set of 12 cancer types (head and neck, esophagus, liver/bile-duct, anus, colon/rectum, bladder, plasma cell neoplasm, stomach, pancreas, ovary, lung, and lymphoma), which make up approximately 63% of annual cancer deaths in the U.S. Stage I-III sensitivity was 43.9% in all cancer types, with increase in detection as cancer stage increased. Tissue of original was predicted in 96% of samples with cancer-like signals and of that group, the tissue of origin localization was accurate in 93%. In conclusion, the researchers indicate that cfDNA sequencing using informative methylation patterns warrants further evaluation in prospective, population-level studies.

Circulating tumor DNA

Signatera

Signatera is a personalized molecular test that detects circulating tumor DNA (ctDNA) in the blood of individuals who have been diagnosed with cancer. The test detects residual disease following surgery to monitor response to treatment and/or detect recurrence after remission. Signatera uses a whole exome sequencing-based, tumor-informed approach to target specific mutations present in tumor tissue.

In a retrospective, single-center cohort study, Fakih et al. (2022) evaluated the comparative surveillance strategies of ctDNA assay (Signatera) with standard radiographic imaging and carcinoembryonic antigen (CEA) levels per NCCN guidelines in individuals with resected colorectal cancer (CRC). Out of 48 individuals with curatively resected CRC, 15 had disease recurrence during surveillance. Confirmation via imaging was made on nine individuals, and positive ctDNA confirmed disease recurrent in 8, CEA levels in 3 individuals and combined imaging with CEA levels in 11 individuals. According to the numbers, ctDNA did not perform better than imaging in detecting recurrence, as sensitivity results were 53.3% (95% CI, 27.4%-77.7%) and 60% (95% CI, 32.9%-82.5%), respectively ($p > .99$). The combination of imaging plus measurement of CEA levels [sensitivity, 73.3% (95% CI, 44.8%-91.1%)] had a numerical advantage compared with ctDNA in identifying recurrence ($p = .55$). In addition, authors noted no significant difference among ctDNA (median, 14.3 months), imaging (median, 15.0 months), or imaging plus measurement of CEA levels (median, 15.0 months) in the time to identify disease recurrence. The study is limited by its small size, a small number of reoccurrences, and short follow-up. The authors concluded that the findings show that ctDNA assay (Signatera) may not supply definitive advantages as a surveillance strategy compared to standard imaging combined measurement of CEA levels when performed per NCCN guidelines. Additional prospective studies focusing on the correlation between low-burden lung recurrence and negative ctDNA findings should be investigated further.

The use of ctDNA as a prognostic biomarker for relapse of metastatic colorectal cancer (mCRC) was the subject of a cohort study by Loupakis et al. (2021). In this study, 112 individuals with mCRC were evaluated. These participants were part of the PREDATOR clinical trial and had undergone resection of metastases with curative intent. In this study, evaluation of the prognostic value of ctDNA was performed by correlating clinical outcomes with molecular residual disease (MRD) status after surgery using a tumor-informed, personalized ctDNA assay (Signatera). MRD positive results were found in 54.4% of the participants after surgery. Of those, 96.7% progressed at the time data collection ended. Positive results were also associated with lower overall survival. At the time of data analysis, 96% of all study participants in the MRD-negative arm of the study were

living, compared with only 52.4% in the MRD-positive arm. For individuals who were MRD-negative in the combined ctDNA analysis at both points in time and did not receive systemic therapy, overall survival rate was 100%. When multivariate analysis was performed, the most significant prognostic factor associated with disease-free survival was ctDNA based MRD status. The researchers concluded that post-operative MRD evaluation is a strong biomarker in individuals with mCRC undergoing metastatic resection and feel that it has potential use in clinical decision-making. Further clinical studies will be needed to support use of this technology in the future.

Magbanua et al. (2021) evaluated the clinical utility of ctDNA to test for risk of metastatic recurrence and predictive ability of pathologic complete response (pCR) for individuals with early breast cancer. A retrospective ancillary ctDNA study was performed on samples that had been prospectively collected from high-risk individuals with early breast cancer that were part of the multicenter neoadjuvant I-SPY2 TRIAL. Eligibility requirements included tumor size ≥ 2.5 cm and stage II/III breast cancer. Participants with de novo metastatic disease were not included in the study. In addition, eligibility was limited to individuals who had received a MammaPrint high score. On pretreatment testing, 73% of participants were ctDNA positive. Those participants who continued to be ctDNA positive 3 weeks after initiation of paclitaxel were significantly more likely to have residual disease after neoadjuvant chemotherapy (NAC) when compared to those who were no longer ctDNA positive. All individuals who achieved pCR after NAT were ctDNA negative. For participants who did not achieve pCR, ctDNA positive results had a significantly increased risk of metastatic recurrence. Notably, participants who were ctDNA negative but who did not achieve pCR still had excellent outcomes. In this study, lack of ctDNA clearance significantly predicted poor response and metastatic recurrence of cancer. Clearance of ctDNA was associated with improved survival. The researchers concluded that personalized testing of ctDNA during NAC may assist with clinical assessment and treatment in early breast cancer. Noted limitations include the inability of the Signatera test to detect new second primary cancers and novel somatic variants that may have arisen during tumor evolution. Further studies are required, including those that simultaneously evaluate ctDNA and circulating tumor cells in the neoadjuvant setting.

Reinert et al. (2019) reported results of a prospective, multicenter cohort study designed to analyze how ctDNA is associated with CRC recurrence. Ultradeep sequencing of plasma cell-free DNA was performed in study participants with CRC before pre- and post-surgery, during and after adjuvant chemotherapy (ACT), and during the surveillance period. The study took place in Demark and evaluated 125 individuals with stages I to III CRC. Plasma samples were obtained prior to surgery, after surgery (day 30) and ongoing every third month for up to 3 years. In the pre-surgery period, ctDNA was detected in 88.5% of participants. Post definitive treatment, ctDNA analysis identified 87.5% of relapses and at post-op day 30, ctDNA-positive participants were 7 times more likely to suffer relapse that those with negative ctDNA results. After ACT, ctDNA participants with positive results were 17 times more likely to relapse. During and after undergoing ACT, monitoring of participants found that 30% of the ctDNA positive individuals were cleared of disease. In the post-therapy period, ctDNA-positive participants were more than 40 times more likely to have a recurrence of their disease than the ctDNA-negative participants. Actionable mutations were found in 81.8% of the relapse samples that were ctDNA-positive. The researchers concluded that ctDNA analysis has potential to be helpful with postoperative management of CRC, in terms of early relapse detection, ACT monitoring and risk stratification. However, the sample size of participants with recurrent CRC in this study was small and analysis was done on multiple different subsets. This study provides a base for further clinical trials investigation the use of ctDNA in management of CRC and other diseases.

Clinical Practice Guidelines

American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP)

Merker et al. (2018) published a joint review from ASCO and CAP assessing the clinical use of circulating tumor DNA (ctDNA). The researchers performed a literature review and identified 1,339 references. Of these references, 390, plus an additional 31 supplied by the researchers, were evaluated. The literature review ultimately included 77 references and stated that while some ctDNA tests have demonstrated clinical validity and utility with specific advanced stage cancer, overall, there is insufficient evidence of clinical validity and utility for the majority of these assays in this stage of cancer. The researchers also noted that there is no evidence of clinical utility and little evidence of clinical validity of ctDNA tests in early-stage cancer, treatment monitoring, or residual disease detection. Likewise, no evidence of clinical validity and utility was demonstrated in the literature review for the use of ctDNA in cancer screening.

National Institute for Health and Care Excellence (NICE)

In 2022, NICE published a Medtech innovation briefing on Signatera for detecting MRD from solid tumor cancers. In summary, the briefing outlines the lack of prospective evidence on the utilization of Signatera in clinical practice or its effect on treatment decisions or clinical outcomes. Additionally, experts advised there is insufficient evidence to support the use of the technology routinely in the NHS. The experts point out their advice is in line with the recommendations from the ESMO on the use of ctDNA. Many ongoing trials may address the gaps in the evidence in the future.

U.S. Food and Drug Administration (FDA)

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

Laboratories that perform genetic tests are regulated under the Clinical Laboratory Improvement Amendments (CLIA) Act of 1988. More information is available at: https://www.fda.gov/medicaldevices/deviceregulationandguidance/ivdregulatoryassistance/ucm124105.htm. (Accessed July 28, 2023)

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Policy History/Revision Information

Date	Summary of Changes

05/01/2024	**Title Change**
	• Previously titled *Molecular Oncology Testing for Cancer Diagnosis, Prognosis, and Treatment Decisions (for Louisiana Only)*
	Coverage Rationale
	• Removed language pertaining to:
	  ○ Companion Diagnostics; refer to the Medical Policy titled *Molecular Oncology Companion Diagnostic Testing (for Louisiana Only)*
	  ○ Hematological cancer testing; refer to the Medical Policy titled *Molecular Oncology Testing for Hematologic Cancer Diagnosis, Prognosis, and Treatment Decisions (for Louisiana Only)*
	• Revised list of unproven and not medically necessary gene expression profiling (GEP), multigene Next Generation Sequencing (NGS) panels, and/or comprehensive genomic profiling (CGP) for molecular testing of solid tumors:
	  ○ Added "whole genome methylation testing for tumors"
	  ○ Removed "Prolaris® Prostate Cancer Test"
	  ○ Replaced "tumor-informed assays (e.g., Invitae Personalized Cancer Monitoring, Signatera™, RaDaR®) and MRD *monitoring for solid tumors* (e.g., Guardant Reveal™)" with "tumor-informed *and tumor-naïve* minimal residual disease (MRD) assays (e.g., Invitae Personalized Cancer Monitoring, Signatera™, RaDaR®, Guardant Reveal™, *Guardant Response™*)"
	Additional Non State-Specific Criteria
	• Added language to indicate this policy applies to tests that have not been granted approval as an FDA cleared or approved Companion Diagnostic
	Lung Cancer
	• Added reference link to the Medical Policy titled *Molecular Oncology Companion Diagnostic Testing*

Date	Summary of Changes
	(for Louisiana Only) Prostate Cancer Gene Expression Profiling (GEP) • Revised language to indicate: ○ The use of the Oncotype DX® Genomic Prostate Score (GPS) is proven and medically necessary for individuals with biopsy-proven, untreated, localized adenocarcinoma of the prostate (no clinical evidence of metastasis or lymph node involvement) when: ▪ Test is ordered by a physician specializing in the treatment of organ confined prostate cancer including surgical oncology/urology, radiation oncology or medical oncology ▪ Results will be used to assist with treatment decision-making when the individual has not yet received treatment for prostate cancer and is a candidate for either active surveillance or definitive therapy and all of the following: – Life expectancy is greater than 10 years – Risk group is one of the following: • Very Low-Risk Prostate Cancer • Low-Risk Prostate Cancer • Favorable Intermediate-Risk Prostate Cancer ○ The use of the Prolaris® Biopsy prostate cancer prognostic test or Decipher® Prostate Biopsy genomic classifier is proven and medically necessary for individuals with biopsy-proven, untreated, localized adenocarcinoma of the prostate (no clinical evidence of metastasis or lymph node involvement) when: ▪ Test is ordered by a physician specializing in the treatment of organ confined prostate cancer including surgical oncology/urology, radiation oncology or medical oncology ▪ Results will be used to assist with treatment decision-making when the individual has not yet received treatment for prostate cancer and is a candidate for either active surveillance or definitive therapy and all of the following: – Life expectancy greater than 10 years – Risk group is one of the following: • Very Low-Risk Prostate Cancer • Low-Risk Prostate Cancer • Favorable Intermediate-Risk Prostate Cancer • Unfavorable Intermediate-Risk Prostate Cancer • High-Risk Prostate Cancer ○ The use of Decipher Prostate RP genomic classifier is proven and medically necessary to inform adjuvant treatment after radical prostatectomy for either of the following: ▪ Adverse features are found (e.g., high-grade disease, Gleason score 8 or higher, extracapsular extension, positive surgical margins, seminal vesicle invasion) ▪ PSA is greater than zero at any point following prostatectomy ○ Molecular screening panel tests for prostate cancer are unproven and not medically necessary due to insufficient evidence of efficacy (e.g., ExoDx™ Prostate Test, My Prostate Score™, Confirm MDx™, Select MDx™) ***Thyroid Cancer or Indeterminate Thyroid Nodule Testing*** • Removed instruction to refer to the criteria for FoundationOne® CDx for all other primary thyroid cancers [not addressed in the policy] **Definitions** • Added definition of: ○ High-Risk Prostate Cancer ○ Unfavorable Intermediate-Risk Prostate Cancer ○ Very High-Risk Prostate Cancer • Removed definition of: ○ Chromosome Microarray Analysis (CMA) ○ Predictive Molecular Markers ○ Prognostic Molecular Markers

Date	Summary of Changes

	• Updated definition of:
	  ○ Favorable Intermediate-Risk Prostate Cancer
	  ○ Liquid Biopsy
	  ○ Low-Risk Prostate Cancer
	  ○ Very Low-Risk Prostate Cancer
	Applicable Codes
	• Added CPT code 0379U, 0420U, 0421U, 0422U, 0424U, 0428U, 0433U, 81457, 81458, 81459, 81462, 81463, and 81464
	• Removed CPT codes 0017M, 0021U, 0050U, 0118U, 0120U, 0171U, 0331U, 0364U, 81228, 81229, 81277, 81425, 81426, 81427, 81450, and 81451
	• Added notation to indicate CPT codes 0379U, 0409U, 0420U, 0421U, 0422U, 0424U, 0428U, 0433U, 81457, 81458, 81459, 81462, 81463, and 81464 are not on the State of Louisiana Medicaid Fee Schedule and therefore may not be covered by the State of Louisiana Medicaid Program
	• Removed list of applicable ICD-10 diagnosis codes
	Supporting Information
	• Updated *Description of Services*, *Clinical Evidence*, and *References* sections to reflect the most current information
	• Archived previous policy version CS152LA.R

Instructions for Use

This Medical Policy provides assistance in interpreting UnitedHealthcare standard benefit plans. When deciding coverage, the federal, state or contractual requirements for benefit plan coverage must be referenced as the terms of the federal, state or contractual requirements for benefit plan coverage may differ from the standard benefit plan. In the event of a conflict, the federal, state or contractual requirements for benefit plan coverage govern. Before using this policy, please check the federal, state or contractual requirements for benefit plan coverage. UnitedHealthcare reserves the right to modify its Policies and Guidelines as necessary. This Medical Policy is provided for informational purposes. It does not constitute medical advice.

UnitedHealthcare may also use tools developed by third parties, such as the InterQual® criteria, to assist us in administering health benefits. The UnitedHealthcare Medical Policies are intended to be used in connection with the independent professional medical judgment of a qualified health care provider and do not constitute the practice of medicine or medical advice.