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

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Instructions for Use

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Related Policies

- Molecular Oncology Companion Diagnostic Testing (for Idaho Only)
- Molecular Oncology Testing for Hematologic Cancer Diagnosis, Prognosis, and Treatment Decisions (for Idaho Only)

Application

This Medical Policy only applies the state of Idaho, including Idaho Medicaid Plus plans.

Coverage Rationale

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

Breast Cancer Gene Expression Profiling (GEP)

The use of one of the following GEP tests – MammaPrint®, Oncotype Dx Breast®, Prosigna® Breast Cancer Prognostic Gene Signature Assay (formerly PAM-50), Breast Cancer Index™ (BCI), and EndoPredict® – is proven and medically necessary when used to inform treatment decisions in individuals with invasive breast cancer in the following situations:

- Newly diagnosed (within the last 6 months) when all the following criteria are met:
  - Lymph node negative (including lymph nodes with micrometastases no greater than 2 mm) or 1-3 positive ipsilateral axillary lymph nodes; and
  - No distant metastases; and
  - Hormone receptor-positive (estrogen receptor positive, progesterone receptor positive, or both); and
  - HER2 receptor negative; and
  - Adjuvant chemotherapy is not precluded due to any other factor (e.g., advanced age and/or significant co-morbidities)

  or
- Currently receiving adjuvant hormonal therapy (e.g., Tamoxifen or an aromatase inhibitor) for a breast cancer when all of the following criteria are met:
  - Hormone receptor-positive (estrogen receptor positive, progesterone receptor positive, or both); and
  - HER2 receptor negative; and
  - Individual and treating physician have had a discussion prior to testing regarding the potential results of the test and determined to use the results to guide a decision regarding extended adjuvant hormonal therapy

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The use of more than one predictive GEP for the same tumor in an individual with breast cancer is unproven and not medically necessary due to insufficient evidence of efficacy.
Note: This does not apply to BCI testing, which can be used once in the evaluation of the role of extended endocrine therapy in a breast cancer that may have already had GEP to determine the role of adjuvant chemotherapy.

Due to insufficient evidence of efficacy, GEP for breast cancer for indications [including ductal carcinoma in situ (DCIS)] or treatment decisions other than those previously described as proven are unproven and not medically necessary. Such tests may include but are not limited to:
- BluePrint
- DCISionRT®
- Oncotype DX Breast DCIS Score® test

Lung Cancer

Multigene molecular profiling (including no more than 50 genes) performed using tumor tissue or via Liquid Biopsy [cell-free DNA (cfDNA) or circulating tumor DNA (ctDNA)] is proven and medically necessary for non-small cell lung cancer.

Prostate Cancer Gene Expression Profiling (GEP)

The use of the Genomic Prostate Score® (GPS) test (previously Oncotype DX® 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™).

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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
- CancerTYPE ID®
- PancraGEN®, PancreaSeq®
- Oncotype DX® colon cancer assay, Colorectal Cancer DSA™, GenefxH℠ Colon (also known as ColDx), OncoDefender™, CRC, ColoPrint®, ColonSentry®
- Blood based colorectal cancer screening tests (e.g., Signal-C, Guardant Shield)
- DecisionDx®-Melanoma, DiffDx™-Melanoma, DecisionDx®-SCC, DermTech PLA™, myPath® Melanoma
- 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™)
- Molecular testing with GEP, multigene NGS panels and/or CGP for tracking MRD in solid tumors
- NavDx®
- Percepta® GSC
- Solid tumor profiling that includes Whole Exome, Whole Genome, or whole transcriptome Sequencing (e.g., Caris MI Tumor Seek™, Caris MI Profile™, Tempus xE, OncoExTra™)
- 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).

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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, v4.2023).

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, v4.2023).

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 (National Cancer Institute [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, v4.2023).

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, v4.2023).

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, v4.2023).

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, v4.2023).

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

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CPT Code	Description
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)
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
0020M	Oncology (central nervous system), analysis of 30000 DNA methylation loci by methylation array, utilizing DNA extracted from tumor tissue, diagnostic algorithm reported as probability of matching a reference tumor subclass
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)
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
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
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)

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CPT Code	Description
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
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 of at least 20 molecular features (e.g., human and/or microbial mRNA), saliva, algorithm reported as positive or negative for signature associated with malignancy
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

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CPT Code	Description
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
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
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
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

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CPT Code	Description
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
0433U	Oncology (prostate), 5 DNA regulatory markers by quantitative PCR, whole blood, algorithm, including prostate-specific antigen, reported as likelihood of cancer
0444U	Oncology (solid organ neoplasia), targeted genomic sequence analysis panel of 361 genes, interrogation for gene fusions, translocations, or other rearrangements, using DNA from formalin-fixed paraffin-embedded (FFPE) tumor tissue, report of clinically significant variant(s)
0452U	Oncology (bladder), methylated PENK DNA detection by linear target enrichment-quantitative methylation-specific real-time PCR (LTE-qMSP), urine, reported as likelihood of bladder cancer
0453U	Oncology (colorectal cancer), cell-free DNA (cfDNA), methylation-based quantitative PCR assay (SEPTIN9, IKZF1, BCAT1, Septin9-2, VAV3, BCAN), plasma, reported as presence or absence of circulating tumor DNA (ctDNA)
0467U	Oncology (bladder), DNA, next-generation sequencing (NGS) of 60 genes and whole genome aneuploidy, urine, algorithms reported as minimal residual disease (MRD) status positive or negative and quantitative disease burden
0478U	Oncology (non-small cell lung cancer), DNA and RNA, digital PCR analysis of 9 genes (EGFR, KRAS, BRAF, ALK, ROS1, RET, NTRK 1/2/3, ERBB2, and MET) in formalin-fixed paraffin-embedded (FFPE) tissue, interrogation for single-nucleotide variants, insertions/deletions, gene rearrangements, and reported as actionable detected variants for therapy selection
0485U	Oncology (solid tumor), cell-free DNA and RNA by next-generation sequencing, interpretative report for germline mutations, clonal hematopoiesis of indeterminate potential, and tumor-derived single-nucleotide variants, small insertions/deletions, copy number alterations, fusions, microsatellite instability, and tumor mutational burden
0486U	Oncology (pan-solid tumor), next-generation sequencing analysis of tumor methylation markers present in cell-free circulating tumor DNA, algorithm reported as quantitative measurement of methylation as a correlate of tumor fraction
0487U	Oncology (solid tumor), cell-free circulating DNA, targeted genomic sequence analysis panel of 84 genes, interrogation for sequence variants, aneuploidy-corrected gene copy number amplifications and losses, gene rearrangements, and microsatellite instability

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CPT Code	Description
0496U	Oncology (colorectal), cell-free DNA, 8 genes for mutations, 7 genes for methylation by real-time RT-PCR, and 4 proteins by enzyme-linked immunosorbent assay, blood, reported positive or negative for colorectal cancer or advanced adenoma risk
0497U	Oncology (prostate), mRNA gene-expression profiling by real-time RT-PCR of 6 genes (FOXM1, MCM3, MTUS1, TTC21B, ALAS1, and PPP2CA), utilizing formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as a risk score for prostate cancer
0498U	Oncology (colorectal), next-generation sequencing for mutation detection in 43 genes and methylation pattern in 45 genes, blood, and formalin-fixed paraffin-embedded (FFPE) tissue, report of variants and methylation pattern with interpretation
0499U	Oncology (colorectal and lung), DNA from formalin-fixed paraffin-embedded (FFPE) tissue, next-generation sequencing of 8 genes (NRAS, EGFR, CTNNB1, PIK3CA, APC, BRAF, KRAS, and TP53), mutation detection
0501U	Oncology (colorectal), blood, quantitative measurement of cell-free DNA (cfDNA)
0507U	Oncology (ovarian), DNA, whole-genome sequencing with 5-hydroxymethylcytosine (5hmC) enrichment, using whole blood or plasma, algorithm reported as cancer detected or not detected
0510U	Oncology (pancreatic cancer), augmentative algorithmic analysis of 16 genes from previously sequenced RNA whole-transcriptome data, reported as probability of predicted molecular subtype
0523U	Oncology (solid tumor), DNA, qualitative, next-generation sequencing (NGS) of single-nucleotide variants (SNV) and insertion/deletions in 22 genes utilizing formalin-fixed paraffin-embedded tissue, reported as presence or absence of mutation(s), location of mutation(s), nucleotide change, and amino acid change
0530U	Oncology (pan-solid tumor), ctDNA, utilizing plasma, next-generation sequencing (NGS) of 77 genes, 8 fusions, microsatellite instability, and tumor mutation burden, interpretative report for single-nucleotide variants, copy-number alterations, with therapy association
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 (e.g., 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 (e.g., 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 (e.g., 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

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CPT Code	Description
81504	Oncology (tissue of origin), microarray gene expression profiling of > 2000 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as tissue similarity scores
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

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

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

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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 certain 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

Breast Cancer

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

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

Harnan and colleagues (2019) conducted a systematic review and economic analysis to determine the efficacy and cost-effectiveness of the tumor profiling tests Oncotype DX, MammaPrint, Prosigna, EndoPredict, and immunohistochemistry 4 (IHC4). Studies included individuals with estrogen receptor-positive (ER+), HER2-, stage I, or II cancer with zero to three positive lymph nodes (LN+). The review included 153 articles on all five tests. In all five tests, the proportions of individuals who were lymph node-negative (LN0) getting endocrine monotherapy, 9% to 33%, were categorized as high-risk, according to the literature. For individuals who were LN+, three tests: Prosigna, EPClin, and IHC4 plus clinical factors (IHC4+C), categorized more (38% to 76%) individuals who were LN+ than those who were LN0 as high-risk according to the studies of endocrine monotherapy. Oncotype DX categorized high-risk in the LN0 and LN+ subsets as equal. Oncotype DX classified more individuals as low-risk in LN+ when compared to other tests (57% in Oncotype DX vs. 4% to 28% in other tests), but worse 10-year distant recurrence/relapse-free survival/distant recurrence/ relapse-free interval outcomes (82% in Oncotype DX vs. 95% to 100% in other tests). An increase of 1% to a decrease of 23% was seen in UK studies and a reduction of 0% to 64% across European studies on the net change of individuals who were recommended chemotherapy or decision pre/posttest. Limitations included gaps in the literature, the risk of bias, and limited data relating to the ability of Oncotype DX and MammaPrint to predict benefits from chemotherapy. Additional long-term studies can show the impacts and changes in chemotherapy decisions for Oncotype DX and MammaPrint. The authors concluded that the evidence indicates that all the tests deliver prognostic data regarding the risk of relapse, although greater variation was seen in individuals with LN+ status than those with LN 0 status.

Oncotype Dx® Breast

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

Nash and colleagues (2023) investigated the benefit of chemotherapy based on RS in younger women (aged 40-50) who were eligible for oncotype testing. Participants were selected from the National Cancer Database (NCBD) and grouped by age, RS, nodal status, and receipt of chemotherapy. A total of 15,422 individuals met inclusion criteria for the study. Of these 43.5% received chemotherapy. Log-rank tests were used to assess for differences between groups and Kaplan-Meier curves compared the unadjusted OS between groups. The analysis revealed that individuals who received chemotherapy were more likely to have higher-stage and higher-grade tumors, tumors that were PR-negative, and higher

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RS ($p < 0.001$ for all). RS was prognostic for OS regardless of nodal status. After adjustment, chemotherapy was associated with a significant improvement in OS only in the pN1 RS 31-50 subgroup ($p = 0.02$). The authors concluded that RS remains prognostic in younger individuals with early-stage HR-positive, HER2- BC. The survival benefit with chemotherapy was only found in those aged 40-50 with pN1 disease and RS of 31-50. As such, chemotherapy decision-making should be especially preference-sensitive in women aged 40-50 with intermediate RS, where survival benefit may not be enhanced for many women.

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

In a 2022 systematic review and network meta-analysis, Davey et al. evaluated the Oncotype DX 21-gene RS for its ability to estimate locoregional recurrence (LRR) in ER+/HER2- BC. The review uncovered 16 articles together with 21,037 individuals. The average RS was 17.1, and the average follow-up was 66.4 months. Employing standard RS cut-offs, 49.7% of individuals had RS < 18 (3,944/7,935), 33.8% had RS 18-30 (2,680/7,935), and 16.5% had RS > 30 (1,311/7,935). Those with RS 18-30 and RS > 30 were significantly more likely to experience LRR than those with RS < 18. Using the TAILORx cut-off, 16.2% of individuals had RS < 11 (1,974/12,208), 65.8% had RS 11-25 (8,036/12,208), and 18.0% with RS > 30 (2,198/12,208). LRR rates were comparable for individuals with RS 11-25; however, those with RS > 25 had a considerable risk of LRR versus those with RS < 11. The authors concluded that RS testing correctly estimates the risk of LRR for individuals being treated with the intent to cure early-stage ER+/HER2- BC. RS testing is a valid method to measure the risk of distant disease recurrence; however, awareness of its ability to predict LRR is significant to create effective locoregional control of the breast and axilla. Future prospective, randomized studies can confirm the predictive value of RS for estimating LRR and the application of RS to create suitable locoregional control in high-risk cases.

In 2021, Kalinsky et al. published the results of a prospective randomized clinical trial (RCT) to find the effect of chemotherapy on invasive disease-free survival in individuals with positive lymph-node disease and determine whether the RS based on the 21 gene assay (Oncotype Dx) influenced the outcome. A total of 5,018 women with hormone-receptor-positive, HER2- BC, 1 to 3 positive axillary lymph nodes, and an RS of 25 or lower were randomly grouped into an endocrine therapy alone subset or a chemotherapy with endocrine (chemoendocrine) therapy subset. The intention-to-treat analysis included the participants who declined the assigned treatment, with 402 (16.2%) participants allocated to chemoendocrine therapy and 144 (5.8%) given to endocrine treatment. The trial did not show a clinically applicable or statistically significant rise in invasive disease-free survival with the addition of adjuvant chemotherapy to endocrine therapy in the global population with the same characteristics. For this trial, 67% of post-menopausal participants had no chemotherapy advantage. Dissimilarity, adjuvant chemotherapy led to a relative growth of 40% in invasive disease-free survival and a relative rise of 42% in distant relapse-free survival (RFS) among premenopausal women. Invasive disease-free survival at five years was 91.9% among post-menopausal women in the endocrine-only group and 91.3% in the chemoendocrine group, with no chemotherapy advantage. In the group of premenopausal women, invasive disease-free survival at five years was 89.0% with endocrine-only therapy and 93.9% with chemoendocrine treatment, with a comparable rise in distant relapse-free survival. The trial showed that between premenopausal women with 1 to 3 positive lymph nodes (N1) and an RS of 25 or less, individuals who received chemoendocrine therapy had a lengthier invasive disease-free survival and distant RFS than those who received endocrine-only treatment. In contrast, post-menopausal women with the same characteristics did not profit from adjuvant chemotherapy.

Hayes published a Molecular Test Assessment addressing the use of the Oncotype DX Breast Recurrence Score for individuals with ER+, HER2-, lymph node positive BC to determine the capability of the test to estimate the risk of distant recurrence and the predict the likelihood of chemotherapy benefit in 2020. For individuals with N1 disease, limited but

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consistent evidence supports the use of the Oncotype DX test for predicting the risk of 9-year distant recurrence, but there is insufficient evidence supporting the test's ability to predict the benefit of chemotherapy. Oncotype DX may improve outcomes for individuals with N1 cancer by lessening the total population of individuals treated with chemotherapy, thereby avoiding detrimental side effects. Insufficient evidence was found to support the use of Oncotype DX testing for estimating the risk of distant recurrence and the potential benefit of chemotherapy for individuals with N2 disease (4 to 9 positive lymph nodes) [Hayes, Oncotype DX Breast Recurrence Score (Genomic Health Inc.) for Lymph Node-Positive Patients, 2020, updated 2023].

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

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

Wang et al. (2019) examined the value of Oncotype Dx when determining the prognosis in female individuals with BC and tumor stage 1-2 (tumor is 20-55 mm), LN+ and no evidence of metastasis (T1-2 N1M0). The study reviewed 4,059 cases to categorize them to prognostic stages IA and IIB and used data derived from the National Cancer Institute's limited use Surveillance, Epidemiology, and End Results (SEER) 18 registry databases, released in November 2017. Cases in the SEER database was linked to RS results from assays performed by Genomic Health. All cases with RS had negative HER2, and the authors selected female ER+ invasive ductal carcinoma (IDC) cases in T1-2N1M0 stage with Oncotype RS results diagnosed between 2004 and 2012. Individuals were categorized into low-risk (RS < 11), intermediate-risk (RS 11-25), and high-risk (RS > 25) groups. The median age of the individuals was 59 years. Of these participants, 2,898 (71.4%) had stage T1 cancer, 1,854 (45.7%) had stage N_{1mic} cancer, 743 (18.3%) had grade 3 cancer, and 3,746 (92.3%) had positive PR status. They were stratified into the RS low-risk group (794, 19.6%), the RS intermediate-risk group (2,667, 65.7%), and 598 (14.7%) were in the RS high-risk group. The high-risk group tended to have younger individuals, larger tumors, a higher percentage of grade 3 disease, negative PR, and more advanced cancer staging. They also had more frequent use of chemotherapy. Otherwise, the RS groups did not differ much in race, N stage, surgery, or radiation. In terms of pathological prognostic stages, there were 2,781 individuals (68.5%) in stage IA, 829 (20.4%) in stage IB, 360 (8.9%) in IIA, and 89 (2.2%) in IIB. The distributions of clinical and pathological characteristics, including BCSS and OS, were compared between RS and pathological staging groups using a variety of statistical analysis. The median follow-up period was 57 months. The results showed a statistically significant correlation ($p < .001$) between the RS groups and pathological stage results. In the low and high-risk RS groups, the BCSS and OS were similar between RS and pathological staging groups. In the intermediate RS group, however, survival rates differed significantly between RS staging and pathological staging. The survival rates were inversely correlated with the escalation of prognostic stages. Similar trends were seen in the high-risk group but were not statistically significant. In this retrospective study, RS was an independent prognosticator for BCSS, and with pathological stage for OS. The authors concluded that Oncotype Dx could complement the prognostic staging system in N+ individuals.

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

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Prosigna Breast Cancer Prognostic Gene Signature Assay (Formerly PAM-50)

The Prosigna Breast Cancer Prognostic Gene Signature Assay (NanoString Technologies, Seattle, WA) is a prognostic BC assay that provides a risk category and numerical score to assess an individual's risk of distant recurrence of disease at 10 years in postmenopausal women with N0 (Stage I or II) or N+ (Stage II), HR+BC. The Prosigna assay measures expression levels of 50 genes using formalin-fixed paraffin-embedded (FFPE) breast tumor tissue diagnosed as invasive breast carcinoma. The assay is not intended for individuals with N2 (Gnant et al., 2013; Parker et al., 2009).

Fitzal et al. 2021 conducted a prospective multicenter RCT [The Austrian Breast and Colorectal Cancer Study Group (ABCSG) 8] to investigate if the PAM50 based 46-gene assay brings prognostic value for the risk of local recurrence of BC. The trial compared five years of adjuvant tamoxifen with sequential therapy involving tamoxifen for two years and then anastrozole for three years in postmenopausal women with endocrine receptor-positive early-stage BC. All participants were regularly followed up every three months for one year, at six-month intervals over the second and third years, and annually afterward. Ribonucleic acid (RNA) was extracted from FFPE blocks from BC excision specimens from the ABCSG-8 trial. Participants were distributed randomly to either the group who received five years of tamoxifen (525 participants) or tamoxifen, followed by anastrozole (509 participants) after surgery group. There were 765 individuals (74%) with a low risk of recurrence (ROR) score (< 57). The existing data showed that the PAM50 ROR score and intrinsic molecular subtypes could detect a low-risk genomic population in individuals with a clinically minimal risk of local recurrence. The PAM50 ROR score is consistently associated with the prospect of disease recurrence. Authors explored if the PAM50 test may predict the value of radiotherapy following breast conservation, using a subgroup of 170 women in the ABCSG-8 trial who did not have adjuvant radiotherapy. The trial suggested that a PAM50-based assay is helpful as a prognostic instrument for local recurrence risk in postmenopausal women with HR+BC treated with endocrine therapy; however, it is not predictive of the benefit of radiotherapy. The trial is limited by its retrospective nature. The authors concluded that a PAM50-based assay brings value for the risk of local recurrence of BC for postmenopausal women with HR+BC treated with endocrine therapy.

MammaPrint® (Also Referred to as the "Amsterdam Signature" or "70-Gene Signature") and BluePrint

MammaPrint (Agendia, Amsterdam, The Netherlands) is a 70-gene expression test to assess BC distant recurrence risk. The assay analyzes tumor tissue (fresh, frozen or FFPE) for expression of 70 genes assumed to be important in cancer metastasis. Based on the test results, MammaPrint may assist individuals considering adjuvant treatments. Individuals are assigned either a low-risk or a high-risk for a distant recurrence. The risk category may be taken into consideration for treatment options.

BluePrint (Agendia, Amsterdam, The Netherlands), a complementary test to MammaPrint, measures the expression of 80 genes to classify the tumor as one of three subtypes. The tumor subtype is used to predict future behavior of the cancer, long term prognosis and response to systemic therapy. Evidence addressing use of Blueprint in conjunction with MammaPrint is insufficient to support clinical utility at this time.

Marin-Liebana et al. published an initial analysis from the DETERMIND study in 2023. DETERMIND is a prospective, open-label, multicenter study evaluating the utility of the MammaPrint/BluePrint (MP/BP) signature related to determining optimal therapy for individuals with operable, clinically high-risk HR+/HER2- early breast cancer (EBC), stage II-IIIA (up to N1) who have received a recommendation for NCT. One hundred sixty-five individuals from 11 centers have been included in this analysis, with data collected at baseline, at the time of MP/BP results and finally at one and three year follow-ups. The first analysis incorporated 99 participants with a median age of 57 years (range 31-85). Ninety-four percent of these were stage II, with 51% cN1. At the time of MP/BP, 37 individuals (37%) were classified as Luminal A, 58 (59%) were Luminal-B, and four presented as a non-Luminal phenotype (3 Basal, 1 HER2). Corresponding with MP/BP results, 44 pts did not receive NCT. In the MP/BP Luminal A group, 35 (95%) did not receive NCT; for 19 of these individuals, it was replaced by NET. Individuals with MP High-Risk results received NCT in 53 cases (85%). MP/BP results significantly increased confidence on the final treatment decision made collaboratively by the treating physicians and participants. The authors concluded that in individuals with clinical high-risk HR+/HER2- EBC, there is a high frequency (35%) of MP/BP Luminal A, who were able to de-escalate NCT. The use of MP/BP also bolstered the decision to administer NCT in the majority (85%) of those with MP High Risk. The authors assert that these findings support the utility of MP/BP in high clinical risk HR+/HER2- EBC to inform neoadjuvant therapy decisions and increase confidence in clinicians and their patients during shared-decision making. The study was sponsored by Agendia, the manufacturer of the MP/BP test, which presents potential bias. Larger, high-quality prospective trials are needed to further validate these findings.

Pellicane et al. (2022) addressed the need for reliable biomarkers to identify individuals with HR+ HER2- BC tumors who are likely to receive benefit from neoadjuvant endocrine therapy (NET) in a recent observational registry trial of 1,091

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individuals with early-stage BC. Participants, who were scheduled to receive neoadjuvant therapy, were prospectively enrolled into the Neoadjuvant Breast Registry Symphony Trial (NBRST), sponsored by Agendia. NBRST compared the prognostic value of the 70-gene risk classifier (MammaPrint) and the 80-gene molecular subtyping signatures (BluePrint) with standard pathological classification methods in response to neoadjuvant treatment. The association of these signatures with clinical response and 5-years outcome of participants who underwent treatment with NET (n = 67) were evaluated in a sub-analysis. Standard of care genomic testing with MammaPrint and BluePrint was performed and participants underwent therapy with NET per their physician's discretion. Primary outcome was pathologic partial response (pPR). Secondary outcomes included distant metastasis-free survival (DMFS) and OS. The researchers defined clinical benefit as a pPR or stable disease (SD) with use of NET. Of individuals with genomically Luminal A-Type tumors, 94.4% displayed clinical benefit (50.0% pPR and 44.4% SD). Ninety-five percent of individuals with Luminal B-Type tumors exhibited benefit (55.0% pPR and 40.0% SD). At 5 year assessment, individuals with genomically Luminal B tumors had substantially worse DMFS (75.6%, 95% CI 50.8-89.1) than those with genomically Luminal A tumors (91.1%; 95% CI 74.8-97.1; p = 0.047). The trend for OS was similar, but was not significant (81.0%, 95% CI 56.9-92.4 and 91.1%, 95% CI 74.8-97.1, respectively; p = 0.13). The authors concluded that individuals with 70-gene signature low risk results and genomically Luminal A tumors who were treated with endocrine therapy alone have excellent outcomes at 5 years. In addition, most individuals with genomically-defined Luminal A- and B-type tumors respond well to NET, which suggests NET may be a safe option for treatment; however, those with genomically Luminal B tumors will also need post-operative chemotherapy or CDK4/6 inhibitors to improve their long-term outcomes. The researchers indicate that genomic classification (defined by the combined use of 70- and 80-gene signatures) is prognostic of long-term outcomes and is related to tumor response, supporting the use of these tests in making neoadjuvant treatment decisions in individuals with early-state HR=+ HER2- BC. This study was observational and the number of individuals receiving NET was limited, so the sample size was small and prevented further subgroup analyses. In addition, NBRST participants receiving NET instead of neoadjuvant chemotherapy despite features associated with high clinical risk were more likely to be older and postmenopausal. Larger, prospective trials, such as the ongoing FLEX trial (NCT030631983), are needed to confirm the findings of this study.

In 2022, Vliek and colleagues published a ten-year follow-up of the observational RASTER study. The prospective RASTER study assessed the tumors of 427 individuals with cTanyN0M0 BC. The study aimed to decide the 70-gene signature (MammaPrint)'s ability to guide adjuvant chemotherapy decisions for individuals with ER+ and HER2- BC. The authors evaluated 310 of the 427 individuals at ten years of follow-up. Of the clinically high-risk individuals, 45 (49%) were classified as genomically low-risk. In this subcategory, at ten years, distant recurrence-free interval (DRFI) was comparable among individuals treated with [95.7% (95% CI 87.7-100)] and without [95.5% (95% CI 87.1-100)] chemotherapy. In the group of clinically low-risk individuals, 56 (26%) were classified as genomically high-risk. For the clinically low-risk group, a variance was seen among the genomically high- and low-risk subgroup after five years, resulting in a 10-year DRFI of 84.3% (95% CI 74.8-95.0) and 93.4% (95% CI 89.5-97.5), respectively. Genomic ultralow-risk individuals' outcomes were a 10-year DRFI of 96.7% (95% CI 90.5-100), primarily (79%) without systemic therapy. Limitations to the RASTER study include the observational nature and the risk of bias. The authors concluded that over ten years, individuals with clinically high-risk, genomic low-risk tumors have excellent results irrespective of the use of chemotherapy. The updated outcomes of the MINDACT trial and RASTER study collectively demonstrate that the data supports the use of the MammaPrint, in ER+, HER2-, and N0, clinically high-risk individuals with BC.

In NBREaST II, a prospective, neoadjuvant study, Göker et al. (2022) measured the treatment response and 5-year survival outcome in the molecular subgroups by combining the MammaPrint and BluePrint. MammaPrint and BluePrint were carried out on 256 individual core needle biopsies (CNB) to quantify chemosensitivity or endocrine sensitivity in the molecular subgroups. The outcomes measured were DMFS, RFS, and BCSS at long-term follow-up. In the group of individuals who received NCT (n = 234), MammaPrint and BluePrint categorized 50 tumors as Luminal A-Type (21%), 110 as Luminal B-Type (47%), 27 as HER2-Type (12%), 47 as Basal-Type (20%). Of individuals that attained a pCR in response to NCT (n = 47), 4% had a MammaPrint Low-Risk result, and 96% had a High-Risk outcome. All BluePrint-defined HER2-Type and Basal-Type tumors had a High-Risk MammaPrint outcome. At five years, DMFS was significantly lower (p = 0.039) in MammaPrint High-Risk tumors (83.8%; 95% CI 77.4-88.6) versus MammaPrint low-risk tumors (91.4%; 95% CI 78.6-96.7). Similar outcomes were seen for 5-year RFS; however, not for BCSS. Limitations to the study include a small sample size, with no differences in 5-year survival when stratifying the cohort into subgroups. The study confirms previous conclusions signifying that MammaPrint and BluePrint can predict chemosensitivity and 5-year results more precisely versus traditional pathological sub-typing, supporting informed decision-making.

Crozier et al. (2022) prospectively collected 139 matched CNB and surgical resection (SR) specimens from women with established EBC registered in the ongoing FLEX study (NCT03053193). The goal was to decide the concordance of MammaPrint and BluePrint results among CNB and SR to safeguard reliable prognostic information that can be apprehended from a CNB. FLEX is an ongoing, multi-institutional prospective study of individuals with Stage I–III EBC. Overall, 121 individuals from the FLEX study database with diagnostic MammaPrint and BluePrint results with matched

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CNB and SR specimens were involved in the study. In total, 50 individuals had High-Risk CNB and SR specimens, and 60 had Low-Risk CNB and SR specimens, resulting in 90.9% total agreement (κ = 0.817), 95.2% negative predictive value (NPV), and 86.2% positive predictive value (PPV). The authors concluded the concordance of BluePrint between CNB and SR to be 98.3%. For more than 97% of individuals in this study, treatment decisions and probable outcomes are precise and consistent based on MammaPrint testing of the CNB. According to the authors, this analysis is the most extensive powered study using prospective real-world numbers to assess the concordance of a genomic assay on matched CNB and SR samples. The limitation of the study is the lack of data maturity, as individual follow-up data is not available to correlate outcomes with MammaPrint and BluePrint results from the CNB and SR samples. The authors concluded that the high concordance rates of MammaPrint and BluePrint result among paired samples strongly support the value of these assays to acquire reliable prognostic data on core biopsy tissue, which can guide prompt and proper treatment decisions.

In 2021, Piccart et al. produced updated results on phase 3 randomized MINDACT trial, including long-term follow-up with an exploratory analysis by age. MINDACT was a randomized, phase 3, multicenter trial conducted in 112 academic and community hospitals in nine countries that enrolled individuals that had confirmed primary invasive BC with N1, no distant metastases, and a WHO performance status of 0-1, and their genomic risk was decided using the MammaPrint 70-gene signature. Enrolled in the trial were 6,693 individuals with a mean follow-up of 8.7 years. The 8-year estimates for DMFS in the intention-to-treat population were 92·0% (95% CI 89·6-93·8) for chemotherapy set against 89·4% who received no chemotherapy. The 8-year DMFS in the exploratory analysis by nodal status in these individuals was 91·7% (95% CI 88·1-94·3) with chemotherapy and 89·2% (85·2-92·2) without chemotherapy in 699 N0 individuals [absolute difference 2·5 percentage points (SE 2·3, 95% CI −2·1 to 7·2)] and 91·2% (87·2-94·0) as opposed to 89·9% (85·8-92·8) for 658 individuals with N1. The exploratory analysis conducted to determine the effects of chemotherapy administration on 8-year DMFS according to age resulted in 93.6% with chemotherapy set against 88.6% without chemotherapy in 464 women aged 50 years or younger and 90.2% vs. 90.0% in 894 women older than 50 years. This long-term follow-up of phase 3 randomized MINDACT trial showed the 70-gene signature's capability of detecting women with high clinical risk, a subgroup, and specific individuals with low genomic risk, with an exceptional DMFS when treated with endocrine therapy by itself. For this group of women, the size of the profit from adding chemotherapy to endocrine therapy continues to be small and is not improved by nodal positivity. The benefit is age-dependent and is solely seen in women under 50; further study is needed in younger women, who may need reinforced endocrine therapy to forego chemotherapy. The authors concluded that MammaPrint ought to be a portion of informed, shared decision-making.

Soliman et al. (2020) conducted a prospective case-only trial (IMPACt) enrolling 452 individuals with state I-II, HR+, HER2- BC to evaluate the variation in treatment decision and physician assurance based on the 70-gene ROR signature and the 80-gene molecular subtype signature (80-GS, BluePrint) in early-stage BC. According to clinical risk assessment via the MINDACT criteria, 63.4% (n = 227/358) of individuals were categorized as low-risk, and 36.6% (n = 131/358) of individuals were classed as high-risk of distant recurrence. For individuals with clinically minimal risk, 77.5% (176/227) were suggested not to have chemotherapy by their doctors, while 62.6% (82/131) of individuals with clinically high-risk were recommended treatment plans that involved chemotherapy. The 70-GS categorized 62.5% (n = 224/358) of individuals as low-risk and 37.5% (n = 134/358) as high-risk. Following the 70-GS results, doctors elected to change the chemotherapy treatment (CT) recommendation in 24.0% (n = 86/358) of all cases. After-70-GS treatment plans were, 88.5% (n = 317/358) agreeing with 70-GS results, 83.6% (n = 112/134) for CT in 70-GS high-risk individuals, 91.5% (n = 205/224) for no CT in 70-GS low-risk individuals. The IMPACt trial displayed that the majority (88.5%) of treatment plans were accordant with 70-GS results, showing that doctors make treatment decisions based on the 70-GS result in clinical practice. Physicians also described a rise in confidence in 72.2% of their suggested treatment plans after receiving the 70-GS results. A limitation of the study is that individuals were enrolled both before and after the MINDACT trial results were published, which may have impacted physicians' decisions for treatment. The authors concluded that these results propose that doctors feel the proper individuals (high-risk) are being presented chemotherapy, and they feel confident sparing 70-GS individuals with low-risk from the high clinical and financial burden of chemotherapy. The trial shows that doctors can avoid overtreatment and the adverse effects of chemotherapy treatments for individuals who are not likely to obtain meaningful clinical benefits.

In 2019, Wuerstlein and colleagues reported on the prospective, observational multicenter WSG-PRIMe study designed to gauge the effect of MammaPrint and BluePrint on adjuvant chemotherapy treatment decisions in individuals with early stage BC specifically to show an overall switch percentage of at least 15% regarding chemotherapy. These individuals had MammaPrint considered as part of their standard clinical procedure. Included in the study were 452 individuals who were HR+ and Her2-. Physicians supplied preliminary treatment recommendations created on clinicopathological factors. Post-test therapy recommendations and actual therapy were documented after prospective risk classification by MammaPrint/BluePrint was revealed. MammaPrint allocated 63.5% of participants to the low-risk group and 36.5% to the high-risk group. In (125/430, 29.1%) of individuals (95% CI 24.8-33.6%), the recommendation transformed from chemotherapy to no chemotherapy or vice versa. Chemotherapy had been recommended to 164 individuals (38.1%) pre-

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test. In 60/164 (36.1%) of the individuals who were recommended chemotherapy, the therapy recommendation converted to the omission of chemotherapy post-test; most (59/60, 98%) of these changes happened in low-risk individuals, according to MammaPrint. On the contrary, deletion of chemotherapy been suggested to 266 individuals (61.9%) pre-test; in 65/266 (24.4%) cases, recommendations converted to chemotherapy post-test; most (64/65, 98.4%) of these modifications happened in MammaPrint high-risk individuals. The physician observance of MammaPrint risk calculation was 92.3% for low-risk and 94.3% for high-risk scores. Three-fourths (n = 59/79, 74.7%) of physicians initially recommending chemotherapy converted to no chemotherapy subsequent low-risk MammaPrint results (72.7% in pN0, 77.1% in pN1); on the contrary, nearly nine tenths (n = 64/72, 88.9%) of physicians originally recommending chemotherapy omission from treatment converted to chemotherapy recommendations following high-risk MammaPrint outcomes (88.1% in pN0, 92.3% in pN1). The authors concluded that the WSG-PRIMe study proves that the use of the gene expression profiles, MammaPrint and BluePrint, has a powerful influence on adjuvant therapy recommendations. The results showed that physicians changed their ultimate recommendation for systemic therapy in 29.1% of cases subsequent MammaPrint testing. The study verified that there is improved, genomically determined individualization of treatment regimens that can lead to a reduced risk of over- or under-treatment of individuals. Overall, the high adherence to genomically determining risk assessment signifies a significant prerequisite for reaching further targeted disease management in early-stage BC.

van Steenhoven et al. (2018) evaluated the ability of 70-GS (MammaPrint) and 80-GS (BluePrint) molecular subtyping to surrogate pathological subtyping (PS) for determining treatment options and prognosis. Between 2013 and 2015, 595 intermediate risk individuals who are ER+ with early-stage BC were studied. HER2 receptor status was determine through routine immunohistochemistry and fluorescent in situ hybridization. The overall concordance between molecular sub-typing and PS for luminal cancers type A and B together was 98%. Individually it was poor, at 64%. The ability of the 80-GS assay to differentiate between luminal, HER2-type and basal-like cancers was limited, and furthermore the concordance between PS and the 70-GS approach was low. The authors concluded that two classification methods had significant disparity in outcomes, resulting in the risk of inadequate treatment. More studies are needed to demonstrate the efficacy of this test.

The randomized, phase 3 clinical MINDACT trial included 6,693 women with early-stage BC with the primary goal to assess whether, among individuals with high-risk clinical features and a low-risk gene-expression profile who did not receive chemotherapy, the lower boundary of the 95% confidence interval for the rate of 5-year survival without distant metastasis would be 92% (i.e., the non-inferiority boundary) or higher. Women at low clinical and genomic risk did not receive chemotherapy, while those at high clinical and genomic risk did receive such therapy. For individuals with discordant risk results, either the genomic risk or the clinical risk was used to determine the use of chemotherapy. The researchers found that among women with early-stage BC who were at high clinical risk and low genomic risk for recurrence, the receipt of no chemotherapy on the basis of the 70-gene signature led to a 5-year rate of survival without distant metastasis that was 1.5 percentage points lower than the rate with chemotherapy. Given these findings, approximately 46% of women with BC who are at high clinical risk might not require chemotherapy (Cardoso et al., 2016).

EndoPredict

EndoPredict (Myriad, Salt Lake City, UT) is a 12-gene real-time genomic test that includes eight disease-relevant genes *BIRC5, UBE2C, DHCR7, RBBP8, IL6ST, AZGP1, MGP* and *STC2*, three RNA normalization genes (*CALM2, OAZ1* and *RPL37A*) and one DNA reference gene (*HBB*). EndoPredict also incorporates information on nodal status and tumor size. Results are given as an “EPclin Risk Score;” a number between 1.1 and 6.2 which relates to cancer recurrence risk.

In a Hayes Molecular Test Assessment, the clinical validity, clinical utility, and analytical validity of EndoPredict were evaluated. The assessment uncovered limited but positive evidence suggesting EndoPredict may estimate the 10-year risk of distant recurrence (DR) for individuals with ER+, HER2-, N0, and early-stage BC; however, it remains unclear if the test can prospectively distinguish low-risk individuals from others or if the test is equally applicable for premenopausal women. There is limited evidence suggesting EndoPredict may estimate the 10-year risk of DR for individuals with ER+, HER2-, N1, and early-stage BC, and conflicting results to determine if the EPclin low-risk group was genuinely associated with a low-risk of DR in this population. For the EndoPredict test to estimate the likelihood of DR 5 to 15 years from diagnosis and the absolute benefit of chemotherapy at ten years for individuals with ER+, HER2-, N0/N1, early-stage cancer, there are limited studies and data to support the test results. No prospectively designed studies were found regarding the clinical validity of EndoPredict; additional studies are necessary to examine diverse demographics and possibly improve health outcomes resulting from the EndoPredict test [Hayes, EndoPredict (Myriad Genetics Laboratories Inc.), 2020, updated 2023].

In the prospective, translational, randomized phase II ABCSG-34 trial directed by Dubsky et al. (2020), the ability of EndoPredict to predict response to NCT or NET was assessed. HR+, HER2- samples were gathered from participants, and EndoPredict testing was completed to produce a 12-gene MS. Participants were randomized to have either NCT or

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NET based on menopausal status, HR expression, grade, and Ki67. The outcome measured was calculated via the residual cancer burden (RCB). Overall, 134 individuals who were HR+ received NCT, and 83 received NET as their preoperative SoC treatment. Out of 134 participants who received NCT, nine had low-risk disease according to the 12-gene MS, and 125 had high-risk disease. The 12-gene MS exhibited strong sensitivity for NCT (100%, 95% CI 89.4%e100%), even though specificity was small (8.9%, 95% CI 4.2%e16.2%). Of the participants treated with NET, 44 out of 83 had low-risk disease, and 39 had high-risk disease. According to the authors, this is the first study that prospectively proves the predictive probability of the 12-gene MS for its response to NET. The RCB 0-I outcome for individuals with NET in the low-risk 12-gene MS subset was 27% in contrast to 7.7% in individuals with high-risk MS. The data presented in this trial shows that the 12-gene MS offers supplementary predictive data beyond the traditional clinicopathologic factors used to evaluate risk and is a valuable instrument preoperatively.

Sestak et al. (2020) retrospectively investigated a cohort of individuals with invasive lobular carcinoma (ILC) from previously conducted clinical trials (ABCSG-6, ABCSG-8, TransATAC). The main objective of the study was to determine the prognostic value of EPclin, either alone or in combination with clinical parameters, for DR in women with ILC. All participants had received 5 years of endocrine treatment as the only adjuvant therapy. Information compiled from the 3 clinical trials included data from 2,630 postmenopausal women with ER+, HER2- BC. As part of that group, 470 (19.5%) had ILC, 1944 (80.5%) had IDC and 216 (8.2%) had another histological subtype. The researchers found that in this study, EPclin was highly prognostic in women with ILC [HR = 3.32 (2.54-4.34), $p < 0.0001$] and provided better prognostic value than the Clinical Treatment Score [CTS; HR = 2.17 (1.73–2.72)]. Further, they found that EPclin was prognostic in women with IDC (n = 1,944) overall [HR = 2.36 (2.11–2.65), $p < 0.0001$], though not to the level of ILC. They concluded that EPclin provided substantial prognostic information and risk stratification for women with ILC. This study was included in Hayes, EndoPredict (Myriad Genetics Laboratories Inc., 2020).

Penault-Llorca et al. (2020) led a prospective single-arm multicenter trial calculating the clinical and psychological influence of EndoPredict use for individuals with ER+HR- localized BC. The trial assessed the quantity of change from the initial adjuvant decision and the last administration of chemotherapy. Secondary measures involved post-test (Day 17) and 1-year patient-reported results. The trial encompassed 203 participants from 25 centers: 201 had an EPclin assessment. Overall, the decision to treat compared to the initial decision was changed for 72/200 (36%, 95% CI 29.3-42.7) individuals. Chemotherapy was first recommended to 48% of individuals, of which only 26% underwent chemotherapy. Chemotherapy was withdrawn in 57 cases (28.4%, 95% CI 22.2-34.8), and in 15 cases (7.5%, 95% CI 3.8-11.2), chemotherapy was added. The choices to change therapy were often associated with the EPclin outcomes. The trial exposed that in individuals with early BC at intermediate risk, using EPclin to back up the treatment choice resulted in a 35.8% change in whether adjuvant chemotherapy was given. The trial shows how the test permits a decrease in centers and physicians' therapy decision heterogeneity. The trial has limitations associated with the non-randomized method, open design, and risk of bias in participant selection. The authors concluded that the EPclin test has an established influence that can reduce adjuvant chemotherapy treatments under ordinary circumstances.

Breast Cancer Index (BCI)

BCI (BioTheranostics, San Diego, CA) is a gene expression-based signature that analyzes the combination of two biomarker panels: the *HOXB13:IL17BR* ratio (H/I) and the Molecular Grade Index (MGI), consisting of the average expression of five cell cycle-associate gene index (*BUB1B, CENPA, NEK2, RACGAP1, RRM2*). The test is performed on a FFPE tissue block and results in a single prognostic score quantifying both the risk of late (5-10 years) and overall (0-10 years) distant recurrence (Sgroi et al., 2022).

The evidence supporting predictive tests for the extension of endocrine therapy in individuals with BC has been limited to date. Woolpert et al. (2023) explored the role of biomarker tests in the prediction of clinical response to extended endocrine therapy in a systematic review and meta-analysis. A total of five studies met eligibility requirements and were included; four investigated the BCI assay in three unique study populations and one explored the predictive ability of Ki67 and progesterone receptor status. The studies focused on BCI reliably demonstrated that the BCI score predicted response to an extension of endocrine therapy in 1,946 combined participants (primarily non-Hispanic white and postmenopausal). The authors recommend further study of an assortment of biomarkers in diverse populations of individuals. Studies by Noordhoek et al. (2021), and Bartlett et al. (2019), previously discussed in this policy, were included in the Woolpert systematic review and meta-analysis.

Hayes evaluated the use of the BCI test for predicting likelihood of benefit from extended endocrine therapy (> 5 years) and estimating risk of late (> 5 years from diagnosis) and cumulative distant recurrence risk over ten years in individuals diagnosed with HR+, N0 or N1 invasive BC who have received 5 years of therapy with primary adjuvant chemoendocrine, primary adjuvant endocrine or primary endocrine therapy. The Hayes assessments found insufficient evidence to support the BCI test for the prediction of likelihood of benefit from extended endocrine therapy or to estimate the risk of late and cumulative distant recurrence risk over ten years in these situations. Further investigation including large, prospective,

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randomized trials examining diverse populations and health outcomes related to use of the BCI test are recommended. The 2022 and 2023 updates to the BCI Molecular Test Assessments indicate no change to the current Hayes ratings [Hayes, Breast Cancer Index (BioTheranostics Inc.) for Lymph Node-Negative Patients, 2020, updated 2023; Hayes, Breast Cancer Index (BioTheranostics Inc.) for Lymph Node-Positive (1-3) Patients, 2020, updated 2022].

Sestak et al. (2018) provided a secondary analysis of data obtained from the Anastrozole or Tamoxifen Alone or Combined RCT, comparing 5-year treatment with anastrozole vs tamoxifen with 10-year follow-up data. The objective was to compare the prognostic value of Oncotype Dx recurrence score, PAM50 based Prosigna ROR, BCI, EndoPredict, Clinical Treatment Score, and 4-marker immunohistochemical score to the Clinical Treatment Score (nodal status, tumor size, grade, age, and endocrine treatment) for distant recurrence for 0 to 10 years and 5 to 10 years after diagnosis. The analysis included 774 post-menopausal women with estrogen positive, HER2- disease. Five hundred and ninety-one had N0 disease. All genomic signature tests provided significantly more information than the clinical treatment score, the RS and the 4 marker immunohistochemical score alone. The most valuable tests were the PAM50 and BCI. In the 183 individuals with N1, there was limited information provided by the molecular tests, and BCI and EndoPredict provided the most value. The authors concluded that the data provided by molecular testing could help oncologists and individuals consider chemotherapy or extended endocrine testing [Included in Hayes EndoPredict (Myriad Genetics Laboratories Inc.), 2020, and Hayes Breast Cancer Index (BioTheranostics Inc.) for Lymph Node-Negative Patients, 2020].

Zhang et al. (2017b) examined the predictive ability of BCI results, when integrated with tumor size and grade Breast Cancer Index Model (BCIN), to accurately identify outcomes in a well annotated retrospective series of N+ individuals. A total of 402 participants with N1 who were treated with adjuvant endocrine therapy with or without chemotherapy using a prespecified model. The primary endpoint was time to DR. BCIN classified 20% of participants as low-risk with a 15-year DR rate of 1.3% and 321 individuals as high-risk with a DR risk of 29%. When the results were unblinded and compared to participant outcome, BCI alone was significantly prognostic ($p < .0001$), and when tumor size was added the prognostic ability was even further improved ($p < .0003$) but only incrementally with adding tumor grade ($p = .01$). Overall, BCIN identified 20% of individuals who were N+ with a limited ROR over 15 years that could avoid extended endocrine treatment. Further studies on combined genomic and clinical algorithmic predictions are needed on N+ individuals. This study is included in Hayes, Breast Cancer Index (BioTheranostics Inc.) for Lymph Node-Positive (1-3) Patients, 2020.

Sestak et al. (2016) conducted a retrospective analysis to examine cross-stratification between BCI and the Oncotype DX RS to directly compare their prognostic accuracy at the individual level. Six hundred and sixty-five individuals with HR+ and LN0 disease were included in this retrospective analysis. The authors concluded that BCI demonstrated increased prognostic accuracy versus RS. Notably, BCI identified subsets of RS low and RS intermediate risk individuals with significant and clinically relevant rates of DR. These results indicate that additional subsets of women with HR+, LN0 BC identified by BCI may be suitable candidates for adjuvant chemotherapy or EET.

Other Breast Cancer Profiling Assays

Gene expression profiling assays for BC treatment other than those previously described, including but not limited to Breast Cancer Gene Expression Ratio (also known as Theros H/I), DCISionRT, Oncotype DX DCIS, the 41-gene signature assay, and the 76-gene "Rotterdam signature" lack sufficient evidence to support clinical utility at this time.

DCISionRT

DCISionRT (Prelude Corporation, Laguna Hills, CA) is a risk assessment test for patients with ductal carcinoma in situ (DCIS) which is designed to quantify an individual's 10-year risk of DCIS recurrence and determine whether radiation therapy would be of benefit. DCISionRT assesses 7 genes along with other clinical risk factors to provide a DCISionRT score ranging from 0 to 10**.** Scores 0-3 are considered low risk and scores 3-10 are considered elevated risk.

In a 2023 systematic review and meta-analysis, Ouattara et al. evaluated women with DCIS who had been treated with breast-conserving surgery (BCS) to determine the impact of adjuvant radiotherapy (RT) on local recurrence (LR) according to risk stratification per molecular signature testing. Five studies including 3,478 women with DCIS who had been treated with BCS were included in this evaluation. A molecular assay was performed for each of the women included. The effect of BCS and RT versus with BCS alone on LR was analyzed. The analysis included both ipsilateral invasive breast events (InvBE) and total breast events (TotBE). Two molecular signature tests were used: the Oncotype Dx DCIS (prognostic of LR) and DCISionRT (prognostic of LR and predictive of RT benefit). In the high risk group, for DCISionRT, pooled hazard ratio of BCS + RT versus BCS was 0.39 (95% CI 0.20-0.77) for InvBE and 0.34 (95% CI 0.22-0.52) for TotBE. In the low-risk group, pooled hazard ratio of BCS + RT versus BCS was significant for TotBE at 0.62 (95% CI 0.39-0.99) but was not significant InvBE [HR = 0.58 (95% CI 0.25-1.32)]. The researchers maintain that molecular signature tests can discriminate high- and low-risk individuals; individuals at high-risk showed a significant benefit of RT for reduction of invasive and in situ LR and individuals at low-risk did not show benefit for prevention of

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recurrence of invasive BC. While molecular signatures may be a promising tool for balancing over- and under-treatment of DCIS, further understanding of the basis of invasive cancer is needed. The study was limited by the lack of data on breast-cancer specific mortality and individual data on recurrence-free survival. Further high quality evaluation, including studies focused on impact on mortality are required.

Hayes (2022) published a Molecular Test Assessment evaluating the use of the DCISionRT test to assist individuals with DCIS and their providers with decision-making regarding the use of BCS alone or BCS plus RT. Hayes found a lack of published evidence to support the use of the DCISionRT test. The 2023 update of this assessment identified only one newly published study that may be considered for addition to the report and an unlikely change in the current Hayes rating of D2. [Hayes, DCISionRT (Prelude Corp.), 2022, updated 2023].

Shah et al. (2021) documented the results of the PREDICT study; a prospective, multi-institutional observational registry designed to evaluate the clinical utility of testing with DCISionRT on clinical recommendations regarding RT for individuals who had undergone BCS for a diagnosis of DCIS. The study included 539 women over the age of 25 who had been treated with BCS for unilateral DCIS. All women were eligible to receive RT and received DCISionRT testing as part of the study. Prior to testing, 69% of all participants had received a recommendation of treatment with RT. After testing with DCISionRT, 46% of those that had previously received recommendation for RT had a change in recommendation to not receive RT. Conversely, for women who were not initially recommended to undergo RT, 35% had a change in recommendation for treatment to include RT. In summary, a change in RT treatment plan was made for 42% of women in the study, with a net reduction in overall RT recommendation of 20%. The elevated DS had the strongest association with an RT recommendation (odds ratio 43.4) compared to other factors such as age, grade, size, and margin status. The authors concluded that DCISionRT testing made a significant difference, including an absolute net decrease in RT recommendations overall in women with DCIS who had undergone BCS, and was the factor most strongly associated with RT recommendations compared with traditional measures used to drive treatment decisions. The authors also noted limitations to the study. One such limitation was the lack of patient or physician-reported outcomes regarding satisfaction or quality (pending at time of publication). In addition, data on recommendations for RT were only based on two points in time; pre-testing and post-testing. Finally, there is a lack of long-term clinical outcomes and data on subsequent resource utilization related to treatment decisions. These items are planned for further evaluation and assessment when longer follow-up data become available. This study was included in the 2022 Hayes DCISionRT (Prelude Corp.) Molecular Test Assessment.

Choosing the optimal treatment approach for individuals diagnosed with DCIS has been a significant challenge and a topic of active research. A major goal is to understand the ROR for DCIS. In a 2020 publication, Weinmann et al. [included in the systematic review and meta-analysis by Ouattara et al. (2023), discussed above, and the Hayes 2022 DCISionRT Molecular Test Assessment] described the results of their external prospective-retrospective clinical validation of DCISionRT, a 10-year recurrence/progression risk assessment test using monoclonal protein markers and clinicopathologic factors (age at diagnosis, palpability, tumor size and surgical margin status) for individuals with DCIS who had undergone BCS. The outcome of the DCISionRT test is called the decision score (DS). Study participants included 455 Kaiser Permanent Northwest members over the age of 25 diagnosed with DCIS and treated with BCS with or without radiotherapy from 1990 to 2007. Kaplan Meier analysis and Cox regression were used to measure the ability of the DS to predict outcomes beyond that of clinicopathology factors. The researchers found a positive association of the DS produced by DCISionRT with total breast event and invasive breast event risk after adjustment for radiotherapy in the Cox regression analysis. Kaplan-Meier analyses showed that elevated-risk DS scores showed more than twice the 10-year risk of total breast events compared to low-risk DS scores. The authors concluded that DS score from DCISionRT test was prognostic for risk of later breast events in this study group. Despite these promising results, the study had some noteworthy limitations. Most study participants with DCIS received adjuvant radiotherapy, so there were fewer BCS without radiotherapy participants in the study to analyze. Statistical power was more limited for assessment of DS associated with invasive BC because approximately half of the total breast events were invasive. In addition, some participants had received endocrine therapy, which may have impacted overall outcomes. While the study indicates elevated DS scores would suggest a preferential radiotherapy benefit, this study design did not assess radiotherapy benefit. In addition, some of the risk difference between radiation treated and nontreated cohorts might be related to the individuals' selection for treatment, since the study was not randomized or rule based. Further research is needed to provide more evidence to support routine DCISionRT testing.

Bremer et al. [2018, included in the systematic review and meta-analysis by Ouattara et al. (2023), discussed above] reported on the development and cross-validation of DCISionRT (PreludeDX). DCISionRT is a risk assessment test that uses a combination of molecular and clinicopathologic factors to generate a biological signature which calculates an individualized DS. The relationship between DS and 10-year risk of invasive breast cancer (IBC) or any ipsilateral breast event (IBE) was assessed in this study. Benefit of radiotherapy was evaluated as a function of DS, by risk group. Study population included 526 individuals diagnosed with a primary DCIS and treated with BCS, with or without radiotherapy,

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from two study sites. The study used archived tissue samples. Treatments for the study participants were neither randomized nor strictly rules based. The researchers found a significant association with IBC and IBE risk. In study participants who had been treated without RT, the DS identified a low group with 10-year IBC risk of 4% (7% IBE) and an elevated risk group with IBC risk of 15% (23% IBE). The elevated risk group received significant RT benefit in analysis of DS and RT by group. In a clinicopathologically low-risk-subset, 42% of participants were reclassified into the elevated risk group by using DS. When an interaction analysis of DS and RT was performed, participants whose DS was elevated had significant RT benefit over baseline. The authors concluded that DS appeared to be prognostic for risk and for predicting benefit of RT for individuals with DCIS status-post BCS and was able to identify a clinically meaningful low-risk group and an elevated 10-year risk group, whose members may receive significant benefit from RT over baseline. However, further clinical validations are required to provide more evidence on the capabilities, both prognostic and predictive, of the biological signature and DS.

Oncotype Dx DCIS

The Oncotype Dx DCIS assay (Genomic Health, Redwood City, CA) uses reverse transcription polymerase chain reaction (PCR) with DNA extracted from excised tumor tissue to assess expression levels of 12 genes. A Breast DS designed to represent the risk of BC recurrence within 10 years of original diagnosis (0 to 100) is then calculated for the individual.

Hayes conducted a Molecular Test Assessment evaluating use of the Oncotype DX Breast DCIS Score test to predict the risk of 10-year local or invasive cancer recurrence and create a baseline consideration of benefit from radiation therapy in women diagnosed with DCIS. The assessment revealed favorable but inadequate evidence supporting the use of the Oncotype DX Breast DCIS Score. Studies with diverse populations are needed to support the premise that the DCIS Score offers prediction for 10-year local recurrence and creates a baseline for consideration of the absolute benefit of treatment with radiation therapy. Additionally, evaluation of clinical outcomes when test results are used for risk assessment and medical management is needed [Hayes, Oncotype DX Breast DCIS Score (Genomic Health Inc.) 2018, updated 2022].

Clinical Practice Guidelines

American Society of Clinical Oncology (ASCO)

In 2022, Andre et al. updated ASCO recommendations regarding the appropriate use of biomarker assay results to inform decisions regarding adjuvant endocrine and chemotherapy in early-stage BC. Evidence for these recommendations was based on information from 24 applicable studies (14 RCTs and 10 prospective-retrospective). The recommendations include the following:

- Oncotype DX, MammaPrint, BCI, and EndoPredict may be used to guide adjuvant endocrine and chemotherapy in postmenopausal individuals or individuals over the age of 50 years with early-stage ER+, HER2- BC that is node-negative or with one to three positive nodes
- BCI and ProSigna may be used in postmenopausal individuals with node-negative ER+, HER2- BC
- For premenopausal individuals, Oncotype DX may be used with node-negative ER+, HER2- BC
- BCI may be offered to individuals with zero to three positive nodes who have undergone five years of endocrine therapy with no evidence of recurrence to aid with decision-making regarding extended endocrine therapy
- No assays are recommended for individuals with HER2+ or triple-negative BC

Evidence from ASCO's review indicates that premenopausal women with one to three positive nodes will show benefit from chemotherapy regardless of genomic assay results. No data supporting the use of genomic tests for informing adjuvant chemotherapy in in individuals with four or more positive nodes was identified. When access to genomic tests is not available, Ki67 in combinations with other parameters or an immunohistochemistry 4 score may be used to assist with decision-making regarding adjuvant therapy. ASCO further recommends the incorporation of factors such as disease stage, comorbidities and patient preference into decision-making.

In 2020, Hassett et al. published recommendations for managing male BC. These recommendations were the result of a review of 26 reports/observational studies by an ASCO Expert Panel which formed the base of evidence on which the recommendations were developed. The panel found that several of the management methods used for men with BC are predominantly the same as those used for women and include the following recommendations regarding molecular testing:

- Gene expression profiling should be used to guide adjuvant treatment decision-making
- Targeted therapy guided by HER2, programmed death ligand 1 (PD-L1), PIK3CA and germline BRCA mutation status may be used for treatment of metastatic/advanced male BC with the same indications and combinations that are routinely offered to women
- Males with BC should be offered genetic counseling and testing for germline mutations

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Andre et al. (2019) published an ASCO Clinical Practice Guideline Update for the use of biomarkers to guide adjuvant therapy for early-stage BC. The update was created by an expert panel that reviewed the results of TAILORx trial along with other published literature on the Oncotype DX assay to assess for evidence of clinical utility. The updated recommendations only refer to individuals with hormone receptor positive, HER2 not overexpressed, axillary N0 early BC and include the following:

- For individuals older than 50 years and whose tumors have Oncotype DX RS less than 26, and for individuals 50 years or younger whose tumors have Oncotype DX RS less than 16, there is little to no benefit from chemotherapy. Clinicians may offer endocrine therapy alone (Type: evidence based; benefits outweigh harms; Evidence quality: high; Strength of recommendation: strong)
- For individuals 50 years or younger with Oncotype DX RS of 16 to 25, clinicians may offer chemoendocrine therapy (Type: evidence based, benefits outweigh harms; Evidence quality: intermediate; Strength of recommendation: moderate)
- Individuals with Oncotype DX RS higher than 30 should be considered candidates for chemoendocrine therapy (Type: evidence based; benefits outweigh harms; Evidence quality: high; Strength of recommendation: strong)
- Based on Expert Panel consensus, oncologists may offer chemoendocrine therapy to individuals with Oncotype DX scores of 26 to 30 (Type: informal consensus; Evidence quality: insufficient; Strength of recommendation: moderate)

Krop et al. (2017) provided an update to the ASCO 2016 guidelines focusing only on MammaPrint. The updated recommendations state that if an individual has ER/PgR–positive, HER2-, N0 BC, the MammaPrint assay may be used in those with high clinical risk per MINDACT categorization. The test should be used to inform decisions on withholding adjuvant systemic chemotherapy due to its ability to identify a good prognosis population with potentially limited chemotherapy benefit. In addition, they recommend if an individual has ER/PgR–positive, HER2-, N+BC, the MammaPrint assay may be used. It should be used for individuals with N1and at high clinical risk per MINDACT categorization to inform decisions on withholding adjuvant systemic chemotherapy due to its ability to identify a good prognosis population with potentially limited chemotherapy benefit. However, such individuals should be informed that a benefit of chemotherapy cannot be excluded, particularly for individuals with greater than one involved lymph node.

European Society for Medical Oncology (ESMO)

Cardoso et al. (2019) described the updated ESMO Clinical Practice Guidelines for early BC. Gene expression profile tests were included in some of the recommendations including:

- Validated gene expression profiles may be used to gain additional prognostic and/or predictive information to complement pathology assessment and help in adjuvant chemotherapy decision making (I, A)
- In cases of uncertainty regarding indications for adjuvant chemotherapy (after consideration of all clinical and pathological factors), expression of uPA-PAI1 (I, A) or gene expression assays, such as MammaPrint (I, A), Oncotype DX (I, A), Prosigna, EndoPredict or BCI, can be used
- Expression of uPA-PAI1 or multigene panels, such as MammaPrint, Oncotype DX, EndoPredict, Prosigna or BCI, may be used in conjunction with all clinicopathological factors to guide systemic treatment decisions for individuals where these decisions are challenging, such as luminal B-like/HER2- and N0/N1 BC (I, A)

Note: Evidence Level I - Evidence from at least one large randomized, controlled trial of good methodological quality (low potential for bias) or meta-analyses of well-conducted randomized trials without heterogeneity; Grade of recommendation A - Strong evidence for efficacy with a substantial clinical benefit, strongly recommended.

National Comprehensive Cancer Network (NCCN) Clinical Guidelines

NCCN BC guidelines indicate that “gene expression assays provide prognostic and therapy-predictive information that complements tumor (T), node (N), distant metastasis (M) and biomarker information. Use of these assays is not required for staging.” The 21-gene assay (Oncotype Dx) is preferred by the NCCN Breast Cancer Panel since it has been clinically validated for the prognosis and prediction of chemotherapy benefit. While other gene expression assays can provide prognostic information, they do not necessarily predict chemotherapy benefit. NCCN notes that the BCI test is predictive of benefit of extended adjuvant endocrine therapy (NCCN Breast Cancer, v5.2023).

NCCN categorizes the primary gene expression assays for consideration of adjuvant systemic therapy in individuals with invasive BC as follows:

Assay	Predictive	Prognostic	NCCN Category of Preference	NCCN Category of Evidence and Consensus
Oncotype DX for pN0	Yes	Yes	Preferred	1

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Assay	Predictive	Prognostic	NCCN Category of Preference	NCCN Category of Evidence and Consensus
Oncotype DX for pN1 (1-3 positive nodes)	Yes	Yes	Postmenopausal: Preferred Premenopausal: Other	1
MammaPrint for pN0 and pN1 (1-3 positive nodes)	Not determined	Yes	Other	2A
Prosigna for pN0 and pN1 (1-3 positive nodes)	Not determined	Yes	Other	2A
EndoPredict for pN0 and pN1 (1-3 positive nodes)	Not determined	Yes	Other	2A
Breast Cancer Index (BCI)	Predictive of benefit of ext. adjuvant endocrine therapy	Yes	Other	2A

National Institute for Health and Care Excellence (NICE)

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

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

Lung Cancer

Liquid biopsy analysis using circulating tumor DNA (ctDNA) or cell-free DNA (cfDNA) is a developing technology that can be used as an alternative to tissue profiling in non-small cell lung cancer (NSCLC). In a systematic review and meta-analysis, Zaman et al. (2023) sought to assess the prognostic value of molecular profiling via ctDNA or cfDNA in NSCLC. Twenty-seven studies including 3,419 individuals were included in the analysis. Eleven studies including 1,359 participants reported on the association of baseline ctDNA with progression-free survival (PFS) and 16 studies including 1,659 participants reported on dynamic changes in ctDNA associated with PFS. The analysis revealed that individuals with negative baseline ctDNA trended towards improved PFS [pooled hazard ratio (pHR) = 1.35; 95% CI: 0.83-1.87; $p < 0.001$; $I2 = 96\%$)] when compared to ctDNA-positive patients. In addition, when early reduction/clearance of ctDNA levels occurred after treatment, individuals showed improved PFS (pHR = 2.71; 95% CI: 1.85-3.65; $I2 = 89.4\%$) in comparison with individuals showing no reduction/persistence in ctDNA levels. Only good and fair quality studies [based on assessment via the Newcastle-Ottawa Scale (NOS)] exhibited improvement in PFS (pHR = 1.95; 95% CI: 1.52-2.38 and pHR = 1.99; 95% CI: 1.09-2.89, respectively); this did not occur in poor quality studies included in the analysis. The authors note that this review and analysis revealed a high level of heterogeneity and publication bias, but despite these limitations, baseline negative ctDNA levels and an early reduction in ctDNA after therapy may be robust prognostic indicators for PFS and OS in individuals who undergo targeted therapies for advanced NSCLC. The authors recommend additional studies including serial ctDNA testing to further support clinical utility in the management of advanced NSCLC.

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 individuals with 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

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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 next-generation sequencing (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,*

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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 fluorescence in situ hybridization (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 1,007 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*, *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

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approach, most often using 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 does not 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

If in the initial diagnostic setting, the feasibility of timely tissue-based testing is uncertain, concurrent cfDNA testing may be helpful for biomarker identification for selection of therapy as long as negative results are considered per the limitations noted above. (NCCN Non-Small Cell Lung Cancer, v5.2023).

Prostate Cancer

Genomic Prostate Score Assay (Formerly Oncotype DX Genomic Prostate Score) Decipher, Prolaris, and Promark

In a 2023 systematic review, Spohn et al. explored the evidence on the use GCs for individuals treated with radiation therapy (RT) and conducted a survey of experts using the Delphi method to address the role of GC use in personalized treatments for the purposes of identifying areas of future clinical research. Initially, a total of 26 studies met inclusion criteria and were sent to a multidisciplinary, international team of experts for review. An updated literature search was performed during the peer-review process time period and an additional 5 studies were identified and sent to the reviewers, for a total of 31. Ongoing clinical trials were also screened and nine studies on GCs use with RT were identified and shared with the expert reviewers as well. There were two rounds of questions; 31 experts completed the first round and 30 completed the second round. When survey results showed ≥ 75% agreement, the question/response was considered relative and included in the qualitative synthesis. The majority of the studies (65%) focused on the Decipher test. The researchers found that the evidence for GCs as predictive biomarkers is primarily focused on the postoperative RT setting, although validation of GCs as prognostic markers in the definitive RT setting is emerging. The experts surveyed used GCs in individuals with extensively metastatic prostate cancer (PCa) (30%), in the postoperative setting (27%), and in newly diagnosed PCa (23%). Of the experts surveyed, 47% do not use GCs in their clinical practice, although the consensus of the experts was that GCs are indeed promising tools for risk-stratification in individuals with primary and oligo-/metastatic PCa in addition to existing classifications. The experts also felt that GCs have potential for use in guiding treatment decisions for RT-field definition and intensification/deintensification over various stages of disease. The study authors postulate that the outcome of this study confirms 1) the value of GCs and 2) the promising evidence that is emerging regarding the utility of GC with respect to RT. The authors recommend ongoing study of GCs as prognostic biomarkers and the predictive ability of GCs for optimization of RT and/or systemic therapy and await the results of prospective clinical trials focused on the role of GCs in the setting of RT which may help to validate the role of GCs for guiding personalized cancer treatment. Publications by Janes et al. (2023) and Feng et al. (2021), discussed below, and Marascio et al. (2020) and Berlin et al. (2019), previously discussed in this policy, were included in the Spohn et al. systematic review.

Participants enrolled in NRG Oncology/RTOG 01-26, a randomized phase 3 trial, comprised the population of an analysis by Spratt et al. (2023) investigating the performance of the 22-gene Decipher GC in individuals with intermediate-risk PCa. This study is the first validation of a biopsy-based GEC that evaluates both prognostic and predictive value using data from a randomized, phase 3 clinical trial of individuals with intermediate risk PCa. The NRG Oncology/RTOG 01-26 trial randomized these individuals to 70.2 Gy versus 79.2 Gy of radiation therapy with no androgen deprivation therapy. With NCI approval, biopsy slides from NRG Oncology/RTOG 01-26 were obtained and RNA was extracted from the highest-grade tumor foci to generate a locked 22-gene GC model. A total of 215 individual samples met quality control standards and were analyzed. The median follow up time was 12.8 years. The primary outcome for this ancillary study was progression of disease, using a composite of biochemical failure, local failure, distant metastases, PCa-specific mortality and use of salvage therapy. Using multivariable analysis, the 22-gene GC was independently prognostic for disease progression [subdistribution hazard ratio (sHR), 1.12; 95% confidence interval (CI), 1.00-1.26; p = .04], biochemical failure (sHR, 1.22; 95% CI, 1.10-1.37; p < .001), distant metastasis (sHR, 1.28; 95% CI, 1.06-1.55; p = .01), and prostate cancer-specific mortality (sHR, 1.45; 95% CI, 1.20-1.76; p < .001). In participants with GC low-risk results, ten-year distant metastasis was 4% compared with 16% in GC high-risk results. The authors contend that the 22-gene Decipher GC improves risk stratification and can help inform treatment decisions in individuals with intermediate-risk

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disease. A limitation of this study was the limited availability of sufficient quality tissue samples which impacted the power of the study and prohibited well-powered subset analyses.

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

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

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

In a 2021 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

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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. Publications by Marascio et al. (2020), Berlin et al. (2019), Kim et al. (2019), Klein et al. (2016), Glass et al. (2016), and Marrone et al. (2015), previously discussed in this policy, were included in this systematic review.

Feng et al. (included in the 2023 Spohn systematic review above) 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 (2021). 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

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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 multicenter 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 1,200 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

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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.

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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 < 10-20], 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

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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 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 1,064 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

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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 (Eastham et al.). 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)

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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.

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, v4.2023) 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 PSA persistence or 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:

<table>
<tr><th>Risk Group</th><th colspan="3">Clinical/Pathological Features</th></tr>
<tr><td>Very low</td><td colspan="3">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</td></tr>
<tr><td>Low</td><td colspan="3">Has all of the following, but does not qualify for very low-risk:
• cT1–cT2a
• Grade Group 1
• PSA < 10 ng/mL</td></tr>
<tr><td rowspan="2">Intermediate</td><td rowspan="2">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</td><td>Favorable intermediate</td><td>Has all of the following:
• 1 IRF
• Grade Group 1 or 2
• < 50% biopsy cores positive (e.g., < 6 of 12 cores)</td></tr>
<tr><td>Unfavorable intermediate</td><td>Has one or more of the following:
• 2 or 3 IRFs
• Grade Group 3
• ≥ 50% biopsy cores positive (e.g., ≥ 6 of 12 cores)</td></tr>
</table>

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Risk Group	Clinical/Pathological Features
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 ≥ 2 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 (NCCN Prostate Cancer Early Detection, v2.2023).

Thyroid Cancer/Indeterminate Thyroid Nodules

Kim et al. (2023) conducted a single-center RCT designed to determine the rate of delayed operation and false negative rate of the Afirma® Gene Sequencing Classifier (GSC) and ThyroSeq® v3 in patients with Bethesda III and IV thyroid nodules who underwent thyroid biopsy between August 2017 and November 2019. Of 176 indeterminate nodules with negative or benign molecular test results, 14 (8%) nodules underwent immediate resection, with no malignancies found on surgical pathology. Nonoperative management with active surveillance was pursued for 162 (92%) nodules with benign or negative test results. The median surveillance was 34 months (range 12-60 months), and 44 patients were lost to follow-up. Of 15 nodules resected during surveillance, one malignancy was found (overall false negative rate of 0.6%). This was a 2.7 cm minimally invasive Hürthle cell carcinoma that initially tested negative with ThyroSeq v3 and underwent delayed resection due to sonographic growth during surveillance. The authors concluded the majority of Bethesda III and IV thyroid nodules with negative or benign molecular test results are stable over three years of follow-up. Study limitations include short-term follow-up and randomization of patients to either Afirma GC or ThyroSeq v3 which did not allow for a comparison of both molecular tests in the same nodule. The authors suggest longer term studies to verify durability of benign/negative molecular test results and to identify the length of time patients need to remain under surveillance.

In 2022, Lee et al. conducted a systematic review and meta-analysis to appraise the diagnostic performance of second-generation molecular tests in diagnosing thyroid nodules with indeterminate fine-needle aspiration biopsy results. Included in the evaluation were 15 studies: seven Afirma Genomic Sequencing Classifier (GSC), six ThyroSeq v3, and two 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. Publications by Livhits et al. (2021) and Endo et al. (2019), previously discussed in this policy, and Steward et al. (2019), discussed below, 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 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%).

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BRAF and *ALK* fusions were 81% and 67%, respectively; the PPV 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 Xpression 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, but the specificity and PPV varied between studies due to the lack of Afirma benign nodules resected to assess test performance. The Hayes report also indicates that the GSC test had better specificity and PPV than the previous version of the test (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. An updated review states the current Hayes rating is unlikely to change from the previous annual rating [Hayes, Afirma Genomic Sequencing Classifier (Veracyte Inc.), 2021, updated 2023].

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 Genomic Classifier (GC) test indicates that there is a very low-quality body of evidence supporting the ability of ThyroSeq v3 to predict malignancy in Bethesda III and IV thyroid nodules. Although the test appears to have high sensitivity and NPV, true accuracy is uncertain because there is a lack of reference standard testing in the majority of samples, especially when the results are negative. In addition, there was insufficient follow-up documented for individuals with ThyroSeq v3 negative results. No studies reporting on the improvement of health outcomes related to the use of ThyroSeq v3 were identified. Overall, Hayes found insufficient evidence for use of the ThyroSeq v3 GC in preoperative assessment of indeterminate thyroid nodules to measure cancer probability or provide prognostic data for clinical management [Hayes, ThyroSeq v3 Genomic Classifier (University of Pittsburgh Medical Center and Sonic Healthcare USA), 2023].

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 precision 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

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28%, the 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%. The authors concluded that 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. The study, however, had limitations; study participants were not consecutively enrolled (they were chosen from a larger population undergoing testing), and approximately 20% of the original cohort was excluded due to no histological diagnosis. In addition, no ethnicity was reported and the study participants were from centers with significant clinical expertise and well-established thyroid nodule imaging, which limits the ability to generalize to larger populations of affected individuals, some of whom may be seen in general practices.

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.

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)

The ATA (Bible et al., 2021) developed a guideline for anaplastic thyroid cancer (ATC) which indicates that no genetic alterations found in ATC are specific for ATC. However, in specific situations, molecular testing may aid with histopathologic diagnosis, which remains the gold standard. Genomic profiling of tumor tissue alone is not sufficient for diagnosing ATC, but the results of this testing may be helpful in differential diagnosis.

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In a 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/PPARγ 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/PPARγ*) 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) published 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 Endocrinology/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 2023 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 may be

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useful for diagnosis of medullary thyroid cancer due to the difficulty of reaching a specific diagnosis with cytology in limited samples. Although past studies have shown that molecular diagnostics do not perform well for oncocytic carcinoma, formerly known as Hürthle cell neoplasms, modern genomic classifiers are promising with regard to these specimens. A requirement for the diagnosis of oncocytic carcinoma 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, but 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 as well, 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, v4.2023).

Melanoma

Cutaneous Melanoma

Several molecular tests designed to assess severity of disease and risk of recurrence/metastases and assist with clinical decision-making regarding the need for biopsy in cases of cutaneous melanoma have been developed. At this time, further studies supporting the accuracy and clinical utility of these tests are needed.

Bailey et al. (2023) conducted a registry study using data from the National Cancer Institutes (NCI) SEER Program to assess the effects of the DecisionDx-Melanoma 31-gene expression profile (31-GEP) test on survival outcomes in patients diagnosed with cutaneous malignant melanoma (CM). Patients with stage I-III CM that had a 31-GEP result between 2016 and 2018, were associated to data from 17 SEER registries (n = 4,687). The ability of the 31-GEP to stratify melanoma-specific survival (MSS) and overall survival (OS) were examined using Kaplan-Meier analysis and the log-rank test. The outcomes between 31-GEP tested patients were matched to those that did not receive the 31-GEP testing. Patients with a 31-GEP class 1A result had higher 3-year MSS and OS than patients with a class 1B/2A or class 2B result (MSS: 99.7% v. 97.1% v. 89.6%, $p < .001$; OS: 96.6% v. 90.2% v. 79.4%, $p < .001$). A class 2B result was an independent predictor of MSS (HR, 7.00; 95% CI, 2.70 to 18.00) and OS (HR, 2.39; 95% CI, 1.54 to 3.70). 31-GEP testing was associated with a 29% lower MSS mortality (HR, 0.71; 95% CI, 0.53 to 0.94) and 17% lower overall mortality (HR, 0.83; 95% CI, 0.70 to 0.99) relative to untested patients. While the clinical use of the test may help providers deliver more personalized clinical management decisions for patients with CM and identify their risk of dying, there were gaps and limitations. Study limitations/gaps included the following: mechanism of action related to better outcomes could not be identified, limited follow-up since the analysis was restricted to 2016-2018, and lack of data related comorbidities and specific treatments. Further robust studies are needed and/or ongoing collaboration with NCI/SEER to identify these gaps.

In a 2023 systematic review [including two Ferris studies (2017, 2018) that were previously discussed in this policy], Thomsen et al. attempted to determine the diagnostic accuracy of tape stripping (TS) for detecting cutaneous malignant melanoma (MM) in suspicious pigmented skin lesions. Ten studies were included. Sensitivity ranged from 68.8% [95% confidence interval (CI) 51.5, 82.1] to 100% (95% CI 91.0, 100). Specificity ranged from 69.1% (95% CI 63.8, 74.0) to 100% (95% CI 78.5, 100). A pooled analysis of five studies testing the RNA markers LINC00518 and PRAME found a sensitivity of 86.9% (95% CI 81.7, 90.8) and a specificity of 82.4% (95% CI 80.8, 83.9). This review had several limitations that included: a lack of information related to the characteristics of the study population, lack of histological examination for TS lesions, potential risk of overlap of patients and no randomized controlled trials that would determine the difference between TS and no-TS in terms of impact to prognosis. The authors indicate that in the studies evaluated, TS was used as a supplement to well-established diagnostic methods such as visual inspection, dermoscopy and clinical photography. Since the overall quality of the studies was low, the reliability of sensitivity and specificity is questionable. Additional high-quality studies are needed to confirm the diagnostic accuracy of PLA testing in cutaneous malignant melanoma.
In their Molecular Test Assessment on the DecisionDX-Melanoma 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 on 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, updated March 2023).

Ludzik et al. (2022) conducted a retrospective case control study evaluating the use of the pigmented lesion assay (PLA). PLA is used to non-invasively detect the presence of three genes associated with melanoma (*LINC00518, PRAME*, and

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TERT) using adhesive patch testing. Patch testing has the potential to reduce the number of unnecessary biopsies. Currently, studies that evaluate the clinical usefulness of this test outside a research setting are lacking. The author's aim in this study was to identify possible barriers that reduce the clinical utility of PLA testing by dermatologists. Data was collected from April 2021 to April 2022, from an academic tertiary-level center evaluating a total of 472 lesions. Genetic analysis failure for *LINC00518* and *PRAME* occurred in 59 or 12.5% of cases and in 300 lesions or 70.9% of cases for *TERT*. In 38.5% of cases, PLA results were discrepant with histopathology. The additional time associated with PLA use independent from the patient's visit was 10-25 minutes. The authors note that this novel non-invasive PLA test for melanoma using an adhesive tape-stripping techniques and gene expression profiling may be a promising technique to reduce unnecessary biopsies and optimize the triage of pigmented lesions. Yet, studies evaluating the clinical value, and possible limitations of these tests in a real-world setting are limited. With the considerable number of discrepancies between PLA test results and histopathology and the number of non-actionable results, the use of this testing remains limited. Additional robust studies are needed to confirm the clinical utility of this test and prevent possible mismanagement of lesions associated with melanoma.

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

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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.

A Molecular Test Assessment by Hayes (2019, updated 2022) focused on the Pigmented Lesion Assay (PLA) (DermTech), a gene expression test that is designed to help rule out melanoma and assist with decision-making regarding the need for biopsy. The assessment indicates that the initial evidence on the PLA test suggests that the use of PLA test results could inform clinical decision-making with respect to surgical biopsy, thereby reducing the number of benign lesions that undergo biopsy in individuals 18 years or older. However, published studies do not address full follow-up of individuals with negative results and most studies were retrospective or simulation design. Additional study is needed to establish whether the test performance is equivalent or superior to current standard of care methods [Hayes, Pigmented Lesion Assay (DermTech), 2019, updated 2022].

Hayes published a Molecular Test Assessment on the myPath Melanoma gene expression test as well. 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 (previously Myriad Genetics test sold to Castle Biosciences in 2021) 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. 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 8x60K 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

Miguez et al. (2023) conducted a retrospective analysis to assess and validate the prognostic value of gene expression profile (GEP) testing in patients with uveal melanoma. There have not been any studies thus far that have predicted metastasis by including tumor size. The authors wanted to determine the prognostic value of combining tumor size with the GEP classification to predict metastases. The results included 337 individuals from three different institutions, eighty-seven demonstrated metastases. The mean follow-up time was 37.2 [standard deviation (SD), 40.2] months for patients with metastases and 55.0 (SD, 49.3) months for those without metastases. Tumors of larger thickness and GEP class 2 (vs. class 1) were associated significantly with increased risk of metastasis. Tumor thickness showed better prognostic usefulness than GEP classification (Wald statistic, 40.7 and 24.2, respectively). Class 2 tumors with a thickness of 7.0 mm or more were associated with increased risk of metastasis than tumors with a thickness of < 7.0 mm [hazard ratio (HR), 3.23; 95% confidence interval (CI), 1.61-6.51], whereas class 1 tumors with a thickness of 9.0 mm or more were

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associated with increased risk of metastasis than tumors with a thickness of < 9.0 mm (HR, 2.07; 95% CI, 0.86-4.99). No difference in metastasis-free survival (MFS) was found between patients with class 1A tumors compared with those with class 1B tumors (p = 0.8). Patients with class 2 tumors showed an observed 5-year MFS of 47.5% (95% CI, 36.0%-62.8%). Study limitations included its retrospective design, the patients were from three different institutions and lastly the tumor size and biopsy techniques varied likely varied among providers. Despite the limitations, the authors indicated that tumor size was the most significant predictor of metastasis and it also provided additional prognostic value independent of GEP classification.

Singh et al. (2022) conducted a retrospective 10-year cohort study to assess the accuracy of the predicted 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 discussed 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.

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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 × 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 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 (v3.2023) indicate that for diagnostic testing there is agreement that any ancillary testing (e.g., CGH, FISH, GEP, SNP arrays, NGS) to differentiate malignant from benign melanocytic neoplasms should be used as an adjunct to clinical and expert dermatopathological examination and that it should be interpreted within the context of their findings.

The guideline further states the following:

- Evidence supporting the incorporation of current GEP tests into melanoma care is currently lacking
- 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
- New and existing GEP tests and other molecular techniques such as ctDNA tests should be compared in prospective studies to evaluate their clinical utility, including multivariable sentinel lymph node biopsy (SLNB) risk prediction models
- Pre-diagnostic noninvasive patch testing may be helpful to guide biopsy decisions for melanocytic neoplasms that are dermoscopically and clinically suspicious for melanoma

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, v1.2023).

Cancers of Unknown Primary (CUP)

Molecular tests intended to guide site-specific treatments in individuals with CUP have been developed. To date, peer-reviewed evidence supporting the use of these tests is insufficient. More high-quality studies addressing accuracy of these tests and data indicating whether they lead to improved outcomes is required.

Ding et al. (2022) conducted a systematic review and meta-analysis of studies investigating the efficacy of site-specific therapy guided by molecular profiling compared to empiric therapy for patients with cancer of unknown primary (CUP).

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GEP was used to identify the tissue of origin in this study. 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% 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). Additionally, when 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 individuals with CUP with more 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.

A study by Wang et al. (2023) sought to evaluate the use of rapid NGS to help identify CUP and associated therapeutic biomarkers that could be employed to guide site-specific therapies. Forty solid tumor samples were evaluated based on initial diagnosis of CUP and NGS testing was performed using the Oncomine Precision Assay GX. Genomic information was used to support a site-specific cancer diagnosis for 6 participants (15%). The most common genetic variations found were KRAS (35%), CDKN2A (15%), TP53 (15%), and ERBB2 (12%). Twenty-three individuals had results identifying actionable molecular-targeted treatments (variations in BRAF, CDKN2A, ERBB2, FGFR2, IDH1, and KRAS). An immunotherapy-sensitizing MMR deficiency was detected in one individual. The authors assert that this study supports the integration of rapid NGS into care for individuals diagnosed with CUP and the viability of using genomic profiling along with diagnostic histopathology and immunohistochemistry for these individuals. They recommend further study including the incorporation of diagnostic algorithms which include genomic profiling to better identify CUP. This study was limited by its retrospective design, small population, and analysis performed in a single institution only. In addition, a relatively small testing panel was used, which may not have captured some genome-wide biomarkers, and no survival or outcome data were evaluated.

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 NGS, 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

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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 is needed. A 2022 update to the original 2018 assessment found no newly published studies meeting inclusion criteria for the Hayes report [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.

Clinical Practice Guidelines

European Society for Medical Oncology (ESMO)

A Clinical Practice Guideline addressing CUP was published by Krämer et al. in 2023. The authors state that pan-cancer NGS can be used in CUP, however, randomized trials assessing the clinical utility of such tests are not yet completed. To date, two randomized trials have failed to demonstrate that GEP-based site-specific therapy is superior to standard empiric therapy. Thus, no recommendation addressing the use of GEP for site-directed therapy in CUP is provided.

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

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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}), v1.2024].

Colorectal Cancer (CRC)

Current evidence addressing the use of molecular testing for predicting risk of CRC recurrence or for CRC screening purposes is insufficient. Additional high-quality studies supporting clinical utility are needed.

In 2023, Rokavec and associates sought to identify and validate a prognostic mRNA expression signature for the stratification of individuals with stage II CRC according to their risk for relapse. From 792 primary stage II CRCs, publicly available mRNA expression profiling data were analyzed to find genes consistently associated with relapse-free survival (RFS). Next, the gene expression signature was validated using NanoString technology and computationally refined on primary colorectal samples from 205 individuals with stage II CRC. Finally, validation of the refined signature was carried out in two independent, publicly available training cohorts comprising 166 individuals with stage II CRC. A 61-gene signature was identified and determined to be highly significantly associated with RFS [HR = 37.08, p = $2.68*10^{-106}$, sensitivity = 89.29%, specificity = 89.61%, and area under the curve (AUC) = 0.937]. Experimental validation and refinement then identified a 15-gene signature that strongly predicted relapse in three separate cohorts: an in-house cohort (HR = 20.4, p = $8.73*10^{-23}$, sensitivity = 90.32%, specificity = 80.99%, AUC = 0.812), publicly available cohort GSE161158 (HR = 5.81, p = $3.57*10^{-4}$, sensitivity = 64.29%, specificity = 81.67%, AUC = 0.796), and publicly available cohort GSE26906 (HR = 7.698, p = $7.26*10^{-8}$, sensitivity = 61.54%, specificity = 78.33%, AUC = 0.752). Pooled cohort values showed that the 15-gene signature test (HR = 4.72, p = $7.76*10^{-25}$, sensitivity = 75%, specificity = 67.44%, AUC = 0.784) was superior to the Oncotype DX colon 7-gene signature test (HR = 2.698, p = 6.3*10-8, sensitivity = 62.16%, specificity = 55.5%, AUC = 0.633), which is currently the most widely used signature for prognostication of stage II colon cancer. The authors assert that they were able to identify and validate a new 15-gene expression signature for prognostication and stratification of individuals with stage II CRC which performed better in the evaluated validation cohorts than currently used clinico-pathologic biomarkers and signatures for stage II colon cancer prognostication. They speculate that this 15-gene expression signature has the potential to improve prognostication and therapy decisions for individuals diagnosed with stage II colon cancer. Further evaluation of the 15-gene signature in additional cohorts is recommended, including a combination of signature analysis and clinico-pathologic parameters, which may improve prognostic sensitivity and specificity. In addition, assessment of the signature and predictive value related to chemotherapy benefit in prospective, randomized controlled studies is required.

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

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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 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.

The ColonSentry test uses quantitative real-time PCR to measure RNA transcript expression of 7 genes using a blood sample. The results are expressed as a ColonSentry score predicting an individual’s risk of CRC related to risk in an average population. Hayes performed a Molecular Test Assessment addressing this technology. Hayes found insufficient evidence to support use of the ColonSentry test for predicting CRC risk, citing limited studies and data and significant limitations in the evidence that does exist. A 2023 update notes no change in the current Hayes Rating of D2. [Hayes, ColonSentry (Stage Zero Life Sciences), 2020, updated 2023].

In a 2018 (updated 2022) Molecular Test Assessment, Hayes found insufficient evidence to support the use of the Oncotype Dx Colon Recurrence Score test. Overall, a very low-quality body of evidence exists for the use of this test in both stage II, mismatch repair proficient colon cancer and stage IIIA/B colon cancer. The most recent update of this assessment in 2022 indicates no anticipated change in the current Hayes Rating of D2. [Hayes, Oncotype DX Colon Recurrence Score test (Genomic Health Inc.), 2018, updated 2022].

Zhang et al. (2017a) retrospectively reviewed the prognostic role of caudal-related homeobox transcription factor 2 (CDX2) expression in patients with stage 1 and stage III metastatic 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. 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.

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Yamanaka et al. (2016, included in the Hayes 2018 Molecular Test Assessment) evaluated the 12-gene Recurrence Score assay (Oncotype Dx Colon Recurrence Score) 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.

In a 2014 evaluation of available data, Heichman reviewed the work of Han et al. (2008) and Marshall et al. (2010, included in the 2020 ColonSentry Hayes Molecular Assessment) that explored the clinical utility of the ColonSentry test and reported that in a case-controlled study of 202 CRC patients and 208 matched healthy controls, a specificity of 70% for distinguishing cancer from healthy controls, and a sensitivity of 72% for identifying CRC was found. Larger, prospective studies are needed to further confirm the performance of this test.

Clinical Practice Guidelines

American Society of Clinical Oncology (ASCO)

In an update to their guideline addressing adjuvant therapy for stage II colon cancer, ASCO (Baxter et al., 2022) notes that their expert panel recognizes the development of tumor-based profiling tools that are designed to provide predictive/prognostic information which can potentially be used in treatment decision-making, but states that these types of tests are not yet ready for routine use. Further evidence of their effectiveness is needed before ASCO will endorse the use of these tools.

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 recommend universal MMR or MSI testing for any individual with a personal history of colon or rectal cancer to 1) identify individuals with Lynch syndrome, 2) to assist with decision-making regarding use of immunotherapy for individuals with metastatic disease and 3) to inform clinical decisions for individuals with stage II disease. The guidelines summarize current data on multigene assays, Immunoscore testing and ctDNA, but the NCCN panel is uncertain regarding the value these tests add, noting insufficient data to recommend use of multigene test panels, Immunoscore or post-surgical ctDNA tests to either estimate risk of recurrence or make determinations regarding adjuvant therapy cancer. The panel encourages clinical trial enrollment to generate further data on these tests. Regarding the use of biomarker testing determining appropriate targeted therapies for treatment of advanced or metastatic CRC, the panel recommends determination of tumor gene status for *KRAS/NRAS* and *BRAF* mutations, as well as *HER2* amplifications and MSI/MMR status (if not previously done). Such testing may be performed for individual genes or as part of an NGS panel. NCCN makes no specific recommendations regarding methodology. (NCCN Colon cancer, v4.2023).

Pancreatic Cancer and Ampullary Adenocarcinoma

There is currently insufficient evidence to support the use of molecular testing for risk assessment or diagnosis of pancreatic cancer. Additional large, high-quality studies are required to evaluate the clinical validity and utility of this technology.

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In 2023, Paniccia and colleagues prospectively investigated the use of NGS of pancreatic cyst fluid in a real-time, multi-institutional group of individuals with pancreatic cysts. A total of 1,887 specimens from 1,832 individuals were tested with the 22-gene PancreaSeq NGS panel. Follow up data was available for 66% (1,216) of participants. Of 251 (21%) individuals with surgical pathology available, mitogen-activated protein kinase/*GNAS* mutations had 90% sensitivity and 100% specificity for a mucinous cyst (PPV, 100%; NPV, 77%). When low-level variants were excluded, the combination of mitogen-activated protein kinase/*GNAS* and *TP53/SMAD4/CTNNB1*/mammalian target of rapamycin alterations had 88% sensitivity and 98% specificity for advanced neoplasia (PPV, 97%; NPV, 93%). With inclusion of cytopathologic evaluation along with PancreaSeq testing, sensitivity improved to 93% and high specificity of 95% (PPV, 92%; NPV, 95%) was preserved. Per the authors, lesser diagnostic performance is found when other methodologies or current pancreatic cyst guidelines (e.g., American Gastroenterology Association and International Association of Pancreatology/Fukuoka guidelines) are used. Out of 965 individuals who did not undergo surgery, none developed malignancy. Postoperative testing with Oncomine found mucinous cysts with *BRAF* fusions and *ERBB2* amplification and advanced neoplasia with CDKN2A alterations. The authors concluded that these results highlight the clinical utility of targeted NGS due to its high sensitivity and high specificity in the diagnosis of mucinous cysts and the detection of advanced neoplasia within a mucinous cyst. This study also expands the number of genomic alterations that are found not only in mucinous cysts but serous cystadenomas and cystic pancreatic neuroendocrine tumors. Although more high-quality studies are required, the data reported from this investigation adds to the existing support for integrating targeted NGS testing into evidence-based pancreatic cyst guidelines. Identified limitations include limited availability of surgical pathology for participants (14%) which represents surgical selection bias, testing selection bias (only pancreatic cyst fluid specimens that were satisfactory for targeted NGS testing were used), and limited follow-up period.

In a prospective, single-arm pilot study, Iwaya et al. (2023) analyzed viability and potential clinical utility of NGS using liquid-based cytology (LBC) samples obtained from endoscopic, ultrasound-guided fine-needle biopsy (EUS-FNB) performed on individuals with pancreatic cancer. Enrolled were 33 individuals with pancreatic cancer who underwent EUS-FNB; of these, samples from 31 individuals were included for DNA extraction/NGS and 30 of these (96.8%) had a sufficient quantity of DNA for analysis. The results of the study showed an overall success rate of 86.7% (n = 26) for use of formalin-fixed paraffin-embedded (FFPE), LBC, or frozen samples. When results were stratified using a variant allele frequency (VAF) > 10% tumor burden, the NGS success rate was 76.7% (n = 23) in FFPE, 83.3% (n = 25) in LBC, and 76.7% (n = 23) in frozen samples. Rates of detection for the primary gene variations were as follows: 86.7% for *KRAS*, 73.3% for *TP53*, 66.7% for *CDKN2A*, 36.7% for *SMAD4*, and 16.7% for *ARID1A*. The highest median value of VAF (23.5%) for *KRAS* and *TP53* was found with LBC. In this study, pancreatic cancer gene variant analysis via NGS was performed effectively using LBC in comparison with FFPE and frozen samples. The authors concluded that EUS-FNB samples are able to provide sufficient amounts of high-quality DNA for NGS analysis (when relatively small gene panels are used). Use of LBC specimens for NGS testing may be an option for genetic testing as a diagnostic or therapeutic approach for individuals with pancreatic cancer. Limitations of this study include the small sample size and inclusion of a single-center only, using a small number of experienced endoscopists. In addition, the individuals in this study were diagnosed by imaging only and no final pathology was performed on resected specimens. The gene panel used did not include *GNAS, VHL* or *RNF43*; as such, the possibility of intraductal papillary mucinous neoplasm (IPMN)-derived pancreatic cancer associated with these genes could not be ruled out.

Rift et al. (2023) evaluated the feasibility and diagnostic accuracy of molecular analysis and subtyping of pancreatic cystic lesions (PCLs) using EUS-guided “through the needle biopsy” (TTNB) sampling in a prospective study. In total, 101 individuals with PCLs > 15 mm in the largest cross-section were included. NGS was used to analyze the EUS-guided TTNB samples for point mutations in tumor suppressors and oncogenes with a 51-gene customized hotspot panel. Histologic diagnosis was used to calculate sensitivity and specificity. A total of 91 participants had residual TTNB samples available for NGS after initial microscopic analysis of the specimens had been performed. Forty-nine of these revealed mutations, most often in *KRAS* and *GNAS*. This indicated an excess frequency of IPMNs in the study population. Sensitivity of 83.7% (95% CI, 70.3-92.7) and specificity of 81.8% (95% CI, 48.2-97.7) were established for the diagnosis of a mucinous cyst and sensitivity of 87.2% (95% CI, 74.2-95.2) and specificity of 84.6% (95% CI, 54.5-98.1) were demonstrated for the diagnosis of an IPMN. The authors concluded that molecular testing performed on TTNB samples yielded high sensitivity and specificity for the diagnosis of mucinous cysts and IPMN. Although TTNB has a risk of adverse effects of approximately 9.9% (which must be carefully considered for each individual’s clinical situation), the use of TTNB specimens is a solid alternative to use of cyst fluid for combined molecular and histologic diagnosis of PCLs. The study was limited by its single-center design and small sample size. In addition, the cohort included mostly low-grade lesions with a majority of IPMNs and a limited surgical cohort. Lastly, no cyst fluid was obtained for NGS analysis and comparison. Further studies focused on characterizing the subgroup of individuals with pancreatic cancer that would derive the greatest benefit from EUS-guided TTNB samples are recommended.

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

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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. In the 2023 annual review, 29 new abstracts were published; however, none met the inclusion criteria set out in the 2022 report. There has been no change in the current rating of D2 and no new application of the technology for the test [Hayes, PancraGEN (Interpace Diagnostics), 2022, updated 2023].

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 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.

Singhi et al. (2018) studied the clinical validity of using pre-operative pancreatic cyst fluid (PCF) for 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 pancreatic cysts. In addition, the combination of *TP53/PIK3CA/PTEN* alterations is a useful preoperative marker for advanced cancer.

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 College of Gastroenterology (ACG)

Elta et al. (2018) created clinical guidelines for the diagnosis and management of pancreatic cysts. The recommendation regarding molecular markers states: “Molecular markers can help identify IPMNs or MCNs. Their use may be considered in cases in which the diagnosis is unclear, and the results are likely to change management.” (Conditional recommendation, very low quality of evidence).

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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 provisional opinion, ASCO notes that despite considerable effort, no biomarkers obtained through non-invasive means (e.g., blood, stool, urine) have been proven effective for early identification of pancreatic cancer in individuals with no symptoms. In addition, there is no evidence supporting clinical utility or validity for the use of ctDNA for pancreatic cancer screening outside the context of clinical trials. ASCO advises that thorough testing and validation of possible biomarkers that could be used in high-risk individuals is needed. (Stoffel et al., 2019)

National Comprehensive Cancer Network (NCCN)

NCCN Pancreatic Adenocarcinoma guidelines include a footnote recommending genetic testing for inherited mutations for individuals with pancreatic cancer and the use of tumor/somatic molecular profiling in cases of metastatic or locally advanced disease when an individual is a candidate for anti-cancer therapy. The use of molecular testing for diagnostics and risk assessment of pancreatic cyst fluid is not addressed. (NCCN Pancreatic Adenocarcinoma, v2.2023).

Other Molecular Oncology Testing for Solid Tumor Cancers

Multi-Cancer Detection Tests [e.g., Galleri (Grail, Inc.)]

Multi-cancer detection (MCD) tests measure biological markers that cancer cells shed in body fluids. Current MCD assays are designed to find and measure the amount of a given substance in a sample (e.g., blood). These tests can check for many different types of cancer stemming from various organs. Currently, MCD tests are being studied in randomized controlled trials to determine the impact of this screening on occurrence of late-stage cancers and mortality. At present, there are no professional medical societies that have issued recommendations on the use of MCD tests for cancer screening (NCI, 2023) and published evidence does not support the use of MCD tests for screening for any type of cancer.

In a recent systematic review (2023), LeeVan & Pinsky evaluated the ability of cell-free-nucleic acid-based MCD tests to predict cancer status. Twenty relevant publications met all inclusion criteria and were evaluated in this review. Most of the included studies reported specificity along with overall sensitivity and many of the studies also reported sensitivity by stage/cancer type. Taken as a whole, the studies in this review reported specificities of 95% or higher and a median sensitivity of 73%. The authors note that the majority of cases of cancer in the studies reviewed were evaluated with MCD tests at diagnosis, which may lead to overestimates of test sensitivity when compared to samples for individuals who are asymptomatic. It was also noted that sensitivity varied by cancer type and typically increased with cancer stage. Ultimately, the researchers recognize the lack of published evidence supporting clinical validity (and clinical utility) of cell-free nucleic acid-based MCD testing and recommend further high-quality studies investigating MCD assay use in populations of asymptomatic individuals, which is the intended-use population for MCD testing. This systematic review includes publications by Klein et al. (2021) and Liu et al. (2020), discussed below.

Schrag et al. (2023) published the results of PATHFINDER, a study funded by GRAIL which examined the feasibility of using MCD for cancer screening. PATHFINDER was a prospective cohort study that was performed in primary care and oncology outpatient centers that were part of seven different United States health networks. The blood of adults aged 50 years or greater was collected and cfDNA was evaluated. If a methylation signature suggesting a cancer diagnosis was found, the predicted cancer origin was used to guide diagnostic assessment. The results were returned to the physicians overseeing each participants care. The chief outcome of this study was the time to further diagnostic testing and the extent of the testing performed that would confirm the presence or absence of cancer. A total of 6,621 individuals had analyzable results and participated in the study. Of these, 1.4% (n = 92) had results which showed detection of a cancer signal. Of the 92 individuals with results showing detection of a cancer signal, 35 (38%) were subsequently diagnosed with cancer (true positives) and 57 (62%) were not diagnosed with cancer (false positives). Diagnostic resolution was achieved in a median of 79 days [interquartile range (IQR) 37-219]: 57 days (33-143) in true-positive and 162 days (44-248) in false-positive participants. Two participants who began diagnostic evaluations prior to receiving MCD results were excluded. The majority of participants had both imaging [30 (91%) of 33 with true-positive results and 53 (93%) of 57 with false-positive results] and laboratory testing [26 (79%) of 33 with true-positive results and 50 (88%) of 57 with false-positive results] and participants with false-positive results had fewer procedures [17 (30%) of 57] than true positive results [27 (82%) of 33]. Surgery was performed in only four participants (one false-positive and three true-positives). The authors assert that this study affords early substantiation to the feasibility of using a single blood test to screen for multiple cancer types. They recognize the need for further large studies demonstrating clinical utility and assessing the impact of

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MCD testing on cancer mortality. Several studies evaluating updated and refined versions of the MCD test originally used in PATHFINDER are in progress at this time.

Another recent study funded by GRAIL assessed the performance of MCD testing in symptomatic individuals who were referred to for specialty evaluation from primary care. Nicholson et al. (2023) conducted a multicenter prospective observational study (SYMPLIFY) in England and Wales. Participants were 18 years of age or older and had been referred from primary care with symptoms that were either non-specific or potentially related to gynecological, lung, or gastrointestinal cancers. A sample of blood was obtained from each participant when they presented for further investigation of their symptoms. A total of 5,461 individuals were included in the final cohort after all exclusionary criteria had been applied (e.g., previous malignancy, cytotoxic or demethylating agents, participation in another trial of a GRAIL MCD test, test errors, lack of final diagnosis, participant withdrawal). Participants were tracked until a diagnosis was reached or for a maximum of nine months. MCD was performed on cfDNA and blinded to clinical outcome. Finally, predictions from the MCD test were compared to the diagnosis obtained via standard processes to determine primary outcomes including overall PPV and NPV, sensitivity and specificity. Final outcomes were measured only in participants who had both a valid MCD test and diagnostic resolution. A total of 368 individuals (6.7%) were found to have a cancer diagnosis and 5,093 (93.3%) did not have a cancer diagnosis. MCD testing identified cancer signals in 323 cases; 244 of those cases were ultimately diagnosed with cancer, indicating a PPV of 75.5% (95% CI 70·5-80.1), NPV of 97.6% (97.1-98.0), sensitivity of 66.3% (61.2-71.1), and specificity of 98.4% (98.1-98.8). The researchers found that sensitivity increased with age and cancer stage [24.2% (95% CI 16.0-34.1) in stage I to 95.3% (88.59-8.7) in stage IV]. When an individual had cancer and a cancer signal was detected by MCD testing, the MCD test's prediction of site of origin was accurate in 85.2% (95% CI 79.8-89.3) of cases. Individuals with symptoms indicating a potential upper gastrointestinal cancer were found to have the highest sensitivity and NPV for the MCD test at 80.4% (95% CI 66.1-90.6) and 99.1% (98.2-99.6), respectively. The authors assert that this study was the first large-scale prospective evaluation of an MCD in a symptomatic population, and its results indicate that the use of MCD testing to assist clinical providers with decision-making regarding the urgency of follow-up and route of referral from primary care is viable. In addition, they feel that data from this study may be used as a foundation for further prospective study on individuals who present to primary care clinics with non-specific symptoms. Further study is recommended to evaluate the impact of MCD testing on use of resources, clinical decision-making and clinical outcomes.

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

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researchers indicate that cfDNA sequencing using informative methylation patterns warrants further evaluation in prospective, population-level studies.

NavDx®

NavDx is a blood test that is meant to detect and measure tumor tissue-modified viral (TTMV) human papillomavirus (HPV) DNA in individuals diagnosed with an HPV-related cancer to confirm tumor HPV genotype, evaluate current treatment response, identify MRD after treatment and potentially detect cancer recurrence earlier than standard follow up surveillance (Naveris, 2023). At present, there is insufficient evidence to support the use of NavDx for use in individuals with HPV-related cancers.

Hayes published a Molecular Test Assessment in 2023 addressing the use of NavDx for the detection and measurement of circulating TTMV-HPV DNA in HPV-related cancer. The assessment found that while multiple studies are currently ongoing, there is presently insufficient evidence to support the use of NavDx for evaluation or management of HPV-related cancers [Hayes, NavDx (Naveris), 2023].

In a 2023 publication, Ferrandino et al. reported on the accuracy of TTMV-HPV DNA testing via liquid biopsy for the diagnosis and monitoring of individuals with HPV-associated oropharyngeal squamous cell carcinoma (OPSCC). In this retrospective, observational cohort study including 399 participants, 163 were in the diagnostic cohort and 290 were in the surveillance cohort. In the diagnostic cohort, 152/163 participants had HPV-associated OPOSCC and 11/163 had HPV-negative OPSCC. For this group, sensitivity of TTMV-HPV DNA in pretreatment diagnosis was 91.5% [95% CI, 85.8%-95.4% (139 of 152 tests)], and specificity was 100% [95% CI, 71.5%-100% (11 of 11 tests)]. In the surveillance cohort, 593 tests were conducted in the 290 participants. Molecularly confirmed pathological recurrences occurred in 23 individuals. The TTMV-HPV DNA test exhibited a sensitivity of 88.4% [95% CI, 74.9%-96.1% (38 of 43 tests)] and specificity of 100% [95% CI, 99.3%-100% (548 of 548 tests)] in identifying recurrences. The PPV was 100% [95% CI, 90.7%-100% (38 of 38 tests)], and the NPV was 99.1% [95% CI, 97.9%-99.7% (548 of 553 tests)]. Median time from positive TTMV-HPV DNA test to pathologic confirmation was 47 (0-507) days. The authors concluded that this cohort study revealed a 100% specificity of the TTMV-HPV DNA assay when used in a clinical setting for both diagnosis and surveillance. Sensitivity, however, was 91.5% for the diagnosis cohort and 88.4% for the surveillance cohort, indicating false negatives in nearly 1 in 10 negative results. Further research in high-quality studies is needed to validate the performance of this assay, after which appropriate use of the assay and clinical usefulness will also need to be established.

Berger et al. (2022) evaluated circulating TTMV-HPV DNA in a population of individuals undergoing posttreatment surveillance for OPSCC in a retrospective case series. A total of 1,076 individuals from 108 sites in the United States were included in the evaluation. All individuals were at least 3 months posttreatment for HPV-driven OPSCC and had undergone at least one TTMV-HPV DNA test during their surveillance period. The results of the TTMV-HPV DNA test were compared with clinical evaluations. In 80 of the 1,076 participants (7.4%), circulating TTMV-HPV DNA was positive. At the time of the first positive surveillance test, 26% (21 of 80) had known recurrence of disease while 74% (59 of 80) were not known to have recurrence. Fifty-five of the 59 individuals not known to have recurrence (93%) were subsequently confirmed to have a recurrence. Two additional participants hade clinically suspicious lesions and two had no evidence of recurrence. The authors state that to date, the overall PPV of TTMV-HPV DNA testing for recurrent disease is 95% (n = 76/80) and the point-in-time NPV is 95% (n = 1,198/1,256). They further indicate that their findings underscore the promise of circulating TTMV-HPV DNA use in the routine care of individuals with OPSCC. The study was limited by its retrospective design and the use of a single surveillance test during the study period when 55% of participants had finished therapy greater than 12 months prior. Additional high-quality studies confirming accuracy of the test and defining the role of TTMV-HPV testing in surveillance setting are required, as well as data substantiating clinical utility.

Signatera™

Signatera is an individualized molecular test that detects circulating tumor DNA (ctDNA) in the blood of individuals who have been diagnosed with cancer. The test assesses molecular residual disease (MRD) following definitive treatment to monitor response and/or detect recurrence after remission. Signatera uses a whole exome sequencing-based, tumor-informed approach to target specific mutations present in tumor tissue (Natera Inc., 2023). Currently, evidence to support the use of Signatera for monitoring response to treatment or for surveillance after treatment is lacking.

In a Molecular Test Assessment [Hayes, Signatera (Natera Inc.), 2023] Signatera was evaluated for use in both monitoring response during treatment and monitoring for recurrence after treatment in individuals with solid tumor cancers. Hayes identified nine studies which assessed the clinical validity of Signatera, but no peer-reviewed articles that reported impact on clinical decision-making or an improvement in outcomes related to the use of Signatera, Significant

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questions exist regarding appropriate selection of individuals for testing and most effective timing of testing. In addition, the studies identified by Hayes had a wide variation in cancer types and treatments and overall quality was poor. At this time, evidence is insufficient to support the use of Signatera for both monitoring response and detecting recurrence. Hayes notes, however, that there are multiple ongoing clinical trials evaluating these outcomes.

In a retrospective, single-center cohort study, Fakih et al. (2022, included in the 2023 Hayes Signatera Molecular Test Assessment) 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 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, included in the 2023 Hayes Signatera Molecular Test Assessment). 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, included in the 2023 Hayes Signatera Molecular Test Assessment) 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 BC. A retrospective ancillary ctDNA study was performed on samples that had been prospectively collected from high-risk individuals with early BC that were part of the multicenter neoadjuvant I-SPY2 TRIAL. Eligibility requirements included tumor size ≥ 2.5 cm and stage II/III BC. 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 BC. 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, included in the 2023 Hayes Signatera Molecular Test Assessment) 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

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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 December 15, 2023)

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Policy History/Revision Information

Date	Summary of Changes
06/01/2025	• New Medical Policy

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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.

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