Molecular Oncology Testing for Solid Tumor Cancer Diagnosis, Prognosis, and Treatment Decisions

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➲ Instructions for Use

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Related Commercial/Individual Exchange Policies

- FDA Cleared or Approved Companion Diagnostic Testing
- Molecular Oncology Testing for Hematologic Cancer Diagnosis, Prognosis, and Treatment Decisions

Community Plan Policy

- Molecular Oncology Testing for Solid Tumor Cancer Diagnosis, Prognosis, and Treatment Decisions

Medicare Advantage Policy

- Molecular Pathology/Molecular Diagnostics/ Genetic Testing

Application

UnitedHealthcare Commercial

This Medical Policy applies to UnitedHealthcare Commercial benefit plans.

UnitedHealthcare Individual Exchange

This Medical Policy applies to Individual Exchange benefit plans.

Coverage Rationale

➲ See Benefit Considerations

This policy applies only to tests that have not been granted approval as a U.S. Food and Drug Administration–cleared or –approved companion diagnostic test.

Breast Cancer

The use of one of the following Gene Expression Profiling (GEP) tests – MammaPrint®, Oncotype DX Breast Recurrence Score®, Prosigna® Breast Cancer Assay, Breast Cancer Index™, 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 one to three 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

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- Adjuvant chemotherapy is not precluded due to any other factor (e.g., advanced age and/or significant comorbidities)

or

- Currently receiving adjuvant hormonal therapy (e.g., tamoxifen or an aromatase inhibitor) for breast cancer when all 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

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 limitation does not apply to Breast Cancer Index 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.

Colorectal Cancer

Blood-based colorectal cancer (CRC) screening using Shield™ is proven and medically necessary when all the following criteria are met:

- The individual agrees to undergo a follow-up colonoscopy if Shield results are abnormal; and
- The individual has no lower gastrointestinal pain, blood in stool, or other signs or symptoms suggestive of colorectal disease; and
- The individual is of average risk for developing CRC, defined as both of the following:
  - No personal history of adenomatous polyps, CRC, or inflammatory bowel disease (e.g., Crohn disease, ulcerative colitis); and
  - No first-degree relative(s) with CRC, adenomatous polyps, familial adenomatous polyposis, or Lynch syndrome (hereditary nonpolyposis CRC); and
- One of the following:
  - The individual is aged 45 to 75 years and has not been screened with Shield or a U.S. Preventive Services Task Force–recommended CRC screening during the recommended screening interval, including but not limited to:
    - Shield in the past 3 years
    - Guaiac-based fecal occult blood test in the past year
    - Fecal immunochemical test in the past year
    - Multitargeted stool DNA test in the past 3 years
    - Colonoscopy in the past 10 years
    - Computed tomography colonography in the past 5 years
    - Flexible sigmoidoscopy in the past 5 years

    or
  - The individual is aged 76 to 85 years and all of the following:
    - Has never been screened for CRC by any method; and
    - Is healthy enough to undergo treatment if CRC is detected; and
    - Does not have comorbid conditions that would significantly limit life expectancy

Lung Cancer

Multigene molecular profiling panels that include no more than 50 genes are medically necessary for non-small cell lung cancer.

Prostate Cancer

The use of the Genomic Prostate Score® test 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, and 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 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

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- 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, and 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 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; or
    - Unfavorable Intermediate-Risk Prostate Cancer; or
    - High-Risk Prostate Cancer

The use of the 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 of 8 or higher, extracapsular extension, positive surgical margins, seminal vesicle invasion); or
- Prostate-specific antigen is greater than 0 at any point following prostatectomy

Anaplastic Thyroid Cancer

Comprehensive Genomic Profiling of confirmed anaplastic thyroid cancer is proven and medically necessary.

Indeterminate Thyroid Nodules

Molecular testing of thyroid nodules [e.g., Afirma® Genomic Sequencing Classifier (GSC), ThyroSeq® V3, ThyGeNEXT®/ThyraMIR®] is proven and medically necessary when all 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

The use of more than one molecular test on a single thyroid nodule is unproven and not medically necessary due to insufficient evidence of efficacy.

Uveal Melanoma

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

- 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

Unproven Molecular Tests

All other molecular oncology testing for solid tumor cancer is unproven and not medically necessary due to insufficient evidence of efficacy, including but not limited to:

- Next-Generation Sequencing panels or Comprehensive Genomic Profiling unless otherwise specified, such as:
  - OncoDEEP
- Testing of breast cancers and ductal carcinoma in situ unless otherwise specified above, such as:
  - BluePrint
  - DCISionRT
  - Oncotype DX Breast DCIS Score
- Testing of thyroid cancer or thyroid nodules unless otherwise specified above, such as:
  - Afirma Xpression Atlas
  - NeoTYPE Thyroid Profile
- Testing for the purpose of identifying tumor origin or primary site, such as:
  - CancerTYPE ID
- Blood-based CRC screening tests unless otherwise specified above, such as:
  - ColoHealth

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  - ColonSentry
  - FirstSight
  - Signal-C
  - Shield for individuals aged < 45 years and > 85 years
- Testing of bladder cancer, including for early detection of bladder cancer, such as:
  - Bladder EpiCheck
  - Cxbladder Triage
  - Cxbladder Monitor
  - Decipher Bladder Genomic Classifier
- Testing of cutaneous cancers and head and neck cancers, including circulating tumor tissue–modified human papillomavirus DNA analysis, such as:
  - DecisionDx-Melanoma
  - DiffDx-Melanoma
  - DecisionDx-SCC
  - DermTech PLA
  - Merlin
  - MyPath Melanoma
  - NavDx
- Testing of prostate cancers, including for early detection of prostate cancer, unless otherwise specified above, such as:
  - Confirm mdx
  - ExoDx Prostate
  - MyProstateScore 2.0
  - ProsTAV
  - Select mdx
- Measurable Residual Disease assays, whether tumor informed or tumor naive, such as:
  - Geneseeq Shielding ULTRA
  - Geneseeq Vanguard
  - Guardant Reveal
  - Haystack MRD
  - Invitae Personalized Cancer Monitoring
  - Oncodetect
  - Personalis NeXT Personal
  - Plasma Detect
  - RaDaR
  - Signatera
- Multicancer detection/screening tests, such as:
  - Cancerguard
  - Galleri
  - Geneseeq Mercury
- Testing of CRCs, such as:
  - Oncotype DX Colon Recurrence Score
  - GeneFx Colon (also known as ColDx)
  - OnkoSight Advanced Colorectal Cancer
- Testing of lung cancers or lung nodules unless otherwise specified above, such as:
  - Percepta Genomic Sequencing Classifier
- Solid tumor profiling that includes Whole-Exome Sequencing, Whole-Genome Sequencing, or whole-transcriptome sequencing, such as:
  - CancerVision
  - Caris Assure
  - Tempus xE
  - Tempus xR
  - OncoExTra
- Tempus Immune Profile Score
- Whole-genome methylation profiling
- EpiSwitch Checkpoint inhibitor Response Test

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Medical Records Documentation Used for Reviews

Benefit coverage for health services is determined by the member specific benefit plan document and applicable laws that may require coverage for a specific service. Medical records documentation may be required to assess whether the member meets the clinical criteria for coverage but does not guarantee coverage of the service requested; refer to the guidelines titled Medical Records Documentation Used for Reviews.

Definitions

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

Favorable Intermediate-Risk Prostate Cancer: Clinical/pathological features must include all the following: no high- or very high-risk group features, grade group 1 or 2, positivity of less than 50% of biopsy cores (i.e., less than six of 12 cores), and one intermediate risk factor [T2b-T2c; prostate-specific antigen (PSA), 10-20 ng/mL; grade group 2 or 3] (NCCN Prostate Cancer, v4.2026).

Gene Expression Profiling: A laboratory test that analyzes messenger RNA 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 the following: does not meet criteria for very high risk but has one or more of the following high-risk features, including T3-cT4, grade group 4 or 5, and a PSA of greater than 20 ng/mL (NCCN Prostate Cancer, v4.2026).

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 the return of cancer (National Cancer Institute, Liquid Biopsy, 2026).

Low-Risk Prostate Cancer: Clinical/pathological features must include all the following but cancer does not qualify for very low risk: PSA of less than 10 ng/mL, grade group 1, and T1-T2a disease (NCCN Prostate Cancer, v4.2026).

Measurable Residual Disease: A very small number of cancer cells or cell contents that may be detectable in the body during and after cancer treatment, even though the affected individual may have no signs or symptoms of disease. Current approaches to the detection of Measurable Residual Disease include real-time quantitative polymerase chain reaction testing, multiparametric flow cytometry, cell-free DNA analysis, circulating tumor DNA quantification, and Next-Generation Sequencing. Measurable Residual Disease is also known as minimal residual disease (National Cancer Institute Dictionary of Cancer Terms, 2026; Yu et al., 2023).

Next-Generation Sequencing: 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, positivity of at least 50% of biopsy cores (i.e., at least six of 12 cores), and two or three intermediate risk factors (T2b-T2c disease, grade group 2 or 3, and PSA 10-20 ng/mL) (NCCN Prostate Cancer, v4.2026).

Very Low-Risk Prostate Cancer: Clinical/pathological features must include all the following: PSA of less than 10 ng/mL; grade group 1; less than three biopsy fragments/cores positive, with no more than 50% cancer in each core; T1c disease; and PSA density of < 0.15 ng/mL/g (NCCN Prostate Cancer, v4.2026).

Whole-Exome Sequencing: Approximately 1% of a person's DNA makes protein. These protein-making sections are called exons. All the exons together are called the exome. Whole-Exome Sequencing 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, 2021).

Whole-Genome Sequencing: Whole-Genome Sequencing 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 noncoding DNA elements (MedlinePlus, 2021).

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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 the member specific benefit plan document and applicable laws that may require coverage for a specific service. The inclusion of a code does not imply any right to reimbursement or guarantee claim payment. Other policies and guidelines may apply.

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

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CPT Code	Description
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)
0244U	Oncology (solid organ), DNA, comprehensive genomic profiling, 257 genes, interrogation for single-nucleotide variants, insertions/deletions, copy number alterations, gene rearrangements, tumor-mutational burden and microsatellite instability, utilizing formalin-fixed paraffin-embedded tumor tissue
0245U	Oncology (thyroid), mutation analysis of 10 genes and 37 RNA fusions and expression of 4 mRNA markers using next-generation sequencing, fine needle aspirate, report includes associated risk of malignancy expressed as a percentage
0250U	Oncology (solid organ neoplasm), targeted genomic sequence DNA analysis of 505 genes, interrogation for somatic alterations (SNVs [single nucleotide variant], small insertions and deletions, one amplification, and four translocations), microsatellite instability and tumor-mutation burden
0262U	Oncology (solid tumor), gene expression profiling by real-time RT-PCR of 7 gene pathways (ER, AR, PI3K, MAPK, HH, TGFB, Notch), formalin-fixed paraffin-embedded (FFPE), algorithm reported as gene pathway activity score
0285U	Oncology, disease progression and response monitoring to radiation, chemotherapy, or other systematic cancer treatments, cell-free DNA, quantitative branched chain DNA amplification, plasma, reported in ng/mL
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, splice-site variants, insertions/deletions, copy number alterations, gene fusions, tumor mutational burden, and microsatellite instability, with algorithm quantifying immunotherapy response score
0405U	Oncology (pancreatic), 59 methylation haplotype block markers, next-generation sequencing, plasma, reported as cancer signal detected or not detected
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

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CPT Code	Description
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
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
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
0534U	Oncology (prostate), microRNA, single-nucleotide polymorphisms (SNPs) analysis by RT-PCR of 32 variants, using buccal swab, algorithm reported as a risk score
0537U	Oncology (colorectal cancer), analysis of cell-free DNA for epigenomic patterns, next-generation sequencing, > 2500 differentially methylated regions (DMRs), plasma, algorithm reported as positive or negative
0538U	Oncology (solid tumor), next-generation targeted sequencing analysis, formalin-fixed paraffin-embedded (FFPE) tumor tissue, DNA analysis of 600 genes, interrogation for single-nucleotide variants, insertions/deletions, gene rearrangements, and copy number alterations, microsatellite instability, tumor mutation burden, reported as actionable variant
0539U	Oncology (solid tumor), cell-free circulating tumor DNA (ctDNA), 152 genes, next-generation sequencing, interrogation for single-nucleotide variants, insertions/deletions, gene rearrangements, copy number alterations, and microsatellite instability, using whole-blood samples, mutations with clinical actionability reported as actionable variant
0549U	Oncology (urothelial), DNA, quantitative methylated real-time PCR of TRNA-Cys, SIM2, and NKX1-1, using urine, diagnostic algorithm reported as a probability index for bladder cancer and/or upper tract urothelial carcinoma (UTUC)
0560U	Oncology (minimal residual disease [MRD]), genomic sequence analysis, cell-free DNA, whole blood and tumor tissue, baseline assessment for design and construction of a personalized variant panel to evaluate current MRD and for comparison to subsequent MRD assessments
0561U	Oncology (minimal residual disease [MRD]), genomic sequence analysis, cell-free DNA, whole blood, subsequent assessment with comparison to initial assessment to evaluate for MRD
0562U	Oncology (solid tumor), targeted genomic sequence analysis, 33 genes, detection of single-nucleotide variants (SNVs), insertions and deletions, copy-number amplifications, and translocations in human genomic circulating cell-free DNA, plasma, reported as presence of actionable variants
0565U	Oncology (hepatocellular carcinoma), next-generation sequencing methylation pattern assay to detect 6626 epigenetic alterations, cell-free DNA, plasma, algorithm reported as cancer signal detected or not detected

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CPT Code	Description
0566U	Oncology (lung), qPCR-based analysis of 13 differentially methylated regions (CCDC181, HOXA7, LRRC8A, MARCHF11, MIR129-2, NCOR2, PANTR1, PRKCB, SLC9A3, TBR1_2, TRAP1, VWC2, ZNF781), pleural fluid, algorithm reported as a qualitative result
0569U	Oncology (solid tumor), next-generation sequencing analysis of tumor methylation markers (> 20000 differentially methylated regions) present in cell-free circulating tumor DNA (ctDNA), whole blood, algorithm reported as presence or absence of ctDNA with tumor fraction, if appropriate
0571U	Oncology (solid tumor), DNA (80 genes) and RNA (10 genes), by next-generation sequencing, plasma, including single-nucleotide variants, insertions/deletions, copy-number alterations, microsatellite instability, and fusions, reported as clinically actionable variants
0572U	Oncology (prostate), high-throughput telomere length quantification by FISH, whole blood, diagnostic algorithm reported as risk of prostate cancer
0578U	Oncology (cutaneous melanoma), RNA, gene expression profiling by real-time qPCR of 10 genes (8 content and 2 housekeeping), utilizing formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reports a binary result, either low-risk or high-risk for sentinel lymph node metastasis and recurrence
0586U	Oncology, mRNA, gene expression profiling of 216 genes (204 targeted and 12 housekeeping genes), RNA expression analysis, formalin-fixed paraffin-embedded (FFPE) tissue, quantitative, reported as log2 ratio per gene
0597U	Oncology (breast), RNA expression profiling of 329 genes by targeted next-generation sequencing and 20 proteins by multiplex immunofluorescence, formalin-fixed paraffin-embedded (FFPE) tissue, algorithmic analyses to determine tumor-recurrence risk score
0611U	Oncology (liver), analysis of over 1,000 methylated regions, cell-free DNA from plasma, algorithm reported as a quantitative result
0612U	Oncology (liver), analysis of over 1,000 methylated regions, cell-free DNA from plasma, algorithm reported as a quantitative result
0613U	Oncology (urothelial carcinoma), DNA methylation and mutation analysis of 6 biomarkers (TWIST1, OTX1, ONECUT2, FGFR3, HRAS, TERT promoter region), methylation-specific PCR and targeted next-generation sequencing, urine, algorithm reported as a probability index for bladder cancer and upper tract urothelial carcinoma
0620U	Oncology (hepatocellular carcinoma), DNA methylation analysis of more than 5,000 sites, whole blood, algorithm reported as positive or negative risk
0630U	Oncology (breast), mRNA, gene expression profiling by microarray of 80 genes (80 content and 465 housekeeping), utilizing formalin-fixed paraffin-embedded tissue (FFPE), algorithm reported as an index that is diagnostic of a molecular subtype (luminal, basal, Her2)
0631U	Oncology (solid tumor), DNA, sequence analysis of 15 genes including BRCA1 and BRCA2 for identification of clonal hematopoiesis, blood, reported as tumor-derived or nontumor-derived
0641U	Oncology (minimal residual disease [MRD]), tumor DNA, next-generation sequencing (NGS), using formalin-fixed paraffin-embedded (FFPE) tissue and blood samples, initial (baseline) assessment
0642U	Oncology (minimal residual disease [MRD]), tumor DNA, next-generation sequencing (NGS), whole blood, comparison to previously performed analyses, reported as trend in circulating tumor DNA (ctDNA) level
0643U	Oncology (genitourinary cancer), cell-free circulating tumor DNA (ctDNA), 200 genes, next-generation sequencing (NGS), interrogation for single-nucleotide variants (SNVs), insertions/deletions, gene rearrangements, copy number alterations, and tumor mutation burden, using urine, identify and report mutations with clinical actionability
0646U	Oncology (molecular residual disease), whole genome sequence analysis, cell-free DNA, whole blood, and formalin-fixed paraffin-embedded (FFPE) tumor tissue DNA, baseline assessment
0647U	Oncology (molecular residual disease), whole genome sequence analysis, cell-free DNA (cfDNA), whole blood, assessment utilizing patient-specific tumor information, reported as negative or percent circulating tumor DNA (ctDNA)
0668U	Oncology (pancreas), DNA, genome sequence with 5-hydroxymethylcytosine (5hmC) enrichment and glycan biomarker analysis, whole blood or plasma, algorithm reported as cancer detected or not detected

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CPT Code	Description
0682U	Oncology (lung cancer), DNA analysis of 1 methylation marker (cg05575921), methylation-sensitive digital PCR, whole blood, algorithm results reported as the 20-year-hazard ratio for lung cancer
0683U	Oncology (lung cancer), DNA analysis of 2 methylation markers (1 content: cg05575921 and 1 normalizing: cg08141395), methylation-sensitive digital PCR, saliva, algorithm results reported as the 20-year-hazard ratio for lung cancer
0686U	Oncology (prostate), mRNA, next-generation sequencing (NGS) gene expression profiling of 22 genes, formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as risk score
0688U	Oncology (colorectal cancer), analysis of minimal residual disease (MRD), next-generation sequencing (NGS), circulating tumor DNA (ctDNA) analysis in whole blood and tumor for baseline assessment to evaluate current MRD status and for comparisons to subsequent MRD assessments
0689U	Oncology (colorectal cancer), analysis of minimal residual disease (MRD) using patient-specific assays, reported as amount of circulating tumor DNA (ctDNA) detection and trend over time
0693U	Oncology (head and neck), circulating tumor DNA (ctDNA), tumor-informed next-generation sequencing (NGS) analysis for 699 genes of patient-specific somatic variants identified from primary tumor tissue, postoperative lymphatic exudate specimen, algorithm reported as presence or absence of ctDNA
0695U	Oncology (central nervous system), low-pass whole genome sequence analysis of cerebrospinal fluid, interrogation for chromosome arm-level and focal losses and gains
0696U	Oncology (solid organ), targeted genomic sequence analysis, formalin-fixed paraffin-embedded (FFPE) tumor tissue, RNA analysis, 350 or more genes for RNA alterations (e.g., gene rearrangements and splice isoforms)
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
81504	Oncology (tissue of origin), microarray gene expression profiling of > 2000 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as tissue similarity scores

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CPT Code	Description
81518	Oncology (breast), mRNA, gene expression profiling by real-time RT-PCR of 11 genes (7 content and 4 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithms reported as percentage risk for metastatic recurrence and likelihood of benefit from extended endocrine therapy
81519	Oncology (breast), mRNA, gene expression profiling by real-time RT-PCR of 21 genes, utilizing formalin-fixed paraffin embedded tissue, algorithm reported as recurrence score
81520	Oncology (breast), mRNA gene expression profiling by hybrid capture of 58 genes (50 content and 8 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a recurrence risk score
81521	Oncology (breast), mRNA, microarray gene expression profiling of 70 content genes and 465 housekeeping genes, utilizing fresh frozen or formalin-fixed paraffin-embedded tissue, algorithm reported as index related to risk of distant metastasis
81522	Oncology (breast), mRNA, gene expression profiling by RT-PCR of 12 genes (8 content and 4 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as recurrence risk score
81523	Oncology (breast), mRNA, next-generation sequencing gene expression profiling of 70 content genes and 31 housekeeping genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as index related to risk to distant metastasis
81524	Oncology (central nervous system tumor), DNA methylation analysis of at least 10,000 methylation sites, utilizing DNA extracted from formalin-fixed tumor tissue, algorithm(s) reported as probability of matching a reference tumor family and class, and MGMT (O-6-methylguanine-DNA methyltransferase) promoter methylation status, if performed
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
S3854	Gene expression profiling panel for use in the management of breast cancer treatment

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Description of Services

Technologies used for molecular profiling of solid tumor cancers vary and can include but are not limited to tests that evaluate variations in the genes, such as chromosome microarray analysis and Next-Generation Sequencing, as well as others that assess the gene products, such as gene expression arrays and microRNA analysis. The amount of genetic material evaluated can range from a single gene to the whole exome or genome of a tumor. For the purposes of this policy, multigene analysis generally refers to a gene panel containing five or more genes, although 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, score, or classifier for potential diagnosis and/or prognosis of disease or to predict the impact of intervention. Results of molecular profiling may assist individuals and health care providers with determining prognosis and selection of more effective and targeted cancer therapies (Chantrill et al., 2015).

Benefit Considerations

Cancer screenings that are eligible under most benefit plans' preventive care services benefit are listed in the Medical Policy titled Preventive Care Services.

Clinical Evidence

Breast Cancer

The Cooper et al. (2025) systematic review evaluated the clinical effectiveness of four gene expression profiling (GEP) tests (Oncotype DX, Prosigna, EndoPredict, and MammaPrint) for guiding adjuvant chemotherapy decisions in individuals with hormone receptor (HR)–positive, human epidermal growth factor receptor 2 (HER2)–negative, early-stage breast cancer (BC) and one to three positive lymph nodes (LNs). The review included 55 studies published up to April 2023, encompassing randomized controlled trials (RCTs), cohort reanalyses, and observational studies. All four tests demonstrated prognostic ability for distant recurrence (DR), with hazard ratios between risk groups often being statistically significant. For example, in reanalyses of trials and cohorts, 10-year freedom from DR ranged from 81% in low-risk groups to 62% in high-risk groups for Oncotype DX, while DR ranged from 79% to 95% in low-risk groups and 54% to 81% in high-risk groups for MammaPrint. Evidence for predicting chemotherapy benefit was limited. The RxPONDER trial found no benefit of chemotherapy in postmenopausal individuals with Oncotype DX recurrence scores (RSs) of 0 to 25 (adjusted hazard ratio, 1.12; 95% CI, 0.82-1.52; p = 0.49), while premenopausal individuals in the same score range experienced a statistically significant benefit (adjusted hazard ratio, 0.64; 95% CI, 0.43-0.95; p = 0.026). A reanalysis of SWOG-8814 suggested greater chemotherapy benefit with higher Oncotype DX scores in postmenopausal individuals, although the results were not consistently significant. Of note, the included studies did not enroll or report outcomes in premenopausal individuals with an RS of > 25. For MammaPrint, the MINDACT trial reported an absolute difference of 1.3% in 8-year distant metastasis–free survival (DMFS), favoring chemotherapy in clinically high-risk, genomically low-risk individuals; however, this was not statistically significant (hazard ratio, 0.84; 95% CI, 0.51-1.37). Decision-impact studies showed that Oncotype DX testing reduced chemotherapy recommendations by 12% to 75%, with larger reductions in low-risk groups. The validity of these findings is supported by the inclusion of two prospective randomized trials (RxPONDER and MINDACT), assessed as a low risk of bias, although potential selection bias in RxPONDER and heterogeneity in retrospective analyses are limitations. Predictive evidence is uncertain, particularly for tests other than Oncotype DX. No studies reported quality-of-life or anxiety outcomes in node-positive populations. Clinical implications include the potential to avoid chemotherapy in postmenopausal individuals with low Oncotype DX scores, reducing treatment-related toxicity; however, caution is warranted for premenopausal individuals who may still benefit from chemotherapy. The absence of data precluded any assessment of the value of chemotherapy in premenopausal individuals in Oncotype DX high-risk groups. Conflicts of interest were disclosed for two authors, including advisory roles and speaker fees from pharmaceutical companies.

The Tappenden et al. (2025) systematic review identified 55 articles evaluating four tumor profiling tests (Oncotype DX, Prosigna, EndoPredict, and MammaPrint) against decision-making without testing to guide adjuvant chemotherapy in individuals with HR-positive/HER2-negative early BC with one to three positive LNs. Across studies, all tests demonstrated prognostic ability for DR, including data from the RxPONDER trial for Oncotype DX and MINDACT trial for MammaPrint, indicating that each assay stratifies relapse risk independently of clinicopathologic factors. Evidence for predicting relative chemotherapy benefit was concentrated on Oncotype DX. A reanalysis of the SWOG-8814 trial suggested increasing benefit at higher RSs, with interaction terms statistically significant in some analyses. The prospective RxPONDER RCT showed no chemotherapy benefit among postmenopausal individuals with RSs of 0 to 25 but showed a statistically significant benefit among premenopausal individuals with RSs of 0 to 25; however, within-range RS-treatment interaction tests in RxPONDER were not significant. For Prosigna and EndoPredict, no predictive data in node-positive populations were identified; MammaPrint did not provide clear prediction of benefit in the node-positive

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subgroup of MINDACT because clinical high-risk/MammaPrint high-risk individuals uniformly received chemotherapy, and effects in the low-risk subgroup were nonsignificant. Decision-impact evidence was available only for Oncotype DX and consistently showed net reductions in chemotherapy recommendations from before the test to post test of approximately 12% to 75%, with larger reductions in lower-risk RS groups. No studies reported health-related quality of life (QOL) or anxiety outcomes for testing in node-positive populations. The authors concluded that all four assays are prognostic for relapse risk, while evidence for predicting chemotherapy benefit is limited and largely confined to Oncotype DX, with menopausal status being a key modifier of observed effects. Important limitations of this study include the scarcity of randomized evidence directly testing prediction across the full RS spectrum; absence of predictive data for Prosigna and EndoPredict in node-positive disease; inability of MINDACT to isolate prediction for MammaPrint in high-risk, node-positive individuals; and availability of decision-impact data only for Oncotype DX. Overall, the findings may support the prognostic utility of all four tests but provide limited, assay-specific evidence of predictive benefit, particularly for Oncotype DX and primarily in relation to menopausal status; well-designed, comparative studies assessing the prediction of chemotherapy benefit in node-positive populations are needed. Publications by Sestak et al. (2020) and Sestak et al. (2018), discussed below, were included in this systematic review and meta-analysis.

Hyams et al. (2024) conducted an assessment and systematic review of the leading commercially available gene expression assays (GEAs) for early-stage BC. The systematic review was intended to build on prior reviews, focusing on the following GEAs: Breast Cancer Index (BCI), EndoPredict, MammaPrint, Oncotype DX, and Prosigna. The reviews assessed each GEA's utility for prognostication and/or prediction of adjuvant therapy benefit. In all, 1,053 articles were identified for inclusion in the review and analysis, and the Tumor Marker Utility Grading System was used to analyze the level of evidence of the studies identified. After review of the dataset, the authors concluded that the five widely commercialized assays all have some high-level evidence supporting prognostic capability in varying subsets of individuals. Most of the tests were validated initially via prospective-retrospective studies, which confirmed their competency for prognostication with a high level of evidence. Several of the GEAs identify low-risk individuals for whom adjuvant chemotherapy is difficult to justify. Two assays (MammaPrint and Oncotype DX) identify those who might avoid even endocrine therapy (ET), although current data remain preliminary. Some of the assays provide late prognostic information that could impact the selection of extended ET (EET). The most commonly used test in this setting, BCI, appears to be predictive of response to antiestrogens. Despite this, several of the GEAs remain unvalidated in certain subpopulations, based on menopausal and LN status. Of the GEAs assessed in this review, just two (MammaPrint and Oncotype DX) have been the focus of large, prospective clinical trials, and only Oncotype DX has a high level of evidence supporting its ability to predict the benefit of adjuvant chemotherapy. Two of three prospective trials of the Oncotype DX assay identified a relationship between chemotherapy benefit and age and/or menopause, underscoring the importance of large, high-quality trials in enhancing clinical care for individuals with BC. Overall, the reviewers asserted that the abundance of recent data from the five most well-known commercial GEAs has provided a basis for comprehensive evaluation of each of the assays and the ability of each to deliver clinically applicable prognostic and/or predictive information.

Griguolo et al. (2022) explored the evidence on the most widely used, commercially available gene expression signatures [Oncotype DX, MammaPrint, Prediction Analysis of Microarray 50 (PAM50), EndoPredict, and BCI] for individuals receiving neoadjuvant therapy for HR-positive/HER2-negative BC. The authors evaluated the data for the association of gene expression signatures and responses to neoadjuvant chemotherapy (NCT) or neoadjuvant ET (NET) and the clinical suggestions from the data to guide clinical decision-making in early HR-positive/HER2-negative 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 GEAs studied. An association between lower risk, based on gene expression signatures, and higher pCR after NET was observed. However, the evidence is limited and based on small, retrospective studies. Larger, prospective trials are needed to confirm results for the use of GEAs in this context. The researchers asserted that the potential use of gene expression signatures to assist with selection of neoadjuvant therapy (chemotherapy vs ET) in early BC merits further exploration.

Harnan et al. (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 (ER)–positive, HER2-, stage I or II cancer with zero to three positive LNs. The review included 153 articles on the five tests. In all five tests, the proportions of individuals who were LN negative (LN0) receiving 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, categorized more (38%-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+ than other tests (57% in Oncotype DX vs 4%-28% in other tests) but identified worse 10-year DR/relapse-free survival (RFS)/DR/relapse-free interval outcomes (82% in Oncotype DX vs 95%-100% in other tests). An increase of 1% to a decrease of 23% was seen in United Kingdom studies as well as a reduction of 0% to 64% across European studies

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on the net change of individuals who were recommended chemotherapy or decision prior to/post test. Limitations include gaps in the literature, risk of bias, and limited data relating to the ability of Oncotype DX and MammaPrint to predict the benefits of chemotherapy. Additional long-term studies can show the impacts of 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 LN0 status.

Sestak et al. (2018) provided a secondary analysis of data obtained from the TransATAC (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 for DR for 0 to 10 years and 5 to 10 years after diagnosis of four commercial RNA expression signatures (Oncotype DX, Prosigna, BCI, and EndoPredict) with a Clinical Treatment Score (CTS; incorporating nodal status, tumor size, grade, age, and endocrine treatment) and an IHC4 score. The analysis included 774 postmenopausal women with estrogen-positive, HER2-negative disease. In the participants with N0 disease (n = 591), Prosigna, BCI, and EndoPredict provided significantly more information than Oncotype DX, CTS, and IHC4 alone. The most valuable tests were PAM50 and BCI. In the 183 participants 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. This study is included in Hayes EndoPredict (Myriad Genetics Laboratories, Inc.) (2020), Hayes Breast Cancer Index (Biotheranostics, Inc.) for Lymph Node–Negative Patients (2020a; updated 2025), and Hayes Breast Cancer Index (Biotheranostics, Inc.) for Lymph Node–Positive Patients (2020b; updated 2025) as well as the Hyams et al. (2024) systematic review discussed above.

Oncotype DX Breast Recurrence Score

The Ashok Kumar et al. (2024) retrospective cohort study used data from the National Cancer Database to evaluate whether adjuvant chemotherapy improves overall survival (OS) in premenopausal individuals with HR-positive, HER2-negative, node-negative BC and an Oncotype DX RS of 16 to 25. The analysis included 15,792 patients aged 18 to 50 years who were diagnosed between 2010 and 2018, all of whom underwent definitive surgery and ET; those receiving NCT were excluded. Patients were stratified by receipt of adjuvant chemotherapy, which was administered to 30.45% of the cohort (n = 4,808). The median RS was 21 in the chemotherapy group and 18 in the nonchemotherapy group. The primary outcome was OS. At 10 years, OS was 96.2% (95% CI, 94.8%-97.3%) with adjuvant chemotherapy vs 91.6% (95% CI, 88.0%-94.2%) without it. After adjustment, omission of chemotherapy was associated with significantly higher mortality (hazard ratio, 1.664; 95% CI, 1.387-1.995; p < 0.0001). Subgroup analyses showed statistically significant benefit in patients with an RS of 21 to 25 (hazard ratio, 1.953; 95% CI, 1.295-2.945), ductal histology (hazard ratio, 1.521; 95% CI, 1.092-2.118), Caucasian race (hazard ratio, 1.655; 95% CI, 1.180-2.322), and age of 41 to 50 years (hazard ratio, 1.732; 95% CI, 1.244-2.411). The study's limitations include its retrospective nature, potential residual confounding, and lack of detailed chemotherapy regimen data. These findings support consideration of adjuvant chemotherapy for premenopausal individuals with node-negative, HR-positive BC and RSs of 21 to 25 and highlight the need for further research to clarify benefit in other subgroups.

Yang et al. (2024b) analyzed data from the TAILORx phase 3, multicenter, randomized trial to determine whether the 21-gene RS independently predicts BC-specific survival (BCSS) and OS in participants with HR-positive, HER2-negative, node-negative BC. Among 8,916 participants aged 23 to 75 years (median, 56 years) with complete clinicopathologic and treatment data, RS was categorized as low (0-10), midrange (11-25), or high (26-100). Participants with an RS of ≤ 10 received ET alone, those with an RS of ≥ 26 received chemoendocrine therapy, and those with midrange scores were randomized to ET or chemoendocrine therapy. The median follow-up was approximately 95 months, and event rates were 1.7% for BCSS, 5.2% for OS, 12.6% for invasive disease–free survival, and 5.6% for recurrence-free interval (RFI). Multivariable Cox regression, adjusting for clinicopathologic factors, showed that compared with low RS, midrange and high RSs were significantly associated with worse BCSS (adjusted hazard ratio, 5.12, 95% CI, 2.09-16.92, p = 0.002 and adjusted hazard ratio, 8.03, 95% CI, 2.91-28.47, p = 0.0002, respectively) and RFI (adjusted hazard ratio, 1.68, 95% CI, 1.23-2.36, p = 0.002 and adjusted hazard ratio, 3.05, 95% CI, 2.02-4.67, p < 0.0001, respectively). A high RS was also associated with poorer disease-free survival (DFS; adjusted hazard ratio, 1.56; 95% CI, 1.20-2.04; p = 0.001) but not OS (adjusted hazard ratio, 1.44; 95% CI, 0.95-2.18; p = 0.09), and a midrange RS was not significantly associated with DFS or OS. Approximately two-thirds of deaths were unrelated to BC, which may explain the lack of OS association. Limitations include the absence of OS significance despite long follow-up and the fact that RS reflects tumor biology rather than competing mortality risks. The authors noted that the RS offers strong prognostic value for disease-specific outcomes, supporting its use in guiding adjuvant therapy decisions while highlighting the need for additional strategies for individuals with high RS who remain at an elevated risk despite chemotherapy.

Sparano et al. (2024) explored the role of Oncotype DX Breast in providing prognostic information for late DR when added to clinicopathologic prognostic factors through an individual-specific meta-analysis. Included in the meta-analysis were

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10,004 women who were enrolled in three trials, which was updated by using the extended follow-up data from the TAILORx trial (including integration of the RS with histological grade, tumor size, and age at surgery for the RSClin tool). Likelihood ratio tests were used to compare Cox models, integrating clinicopathologic factors and the RS. In addition, external validation of the prognosis for DR in years 0 to 10 and 5 to 10 was performed in an independent cohort of 1,098 women from a real-world registry. The results showed that RSClin delivered substantially more prognostic data than either the clinicopathologic factors (Δ likelihood ratio chi-square, 86.2; p < 0.001) or RS alone (Δ likelihood ratio chi-square, 131.0; p < 0.001). The model was predictive in an independent cohort for DR 10 years after diagnosis (standardized hazard ratio, 1.56; 95% CI, 1.25-1.94), associated with late DR risk between 5 and 10 years after diagnosis (standardized hazard ratio, 1.78; 95% CI, 1.25-2.55), and near the observed 10-year DR risk (Lin concordance, 0.87) and 5- to 10-year DR risk (Lin concordance, 0.92). The limitations of the analysis include the risk of limited external generalizability, as it was developed in those who met the inclusion criteria for the associated trials. The authors concluded that the 21-gene RS is prognostic for both DR and OS in individuals with early BC. In addition, they suggested that a model integrating the 21-gene RS and an individual's clinicopathologic factors improved estimates of DR risk compared with either the 21-gene RS or clinicopathologic factors used alone and, additionally, assisted with stratification of late DR risk.

Nash et al. (2023) investigated the benefit of chemotherapy based on RS in younger women (age 40-50 years) who were eligible for Oncotype DX testing. Individuals were selected from the National Cancer Database and grouped by age, RS, nodal status, and receipt of chemotherapy. A total of 15,422 individuals met the inclusion criteria for the study. Of them, 43.5% received chemotherapy. Log-rank tests were used to assess 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 progesterone receptor (PR) negative, and a higher RS (p < 0.001 for all). RS was prognostic for OS, regardless of nodal status. After adjustment, chemotherapy was associated with a significant improvement in OS only in the pN1 RS of 31 to 50 subgroup (p = 0.02). The authors concluded that RS remains prognostic in younger individuals with early-stage, HR-positive, HER2-negative BC. The survival benefit with chemotherapy was only found in those aged 40 to 50 years with pN1 disease and an RS of 31 to 50. As such, chemotherapy decision-making should be especially preference sensitive in women aged 40 to 50 years who have an intermediate RS, in whom survival benefit may not be enhanced.

Li et al. (2023) retrospectively evaluated 35,137 patients with T1-2N1M0 and ER+/HER2- BC from the Surveillance, Epidemiology, and End Results (SEER) Oncotype DX database to evaluate patterns in practice related to the use of the RS for decision-making regarding chemotherapy and survival outcomes in these patients. Both BCSS and OS were included in the assessment. In this study, older age, lower tumor grade, T1 stage, fewer positive LNs, and PR-positive disease (all p < 0.05) were all associated with the 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 that was significantly associated with chemotherapy treatment in the group that did not receive the test. In patients who underwent testing, the probability of chemotherapy was 30.8%; in the group that did not undergo the 21-gene test, the probability of chemotherapy was higher at 64.1%. Based on a multivariate prognostic analysis, use of the 21-gene test was associated with both improved BCSS (p < 0.001) and OS (p < 0.001) compared with patients who did not receive the test. From these data, the authors concluded that the 21-gene assay is related to lower rates of adjuvant chemotherapy use and improved survival outcomes. They indicated their support for the use of the 21-gene assay in individuals with ER+/HER2- BC with N1 disease.

The Davey et al. (2022) systematic review and network meta-analysis evaluated the Oncotype DX 21-gene RS for its ability to estimate locoregional recurrence (LRR) in ER+/HER2- BC. The review encompassed 16 articles, representing 21,037 individuals. The average RS was 17.1, and the average follow-up was 66.4 months. With standard RS cutoffs, 49.7% of individuals had an RS of < 18 (n = 3,944), 33.8% had an RS of 18 to 30 (n = 2,680), and 16.5% had an RS of > 30 (n = 1,311). Those with an RS of 18 to 30 and an RS of > 30 were significantly more likely to experience LRR than those with an RS of < 18. Using the TAILORx cutoff, 16.2% of individuals had an RS of < 11 (n = 1,974), 65.8% had an RS of 11 to 25 (n = 8,036), and 18.0% had an RS of > 30 (n = 2,198). LRR rates were comparable for individuals with an RS of 11 to 25; however, those with an RS of > 25 had a considerable risk of LRR vs those with an RS of < 11. The authors concluded that RS testing correctly estimates the risk of LRR in individuals being treated with the intent to cure in early-stage, ER+/HER2- BC. RS testing is a valid method to assess 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.

Kalinsky et al. (2021) published the results of a prospective RCT to demonstrate the effect of chemotherapy on invasive disease–free survival in participants with positive LN disease and determine whether the RS [based on the 21-gene assay (Oncotype DX)] influenced the outcome. Overall, 5,018 women with HR-positive, HER2- BC, one to three positive axillary LNs, and an RS of ≤ 25 were randomly assigned to either an ET-alone cohort or a chemotherapy with endocrine

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(chemoendocrine) therapy cohort. The intent-to-treat analysis included the participants who declined the assigned treatment, with 402 participants (16.2%) assigned to chemoendocrine therapy and 144 (5.8%) assigned to ET alone. The trial did not show a clinically applicable or statistically significant rise in invasive disease–free survival with the addition of adjuvant chemotherapy to ET in the global population with the same characteristics. In the 67% of participants who were postmenopausal, no chemotherapy advantage was found, while adjuvant chemotherapy led to a relative increase of 40% in invasive disease–free survival and a relative increase of 42% in distant relapse–free survival in premenopausal women. Invasive disease–free survival at 5 years was 91.9% among postmenopausal 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 5 years was 89.0% with endocrine-only therapy and 93.9% with chemoendocrine treatment, with a comparable rise in distant relapse–free survival. Per the authors, the trial showed that among premenopausal women with one to three positive LNs (N1) and an RS of ≤ 25, participants who received chemoendocrine therapy had a lengthier invasive disease–free survival and distant relapse–free survival than those who received endocrine-only treatment. In contrast, postmenopausal women with the same characteristics did not experience benefit from adjuvant chemotherapy.

Hayes published a Molecular Test Assessment (2020; updated 2023) addressing the use of the Oncotype DX Breast RS in individuals with ER+, HER2-, LN-positive BC to determine the capability of the test to estimate the risk of DR and predict the likelihood of chemotherapy benefit. For individuals with N1 disease, limited but consistent evidence supports the use of the Oncotype DX test for predicting the risk of 9-year DR, but there is insufficient evidence supporting the test's ability to predict the benefit of chemotherapy. Oncotype DX may improve outcomes in individuals with N1 cancer by lessening the total population of individuals treated with chemotherapy, resulting in avoidance of detrimental side effects. Insufficient evidence was found to support the use of Oncotype DX testing for estimating the risk of DR and the potential benefit of chemotherapy in individuals with N2 disease (four to nine positive LNs).

A Hayes (2020; updated 2023) Molecular Test Assessment evaluated the Oncotype DX Breast RS as a prognostic indicator for 9-year distant BC recurrence and predictive indicator for chemotherapy benefit in individuals diagnosed with ER+, HER2-, N0 invasive BC. The evidence presented in the assessment suggests that the Oncotype DX test can estimate the risk of DR and the likely benefit of chemotherapy for guiding proper treatment decisions for individuals, thus impacting provider management and decisions related to therapy. An 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 RS-based treatments are needed as well.

Poorvu et al. (2020) evaluated women who were less than 40 years of age and had 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 participants, and the RS assay was performed either clinically (n = 189) or on banked specimens (n = 320). The median follow-up time was 6 years. Of the 509 participants, 300 (59%) had N0 BC, and 195 of them had an RS of 11 to 25; 86 of them received chemotherapy. The 6-year DR-free survival (DRFS) varied by the RS, with an RS of < 11 associated with 94.4% N0 and 92.3% N1. In those with an RS between 11 and 25, DRFS was 96.9% N0 and 85.2% N1. In those with an RS of > 26, DRFS was 85.1% N0 and 71.3% N1. The researchers concluded that the assay is prognostic for women under 40 years of age with N0 and limited N1.

Wang et al. (2019) examined the prognostic value of Oncotype DX in female individuals with BC and tumor stage 1 to 2 (tumor size, 20-55 mm), LN+ disease, and no evidence of metastasis (T1-2 N1M0). The study reviewed 4,059 cases to categorize them to prognostic stages IA and IIB, using data derived from the National Cancer Institute's limited-use SEER 18 registry databases released in November 2017. Cases in the SEER database were linked to RS results from assays performed by Genomic Health. All cases with RS were HER2 negative. The authors selected female individuals with ER+ invasive ductal carcinoma (IDC) cases in T1-2N1M0 stage with Oncotype DX results obtained 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 individuals, 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 the RS high-risk group [598 (14.7%)]. The high-risk group tended to have younger individuals, larger tumors, a higher percentage of grade 3 disease, PR- status, and more advanced cancer staging. They also had more frequent use of chemotherapy. The RS groups were well matched in race, n stage, surgery, and 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 ($p < 0.001$) between the RS groups and pathological stage results. In the low- and high-risk RS groups, BCSS and OS were similar between RS and pathological staging groups. However, in the intermediate RS group, 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

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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 individuals with N+ disease.

Prosigna Breast Cancer Assay

Fitzal et al. (2021) conducted a retrospective analysis of prospectively collected data from the randomized ABCSG-8 trial, which enrolled postmenopausal individuals with endocrine receptor–positive, early-stage BC between 1996 and 2004. Eligible individuals in this analysis were those who underwent breast-conserving surgery, had available formalin-fixed paraffin-embedded (FFPE) tumor blocks, and received adjuvant ET; individuals who underwent mastectomy were excluded. Of 1,620 available tumor blocks, 1,478 passed quality control, and 1,204 individuals who had breast-conserving surgery were included, with 1,034 receiving radiotherapy (RT). Local recurrence was defined as in-breast or chest-wall recurrence, with regional recurrence, DR, second malignancies, and death treated as competing risks. The exposure of interest was Prosigna, also known as the PAM-50–based 46-gene risk-of-recurrence (ROR) score. The primary outcome was cumulative incidence of local recurrence at 5 and 10 years. After a median follow-up of 10.8 years, 23 local recurrences were observed, giving an overall local recurrence rate of 2.2%. Individuals with ROR scores below 57 (n = 765) had very low recurrence rates: 0.1% at 5 years compared with 2.2% in the high-risk group and 0.9% at 10 years compared with 3.8% in the high-risk group. Higher ROR scores were associated with increased recurrence risk. Tumors classified as luminal B also showed higher recurrence risk than luminal A, with 5-year rates of 1.7% vs 0.1% and 10-year rates of 3.2% vs 1.0%. In the subgroup of 170 individuals who did not receive RT, those classified as high risk had 5- and 10-year recurrence rates of 14.6% and 20.8% compared with 1.6% and 5.0% in the low-risk group. However, interaction testing showed no evidence that PAM-50 predicted benefit from RT. These results suggest that the PAM-50 ROR score identifies a subgroup of postmenopausal individuals with endocrine receptor–positive disease who have a very low long-term risk of local recurrence after breast-conserving surgery and ET, although the value of the score as a predictive tool for RT benefit remains unknown. Limitations of this study are its retrospective analytical design, few local recurrence events, and the need for external validation of the locally derived cutoff value.

MammaPrint and BluePrint

The Huppert et al. (2025) phase 2 analysis from the I-SPY2 trial evaluated pCR and DRFS in 379 participants with HR+, HER2-negative, high-risk, early-stage BC treated with NCT with or without investigational agents or immunotherapy. Eligible participants were aged ≥ 18 years with stage II to III disease who had undergone molecular risk testing using the 70-gene MammaPrint assay and been assigned a status of either MP-High1 (0-0.57) or MP-High2 (< -0.57). Between March 2010 and November 2016, participants were randomized to receive weekly paclitaxel alone or combined with one of eight investigational agents, followed by doxorubicin and cyclophosphamide. The overall pCR rate was 17%, with the highest rate observed in the pembrolizumab arm (30%). pCR was significantly more frequent in participants with stage II disease (21% vs 9%; p = 0.0013), ductal histology (19% vs 11%; p = 0.049), low ER positivity (≤ 66% vs > 66%; 35% vs 9%; p = 3.4E-09), MP-High2 vs MP-High1 (31% vs 11%; p = 1.1E-05), and BP-Basal vs BP-Luminal subtype (34% vs 10%; p = 1.62E-07). At a median follow-up of 4.8 years, participants who achieved pCR had significantly better DRFS (hazard ratio, 0.12; 95% CI, 0.03-0.51; p = 5E-04). Among those who did not achieve pCR, DRFS was significantly worse in participants with MP-High2 (hazard ratio, 0.49; 95% CI, 0.30-0.82; p = 0.0051) and the BP-Basal subtype (hazard ratio, 0.43; 95% CI, 0.26-0.7; p = 5E-04). Limitations include the lack of prospective validation of biomarkers, limited follow-up duration, and incomplete data on adjuvant therapies. The study's adaptive, randomized design and robust biomarker profiling enhance internal validity, although subgroup sample sizes and overlapping molecular features may affect reliability. The author disclosures include consulting fees, institutional research funding, and patent holdings.

Rastogi et al. (2024) examined MammaPrint for predicting the benefit of extended letrozole therapy (ELT) in 1,866 individuals with early-stage BC in the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-42 trial. The individuals were randomly assigned to receive either ELT or a placebo. The primary outcome measure was DR; the secondary end points included DFS and BC-free interval (BCFI). Tumors were classified as either MammaPrint high risk (MP-HR) or low risk (MP-LR). The MP-LR tumors were further subcategorized as ultra-low risk or low non–ultra-low risk. The researchers found no statistically significant difference in ELT benefit for DR between MP-HR and MP-LR (interaction p = 0.38). MP-LR tumors (n = 1,160) revealed a statistically significant 10-year benefit of 3.7% for DR (hazard ratio, 0.43; 95% CI, 0.25-0.74; p = 0.002), while MP-HR tumors (n = 706) displayed a nonsignificant 2.4% benefit (hazard ratio, 0.65; 95% CI, 0.34-1.24; p = 0.19). The 10-year ELT benefit was significant for DFS (7.8%) and BCFI (7.0%) for MP-LR tumors, while MP-HR tumors did not show significant benefit (interaction DFS: p = 0.015; BCFI: p = 0.006). The 10-year ELT benefit was significant and more distinct in non–ultra-low-risk (n = 908) tumors: 4.0% for DR, 9.5% for DFS, and 7.9% for BCFI. The benefit in ultra-low-risk (n = 252) tumors was not significant: 3% for DR, 1.8% for DFS, and 4.1% for BCFI in an exploratory analysis. The study's limitations include its prospective-retrospective design and lack of multiplicity adjustment for secondary and exploratory analyses. The authors concluded that the primary hypothesis of the predictive capability of MammaPrint on DR was not verified; however, the secondary end point results indicated that MammaPrint is predictive of

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ELT response, and it detected a subset of people with early-stage, HR-positive BC (MP-LR) that showed better outcomes with ELT. These findings could expand the clinical utility of MammaPrint beyond just prognostic indication because they provide the initial data that suggest that MammaPrint may be predictive of ELT benefit. The authors recommended further study that incorporates clinicopathologic characteristics to further optimize the selection of appropriate individuals for treatment. They suggested that the confirmation of the utility of MammaPrint will allow individuals in specific categories (postmenopausal with HR+ BC) to avoid unneeded treatment while helping to identify individuals who require ELT.

van't Veer et al. (2024) performed a secondary analysis of the IDEAL RCT to establish the utility of MammaPrint, a 70-gene expression ROR assay, in the identification of those participants with early-stage BC in the IDEAL trial who could benefit from 5 years of treatment with letrozole vs 2.5 years of the same treatment. In this study, the researchers assessed postmenopausal women with HR+, early-stage BC who had been assigned to either 2.5 or 5 years of EET. A follow-up assessment occurred 10 years after randomization. To carry out the analysis, MammaPrint was used to classify tumors as high, low, or ultra-low risk. After 5 years of ET, participants were randomized to 2.5 or 5 years of EET with letrozole. The primary end point was DR; Cox proportional hazard regression models and likelihood ratios were used to analyze the interaction between treatment and GEA. Adverse event incidence and treatment adherence were also measured. Of the 515 women included [mean (SD) age at randomization, 59.9 (9.5) years], 265 were in the 2.5-year treatment arm, and 250 were in the 5-year treatment arm. A total of 223 (43.3%) of those with MP-LR tumors had a significant absolute benefit of 10.1% for DR (hazard ratio, 0.32; 95% CI, 0.12-0.87; p = 0.03). Treatment interaction was not significant for DR. Of those with either MP-HR tumors [259 (50.3%)] or ultra-low-risk tumors [33 (6.4%)], 5 years vs 2.5 years of EET had no statistical association with improved benefit for DR. The rate of adverse events and treatment discontinuation rates were similar among the different MammaPrint risk groups in each treatment arm. The study is limited by its retrospective design and the inclusion of a subset of participants due to limited tissue sample availability, which prevented analyses from being adjusted for covariates. In addition, the study participants were of limited racial diversity, only participants who were postmenopausal were included, and the sample size for the ultra-low-risk group was small. Lastly, nonsignificant treatment-by-risk interactions were observed for DR and BCFI, potentially due to low event rates seen in the translational cohort. The authors concluded that MammaPrint was able to detect participants with low-risk tumors who may benefit from 5-year vs 2.5-year EET, which suggests that MammaPrint may have utility beyond guiding neoadjuvant and adjuvant chemotherapy decisions; it may guide decisions regarding adjuvant ET as well. They suggested additional study of MammaPrint for use as a biomarker to determine EET benefit, specifically focusing on populations that include premenopausal women.

Marín-Liébana et al. (2023) published an initial analysis from the DETERMIND study. DETERMIND is a prospective, open-label, multicenter study evaluating the utility of the MammaPrint/BluePrint (MP/BP) signature for determining optimal therapy for individuals with operable, clinically high-risk, HR+/HER2-, early-stage BC, stage II to IIIA (up to N1), who have received a recommendation for NCT. Overall, 165 participants from 11 centers were included in this analysis, with data collected at baseline, at the time of MP/BP results, and at subsequent 1- and 3-year intervals. The first analysis incorporated 99 participants who had a median age of 57 years (range, 31-85 years). Of them, 94% were stage II, and 51% were cN1. At the time of MP/BP, 37 participants (37%) were classified as subtype luminal A, 58 (59%) were subtype luminal B, and four presented as a nonluminal phenotype (three basal, one HER2). Corresponding to MP/BP results, 44 participants did not receive NCT. In the MP/BP luminal A group, 35 (95%) did not receive NCT. In 19 of these participants, it was replaced by NET. Participants with MP-HR results received NCT in 53 cases (85%). MP/BP results significantly increased confidence in the final treatment decision made collaboratively by the treating physicians and participants. The authors concluded that in individuals with clinical high-risk, HR+/HER2-, early-stage BC, there is a high frequency (35%) of the MP/BP luminal A subtype and an ability to de-escalate NCT. The use of MP/BP also bolstered the decision to administer NCT in the majority (85%) of those with MP-HR. The authors asserted that these findings support the utility of MP/BP in high-clinical-risk, HR+/HER2-, early-stage BC to inform neoadjuvant therapy decisions and increase confidence 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 NET in a recent observational registry trial of 1,091 participants with early-stage BC. Participants who were scheduled to receive neoadjuvant therapy were prospectively enrolled into NBRST (Neoadjuvant Breast Registry Symphony Trial), which was sponsored by Agendia. NBRST compared the prognostic value of MammaPrint and BluePrint with standard pathological classification methods in response to neoadjuvant treatment. The association of these signatures with clinical response and 5-year outcomes in participants who underwent treatment with NET (n = 67) were evaluated in a subanalysis. The primary outcome was pathological partial response (pPR). The secondary outcomes included DMFS and OS. The researchers defined clinical benefit as a pPR or stable disease with the use of NET. Of participants with genomically luminal A–type tumors, 94.4% experienced clinical benefit (50.0% pPR and 44.4% stable disease). Overall, 95% of participants with luminal B–type tumors experienced benefit (55.0% pPR and 40.0% stable disease). At the 5-year assessment, participants with genomically luminal B tumors had substantially worse

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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 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 participants with MP-LR results and genomically luminal A tumors who were treated with ET alone had excellent outcomes at 5 years. In addition, most participants with genomically defined luminal A– and luminal B–type tumors responded well to NET, which suggests that NET may be a safe option for treatment; however, those with genomically luminal B tumors will also need postoperative chemotherapy or cyclin-dependent kinase 4/6 inhibitors to improve their long-term outcomes. The researchers indicated that genomic classification (defined by the combined use of MammaPrint and BluePrint) 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-stage, HR+/HER2- BC. This study was observational, and the number of participants receiving NET was limited, so the sample size was small and prevented further subgroup analyses. In addition, NBRST participants receiving NET instead of NCT, 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.

Vliek et al. (2022) published a 10-year follow-up of the observational RASTER study. The prospective RASTER study assessed the tumors of 427 participants with clinical stage cTanyN0M0 BC. The study aimed to evaluate the ability of the MammaPrint 70-gene signature to guide adjuvant chemotherapy decisions in participants with ER+ and HER2- BC. The authors evaluated 310 of the 427 participants at 10 years of follow-up. Of the clinically high-risk participants, 45 (49%) were classified as genomically low risk. In this subcategory, at 10 years, the DR-free interval (DRFI) was comparable among participants treated with (95.7%; 95% CI, 87.7%-100%) or without (95.5%; 95% CI, 87.1%-100%) chemotherapy. In the group of clinically low-risk participants, 56 (26%) were classified as genomically high risk. In the clinically low-risk group, a variance was seen among the genomically high- and low-risk subgroups after 5 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 ultra-low-risk participants' outcomes were a 10-year DRFI of 96.7% (95% CI, 90.5%-100%), primarily (79%) without systemic therapy. Limitations of the RASTER study include the observational nature and risk of bias. The authors concluded that over 10 years, individuals with clinically high-risk, genomically 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 support the use of MammaPrint in ER+, HER2-, N0, clinically high-risk individuals with BC.

Göker et al. (2022) measured the treatment response and 5-year survival outcome in the molecular subgroups by combining MammaPrint and BluePrint in NBREaST II, a prospective neoadjuvant study. MammaPrint and BluePrint were carried out in 256 individual core-needle biopsies (CNBs) to quantify chemosensitivity or endocrine sensitivity in the molecular subgroups. The outcomes measured were DMFS, RFS, and BCSS at the long-term follow-up. In the group of participants 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%), and 47 as basal type (20%). Of participants who attained a pCR in response to NCT (n = 47), 4% had an MP-LR result, and 96% had an MP-HR result. All BluePrint-defined HER2-type and basal-type tumors had an MP-HR result. At 5 years, DMFS was significantly lower (p = 0.039) in MP-HR tumors (83.8%; 95% CI, 77.4%-88.6%) vs MP-LR tumors (91.4%; 95% CI, 78.6%-96.7%). Similar outcomes were seen for 5-year RFS but not for BCSS. Limitations in the study include a small sample size, with no differences in 5-year survival when stratifying the cohort into subgroups. The study confirmed previous conclusions that MammaPrint and BluePrint can predict chemosensitivity and 5-year results more precisely than traditional pathological subtyping and support informed decision-making.

Crozier et al. (2022) prospectively collected 139 matched CNB and surgical resection (SR) specimens from women with established early-stage BC registered in the ongoing FLEX study (NCT03053193), a multi-institutional prospective study in participants with stage I to III, early-stage BC. The goal was to define the concordance of MammaPrint and BluePrint results among CNBs and SR to safeguard reliable prognostic information that can be apprehended from a CNB. Overall, 121 participants from the FLEX study database with diagnostic MammaPrint and BluePrint results with matched CNB and SR specimens were involved in the study. In total, 50 participants 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), a 95.2% negative predictive value (NPV), and an 86.2% positive predictive value (PPV). The authors concluded that the concordance of BluePrint between CNBs and SR was 98.3%. In more than 97% of participants in this study, treatment decisions and probable outcomes were precise and consistent based on MammaPrint testing of the CNB. According to the authors, this analysis was 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 are 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 among paired samples strongly supported the value of these assays to acquire reliable prognostic data on core biopsy tissue, which can guide prompt and proper treatment decisions.

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Piccart et al. (2021) produced updated results from the 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 participants with confirmed primary invasive BC with N1, no distant metastases, and a World Health Organization performance status of 0 to 1. Their genomic risk was decided using the MammaPrint 70-gene signature. Enrolled in the trial were 6,693 participants, 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 and 89.4% for no chemotherapy. The 8-year DMFS in the exploratory analysis by nodal status in these participants was 91.7% (95% CI, 88.1%-94.3%) with chemotherapy and 89.2% (95% CI, 85.2%-92.2%) without chemotherapy in 699 N0 participants (absolute difference, 2.5 percentage points; standard error, 2.3; 95% CI, -2.1 to 7.2) and 91.2% (95% CI, 87.2%-94.0%) as opposed to 89.9% (95% CI, 85.8%-92.8%) in 658 participants with N1. The exploratory analysis of the effects of chemotherapy administration on 8-year DMFS according to age resulted in 93.6% with chemotherapy and 88.6% without chemotherapy in 464 women, who were aged 50 years or younger, and 90.2% vs 90.0% in 894 women who were older than 50 years of age. This long-term follow-up of the phase 3 randomized MINDACT trial showed MammaPrint's ability to identify a subgroup of participants with high clinical risk and specific participants with low genomic risk but with an exceptional DMFS when treated with ET by itself. In this group, the benefit of adding chemotherapy to ET continues to be small and is not improved by nodal positivity. The benefit is age dependent and is solely seen in women who are under age 50 years; further study is needed in younger women, who may need reinforced ET to forego chemotherapy. The authors concluded that MammaPrint should be a portion of informed, shared decision-making.

Soliman et al. (2020) conducted a prospective case-only trial (IMPACt) that enrolled 452 participants with stage I to II, HR+, HER2- BC to evaluate the variation in treatment decisions and physician confidence based on MammaPrint and BluePrint in early-stage BC. According to clinical risk assessment via the MINDACT criteria, 63.4% (n = 227) of participants were categorized as low risk, and 36.6% (n = 131) of participants were classified as at high risk of DR. Of the 227 participants categorized as clinically low risk, 77.5% (n = 176) were counseled against chemotherapy by their physicians. Of the 131 participants categorized as clinically high risk, 62.6% (n = 82) were recommended chemotherapy. In contrast, MammaPrint categorized 62.5% (n = 224) of participants as low risk and 37.5% (n = 134) as high risk. Following the MammaPrint results, physicians elected to change their recommendation regarding chemotherapy for 86 participants. After MammaPrint, the clinical risk assessment–based treatment plans were in 88.5% (n = 317) agreement with MammaPrint results, 83.6% (n = 112) for chemotherapy in MP-HR participants, and 91.5% (n = 205) for no chemotherapy in MP-LR participants. The IMPACt trial results demonstrated that physicians make treatment decisions based on MammaPrint results in clinical practice. Physicians also described increased confidence in 72.2% of their suggested treatment plans after receiving the MammaPrint results. A limitation of the study is that participants were enrolled both before and after the MINDACT trial results were published, which may have impacted physicians' decisions for treatment. The authors concluded that physicians feel that the proper, high-risk individuals are being provided chemotherapy and that they feel confident forgoing chemotherapy in individuals with low-risk MammaPrint results.

Wuerstlein et al. (2019) reported on the prospective, observational, multicenter WSG-PRIMe study designed to gauge the effect of MammaPrint and BluePrint on adjuvant chemotherapy treatment decisions in participants with early-stage BC, specifically to show an overall switch percentage of at least 15% regarding chemotherapy. These participants had MammaPrint, which was considered part of their standard clinical procedure. Included in the study were 452 participants with HR+ and HER2- cancer. Physicians supplied preliminary treatment recommendations based on clinicopathologic factors. Posttest therapy recommendations and actual therapy were documented after prospective risk classification by MP/BP was revealed. MammaPrint allocated 63.5% of participants to the low-risk group and 36.5% to the high-risk group. In 125 of 430 participants (29.1%; 95% CI, 24.8%-33.6%), the recommendation transformed from chemotherapy to no chemotherapy or vice versa. Chemotherapy had been recommended to 164 participants (38.1%) prior to the test. In 60 of the 164 participants (36.1%) 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 participants, according to MammaPrint. On the contrary, deletion of chemotherapy was suggested for 266 participants (61.9%) prior to the test. In 65 of 266 cases (24.4%), recommendations converted to chemotherapy post test; most [64/65 (98.4%)] of these modifications happened in MP-HR participants. The physician observance of MammaPrint risk calculation was 92.3% for low-risk and 94.3% for high-risk scores. Three-fourths [59/79 (74.7%)] of physicians initially recommending chemotherapy converted to no chemotherapy subsequent to low-risk MammaPrint results (72.7% in pN0; 77.1% in pN1); on the contrary, nearly nine-tenths [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 GEPs 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 to 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 undertreatment 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.

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van Steenhoven et al. (2018) evaluated the ability of MammaPrint and 80-GS to surrogate pathological subtyping for determining treatment options and prognosis. Between 2013 and 2015, 595 intermediate-risk individuals with ER+, early-stage BC were studied. HER2 receptor status was determined through routine immunohistochemistry and fluorescence in situ hybridization (FISH). The overall concordance between molecular subtyping and pathological subtyping for luminal cancers type A and B together was 98%. Individually it was poor, at 64%. The ability of BluePrint to differentiate between luminal, HER2-type, and basal-like cancers was limited, and furthermore, the concordance between pathological subtyping and the MammaPrint 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 these tests.

The Cardoso et al. (2016) randomized, phase 3 clinical MINDACT trial included 6,693 women with early-stage BC, with the primary goal to assess whether, among participants with high-risk clinical features and a low-risk GEP who did not receive chemotherapy, the lower boundary of the 95% CI for the rate of 5-year survival without distant metastasis would be 92% (i.e., the noninferiority 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. In participants 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 MammaPrint 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.

EndoPredict

The Penault-Llorca et al. (2024) prospective cohort analysis evaluated the prognostic value of the EndoPredict test in 768 participants with ER-positive, HER2-negative, early BC screened for the randomized, double-blinded, phase 3 UNIRAD trial, which was conducted across multiple centers between May 2015 and March 2020. Overall, 609 participants had PR-positive disease. All participants had undergone complete tumor resection and had no detectable metastases at inclusion. The EndoPredict test placed 663 participants into the EPclin high-risk cohort and 105 participants into the low-risk cohort. The EPclin score was significantly associated with both DFS and DMFS. The 60-month relapse rate was 0% in the EPclin low-risk group and 7% in the EPclin high-risk group (hazard ratio for continuous EPclin score, 1.87; 95% CI, 1.4-2.5; $p < 0.0001$). In a multivariate analysis adjusting for tumor size, nodal status, and grade, EPclin remained independently prognostic for DFS (hazard ratio, 1.52; 95% CI, 1.09-2.13; $p = 0.0141$) and DMFS (adjusted hazard ratio, 8.10; 95% CI, 1.11-59.1; $p < 0.0001$). Subgroup analysis by EPclin quartiles showed a stepwise increase in relapse risk, with the highest risk in participants with scores of ≥ 4.8. Limitations include the enrichment of high-risk participants due to trial eligibility criteria, widespread use of chemotherapy, and insufficient follow-up to assess late recurrences. Author disclosures include advisory roles, funding, and travel support from multiple pharmaceutical companies.

A Hayes Molecular Test Assessment (2020; updated 2023) evaluated the clinical validity, clinical utility, and analytical validity of EndoPredict. The assessment uncovered limited but positive evidence suggesting that EndoPredict may estimate the 10-year risk of DR in individuals with ER+, HER2-, N0, 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 that EndoPredict may estimate the 10-year risk of DR in individuals with ER+, HER2-, N1, 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 10 years in 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.

The Dubsky et al. (2020) prospective, translational, randomized, phase 2 ABCSG-34 trial assessed the ability of EndoPredict to predict response to NCT or NET. HR+, HER2- samples were gathered from participants, and EndoPredict testing was completed to produce a 12-gene molecular score. Participants were randomized to have either NCT or NET, based on menopausal status, HR expression, grade, and Ki67. The outcome measured was calculated via the residual cancer burden. Overall, 134 participants who were HR+ received NCT, and 83 received NET as their preoperative standard-of-care treatment. Of 134 participants who received NCT, nine were considered to have EPclin low-risk disease, and 125 were considered to have EPclin high-risk disease. EndoPredict exhibited strong sensitivity for NCT (100%; 95% CI, 89.4%-100%), even though the specificity was low (8.9%; 95% CI, 4.2%-16.2%). Of the participants treated with NET, 44 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 value of the EPclin score to predict response to NET. The residual cancer burden 0 to I outcome in participants with NET in the EPclin low-risk group was 27% compared with 7.7% in participants with EPclin

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high-risk scores. The data presented in this trial showed that EndoPredict offers supplementary predictive data beyond the traditional clinicopathologic factors used to evaluate risk and is a valuable instrument prior to operation.

Sestak et al. (2020) retrospectively investigated a cohort of patients with invasive lobular carcinoma (ILC) from previously conducted clinical trials (ABCSG-6, ABCSG-8, and 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 patients had received 5 years of endocrine treatment as the only adjuvant therapy. Information compiled from the three clinical trials included data from 2,630 postmenopausal women with ER+, HER2- BC. As part of that group, 470 (19.5%) had ILC, 1,944 (80.5%) had IDC, and 216 (8.2%) had another histological subtype. The researchers found that EPclin was highly prognostic in women with ILC (hazard ratio, 3.32; 2.54-4.34; $p < 0.0001$) and provided better prognostic value than the CTS (hazard ratio, 2.17; 1.73-2.72). Further, they found that EPclin was prognostic in women with IDC (n = 1,944) overall (hazard ratio, 2.36; 2.11-2.65; $p < 0.0001$), although 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 the Molecular Test Assessment by Hayes and in the systematic review by Hyams et al., 2024.)

Breast Cancer Index

The O'Regan et al. (2025) prognostic study used a prospective-retrospective design in the phase 3 Tamoxifen and Exemestane Trial (TEXT) and phase 3 Suppression of Ovarian Function Trial (SOFT) to evaluate BCI as a predictive biomarker for benefit from exemestane plus ovarian function suppression (OFS) vs tamoxifen plus OFS and to validate its prognostic performance. Premenopausal individuals with HR-positive, early-stage BC were randomized to receive 5 years of tamoxifen plus OFS or exemestane plus OFS. The analysis included 1,782 individuals from TEXT (median follow-up, 13 years) and 2,896 from the combined TEXT and SOFT cohorts (n = 1,782 and n = 1,114, respectively). The primary end point was BCFI, and the secondary end point was DRFI. In TEXT, individuals with BCI *HOXB13*:*IL17BR* (H/I)–low tumors (n = 1,034) had a 12-year absolute BCFI benefit of 6.6% with exemestane plus OFS compared with tamoxifen plus OFS (hazard ratio, 0.61; 95% CI, 0.44-0.85), while those with BCI H/I–high tumors (n = 748) had a similar 6.3% benefit (hazard ratio, 0.78; 95% CI, 0.57-1.07; p for interaction = 0.29). In the combined cohort, BCI H/I–low tumors showed a 5.7% benefit (hazard ratio, 0.65; 95% CI, 0.50-0.83), and BCI H/I–high tumors showed a 4.2% benefit (hazard ratio, 0.86; 95% CI, 0.67-1.09; p for interaction = 0.12). Subgroup analyses by age, nodal status, and chemotherapy use revealed consistent benefit for BCI H/I–low tumors but variable effects for BCI H/I–high tumors. BCI and BCI N+ continuous indices were significantly prognostic for DRFI in node-negative (hazard ratio, 1.27; 95% CI, 1.11-1.44; $p < 0.001$) and node-positive disease (hazard ratio, 1.58; 95% CI, 1.21-2.05; $p < 0.001$), with a 12-year DRFI of 96.3%, 90.3%, and 84.9% for low-, intermediate-, and high-risk N0 groups, respectively. The study's limitations include the lack of OFS-alone or tamoxifen-alone arms in TEXT, differences in trial designs, and exploratory subgroup analyses. The findings indicate that BCI H/I status did not clearly predict incremental benefit of exemestane vs tamoxifen when combined with OFS, although BCI remains prognostic for DR. Clinically, these results suggest that while the BCI may help identify individuals at higher ROR, its role in guiding choice between OFS-containing regimens requires further validation. Conflicts of interest include author relationships with pharmaceutical companies and Biotheranostics, Inc., which funded the translational research.

The Bartlett et al. (2024) study was a retrospective analysis of tumor samples from postmenopausal patients enrolled in the prospective phase 3 TEAM trial, which compared 5 years of aromatase inhibitor monotherapy with sequential tamoxifen followed by an aromatase inhibitor. Of 9,766 eligible patients, 3,544 with HR-positive disease and available RNA were included, comprising 1,519 with N0 and 2,025 with node-positive (N1) status. Patients who had received NCT were excluded. The primary end point was time to DR, evaluated overall (0-10 years) in those without chemotherapy, and late DR (5-10 years) in those who were disease free for 5 years. Scores for both the BCI and newly validated prognostic model BCIN+ (BCI Node-Positive) were calculated using prespecified and optimized cutoff points, and analyses were adjusted for age, tumor size, grade, and treatment, where applicable. BCI significantly stratified N0 patients without chemotherapy into low-, intermediate-, and high-risk groups for overall DR, with 10-year rates of 7.8%, 14.1%, and 23.5%, respectively; multivariate hazard ratios were 2.16 (95% CI, 1.32-3.52) for intermediate and 3.89 (95% CI, 2.42-6.24) for high risk vs low risk ($p < 0.001$). For N1 patients, BCIN+ separated low- and high-risk groups, with 10-year DR rates of 10.1% and 24.6% and a hazard ratio of 2.62 (95% CI, 1.72-3.98; $p < 0.001$). Among 2,910 patients who were disease free at 5 years, late DR rates were 5.4% vs 9.3% for N0 (hazard ratio, 2.25; 95% CI, 1.30-3.88; $p = 0.004$) and 4.8% vs 12.2% for N1 (hazard ratio, 2.67; 95% CI, 1.53-4.63; $p < 0.001$). Optimized cutoff points further reduced late DR risk in low-risk groups to 3.8% for N0 and 2.7% for N1, with corresponding hazard ratios of 2.63 (95% CI, 1.36-5.12) and 4.34 (95% CI, 2.03-9.28). These findings were consistent in HER2 subsets. The study's limitations include its retrospective nature. Conflicts of interest were reported by several authors, including funding and patents related to the BCI. The results suggest that the BCI and BCIN+ may provide independent prognostic information beyond clinicopathologic factors, supporting their use to guide decisions on chemotherapy at diagnosis and EET at 5 years. (This study is included in the Molecular Test Assessments by Hayes, 2020a, updated 2025, and Hayes, 2020b, updated 2025.)

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The Mamounas et al. (2024) prospective-retrospective translational study evaluated the predictive utility of the BCI for ELT benefit in postmenopausal individuals with HR-positive, early-stage BC enrolled in the NSABP B-42 trial. Of 3,903 eligible individuals, 2,178 were included in the translational cohort based on availability of tumor tissue and consent. All had completed 5 years of ET and were randomized to receive either 5 additional years of letrozole or placebo. The primary end point was RFI, with DR, DFS, and BCFI as secondary end points. Individuals were stratified by BCI H/I status into high- (n = 971) and low- (n = 1,207) risk groups. In the overall cohort, ELT showed a nonsignificant absolute 10-year RFI benefit of 1.6% (hazard ratio, 0.77; 95% CI, 0.57-1.05; p = 0.10), with no significant interaction between BCI H/I status and ELT for RFI (p interaction = 0.56). A time-dependent analysis of DR revealed a statistically significant benefit of ELT after 4 years in the BCI H/I–high group (hazard ratio, 0.29; 95% CI, 0.12-0.69; p < 0.01) but not in the BCI H/I–low group (hazard ratio, 0.68; 95% CI, 0.33-1.39; p = 0.29). Subgroup analyses showed that BCI H/I–high individuals with node-positive disease derived a significant 10-year absolute DR benefit of 5.6% (hazard ratio, 0.28; 95% CI, 0.10-0.77; p < 0.01), while those with prior tamoxifen therapy had a 4.3% benefit (hazard ratio, 0.16; 95% CI, 0.04-0.76; p < 0.01). In HER2-negative individuals, a significant ELT-by-BCI H/I interaction was observed after 4 years (p interaction = 0.04), with BCI H/I–high individuals having a 4.8% benefit (hazard ratio, 0.22; 95% CI, 0.08-0.60; p < 0.01). Limitations include the retrospective nature of biomarker analysis, low event rates in subgroups, and lack of adjustment for multiple comparisons. Author disclosures include employment, stock holdings, and patents as well as funding from pharmaceutical companies. (This study is included in the Molecular Test Assessment by Hayes, 2020a, updated 2025.)

The O'Regan et al. (2024) prospective-retrospective translational study assessed the predictive and prognostic performance of the BCI H/I in premenopausal individuals (n = 1,687) with HR-positive, early-stage BC enrolled in SOFT. Individuals were randomized to receive 5 years of adjuvant tamoxifen alone, tamoxifen plus OFS, or exemestane plus OFS. BCI testing was performed blinded to clinical data and outcomes. The primary end point was BCFI, and the secondary end point was DRFI. Tumors were classified as BCI H/I low (n = 972) or BCI H/I high (n = 715). Individuals with BCI H/I–low tumors experienced statistically significant 12-year absolute BCFI benefits from exemestane plus OFS (11.6%; hazard ratio, 0.48; 95% CI, 0.33-0.71) and tamoxifen plus OFS (7.3%; hazard ratio, 0.69; 95% CI, 0.48-0.97) compared with tamoxifen alone. In contrast, individuals with BCI H/I–high tumors did not benefit from either regimen (exemestane plus OFS: -0.4%, hazard ratio, 1.03, 95% CI, 0.70-1.53; tamoxifen plus OFS: -1.2%, hazard ratio, 1.05, 95% CI, 0.72-1.54; p for interaction = 0.006). Subgroup analyses showed consistent predictive performance of BCI H/I across individuals with or without prior chemotherapy, those with node-negative or node-positive disease, and those younger or older than 40 years. In the *ERBB2*-negative subset (n = 1,442), BCI H/I–low tumors showed significant benefit from exemestane plus OFS (13.2%; hazard ratio, 0.39; 95% CI, 0.25-0.60) and tamoxifen plus OFS (7.4%; hazard ratio, 0.64; 95% CI, 0.44-0.93), while BCI H/I–high tumors did not. BCI was also significantly prognostic for DRFI in node-negative individuals (n = 1,110; p = 0.004), with a 12-year DRFI of 95.9% in low-risk, 90.8% in intermediate-risk, and 86.3% in high-risk groups. The results suggest that BCI H/I may help identify premenopausal individuals who are most likely to benefit from OFS-containing ET and potentially guide treatment intensity and duration. Limitations of the study include the retrospective nature of biomarker analysis, exploratory subgroup analyses, and small sample sizes in some subgroups. Author disclosures include employment, patents, consulting fees, and funding from pharmaceutical companies. (This study is included in the Molecular Test Assessments by Hayes, 2020a, updated 2025, and Hayes, 2020b, updated 2025.)

The Sanft et al. (2024) prospective, multicenter registry study evaluated the impact of the BCI on EET decision-making in participants with stage I to III, HR-positive BC who had completed 4 to 7 years of adjuvant ET and remained recurrence free. Participants with noninvasive tumors or disease involving the chest wall or skin were excluded. Enrollment began in April 2021, and this analysis included 843 participants with completed physician questionnaires and 823 with completed participant questionnaires. The cohort was predominantly postmenopausal (88.4%), with a mean age of 65 years; most tumors were T1 (74.7%), grade 2 (53.4%), and node negative (76.0%). Prior ET consisted of aromatase inhibitor monotherapy in 73.4%, tamoxifen in 14.5%, and a sequential tamoxifen-aromatase inhibitor in 11.7%. BCI testing classified 61.3% as low likelihood and 38.7% as high likelihood to benefit from EET and 47.1% as low risk vs 52.9% as high risk for late DR. Following BCI testing, physicians changed EET recommendations in 40.1% of cases (p < 0.0001), with 63.3% of these changes from recommending EET to not recommending it and 36.7% in the opposite direction. Overall, EET recommendations decreased from 56.8% prior to the BCI to 45.7% post BCI (p < 0.0001). Among participants classified as BCI H/I high, recommendations for EET increased from 62.1% to 91.2%, whereas in those classified as H/I low, recommendations decreased from 53.6% to 18.3%. Physician confidence improved significantly, with high confidence rising from 58.2% to 80.5% (p < 0.0001). Participants' comfort with their decision increased from 70.8% to 84.0%, and 45.1% changed their EET preference, most often decreasing preference when the BCI indicated a low likelihood of benefit. Concerns about cost, drug safety, and benefit of EET were significantly reduced (p < 0.0001, p = 0.0014, and p = 0.0002, respectively). Limitations include reliance on self-reported questionnaires and some missing data, although rates were low (< 4%). Potential bias from self-reporting and industry affiliations disclosed by several authors may affect reliability. These findings suggest that incorporating the BCI into practice can personalize EET decisions, reduce overtreatment, and improve confidence and satisfaction for both physicians and individuals. (This study is included in the Molecular Test Assessments by Hayes, 2020a, updated 2025, and Hayes, 2020b, updated 2025.)

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The Woolpert et al. (2024) systematic review and meta-analysis explored the role of biomarker tests in the prediction of clinical response to EET. A total of five studies met the eligibility requirements and were included; four investigated the BCI assay in three unique study populations, and one explored the predictive ability of Ki67 and PR status. The studies that were focused on the BCI reliably demonstrated that the BCI score predicted response to an extension of ET in 1,946 combined individuals (primarily non-Hispanic White and postmenopausal). The authors recommended further study of an assortment of biomarkers in diverse populations. Studies by Noordhoek et al. (2021) and Bartlett et al. (2019), previously discussed in this policy, were included in the Woolpert et al. systematic review and meta-analysis.

Hayes (2020a; updated 2025) evaluated the use of the BCI test for predicting the likelihood of benefit from EET (greater than 5 years) and estimating the risk of late (greater than 5 years from diagnosis) and cumulative DR risk over 10 years in individuals diagnosed with HR+, N0 or N1, invasive BC treated with 5 years of primary adjuvant ET or primary ET. The Hayes assessment found insufficient evidence to support the BCI test for the prediction of likelihood of benefit from EET or to estimate the risk of late and cumulative DR risk over 10 years in these situations.

In individuals with HR+, N1, invasive BC, Hayes (2020b; updated 2025) again found the evidence supporting the use of the BCI for predicting the benefit of EET or estimating the risk of DR in individuals treated with 5 years of primary adjuvant chemotherapy to be lacking. Further investigations, including large, prospective, randomized trials examining diverse populations and health outcomes related to the use of the BCI test, were recommended.

Other Breast Cancer Assays

GEP assays for BC or ductal carcinoma in situ (DCIS), other than those previously described, including but not limited to DCISionRT and Oncotype DX Breast DCIS Score, lack sufficient evidence to support clinical utility at this time.

The Dickinson et al. (2024) systematic review and meta-analysis examined 37 clinical studies that included 4,264 female individuals with metastatic or stage IV BC to evaluate whether baseline circulating tumor DNA (ctDNA) alterations were associated with survival outcomes [OS, progression-free survival (PFS), or DFS]. Among the 3,162 records screened, 75 distinct survival analyses were extracted from the 37 included articles. These studies varied in design (54% prospective), BC subtype, timing of blood collection, ctDNA detection method [67% next-generation sequencing (NGS) and 29% digital polymerase chain reaction], and targeted mutations. Across all analyses, detection of a ctDNA alteration at baseline was associated with worse survival, with a pooled hazard ratio of 1.40 (95% CI, 1.22-1.58; $p < 0.001$). Subgroup analyses showed consistent associations across survival end points, including OS (hazard ratio, 1.44; 95% CI, 1.24-1.65), PFS (hazard ratio, 1.31; 95% CI, 1.07-1.55), and DFS (hazard ratio, 1.56; 95% CI, 1.22-1.89). *TP53* alterations demonstrated a clear association with reduced survival (hazard ratio, 1.58; 95% CI, 1.34-1.81), while *ESR1* alterations showed a smaller, nonsignificant effect (hazard ratio, 1.28; 95% CI, 0.96-1.60). *PIK3CA* alterations were not associated with reduced survival (hazard ratio, 1.19; 95% CI, 0.85-1.53). Significant associations were also seen in combined analyses of less common variants such as *ERBB2*, *KRAS*, *FGFR*, and *PTEN* (hazard ratio, 1.82; 95% CI, 1.26-2.39). Associations persisted across prospective and retrospective designs and were consistent regardless of ctDNA detection method or blood tube type. Publication bias was not evident. The authors noted methodological limitations, including heterogeneity in prior treatments and the absence of male individuals. The results suggest that detection of specific baseline ctDNA alterations, particularly *TP53*, may help characterize prognosis in metastatic BC, although ctDNA remains a prognostic rather than actionable biomarker at this stage.

The Chen et al. (2024) systematic review and meta-analysis examined long-term outcomes in individuals with low-risk DCIS across different treatment strategies, including studies that incorporated genomic assays for risk stratification. The review included 33 studies (one RCT, a pooled analysis of four RCTs, and 31 observational cohorts), encompassing 47,696 individuals diagnosed between 1950 and 2018. Low-risk DCIS was defined variably, most often by low or intermediate grade and small tumor size and, in some studies, by genomic assays such as DCISionRT (decision score ≤ 2.8 without residual risk subtype) and Oncotype DX (DCIS score < 39). The primary outcome was an invasive ipsilateral breast tumor event (IBTE) at 5 and 10 years; the secondary outcomes included DCIS-IBTE, total IBTE, contralateral BC, and BCSS. Pooled IBTE rates were 3.3% (95% CI, 1.3%-8.1%) at 5 years and 5.9% (95% CI, 3.8%-9.0%) at 10 years. Surgery significantly reduced IBTE compared with no surgery at 5 years (3.5% vs 9.0%; $p = 0.003$) and 10 years (6.4% vs 22.7%; $p = 0.008$). Among individuals undergoing breast-conserving surgery (BCS), adjuvant RT (ART) further lowered IBTE risk at 5 years (1.3% vs 3.5%; $p < 0.001$) and 10 years (3.9% vs 6.9%; $p = 0.004$). Studies using DCISionRT and Oncotype DX demonstrated similar trends: in one Oncotype DX cohort, the 10-year total IBTE was 4.1% with BCS plus RT vs 10.6% with BCS alone (95% CI, 1.09%-2.13%; $p = 0.0141$). The pooled 10-year BCSS was 98.8% (95% CI, 95.3%-99.7%). No studies assessed ET outcomes in relation to genomic risk scores, and publication bias was detected for some outcomes. Most studies were retrospective, with moderate-quality scores, introducing potential threats to validity such as heterogeneity in risk definitions and the lack of standardized follow-up. These findings suggest that surgery and RT reduce progression risk in low-risk DCIS and that genomic assays may help identify individuals who benefit most from

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adjuvant therapy, although prospective trials are needed to confirm these observations. (Weinmann et al., 2020, and Bremer et al., 2018, previously cited in this policy, are included in this systematic review.)

The Ouattara et al. (2023) systematic review and meta-analysis evaluated women with DCIS who had been treated with BCS to determine the impact of ART on local recurrence according to risk stratification per molecular signature testing. Five studies, which included 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 plus RT vs BCS alone on local recurrence was analyzed. The analysis included both ipsilateral invasive breast events (InvBEs) and total breast events (TotBEs). Two molecular signature tests were used: the Oncotype DX DCIS (prognostic of local recurrence) and DCISionRT (prognostic of local recurrence and predictive of RT benefit). In the high-risk group, for DCISionRT, the pooled hazard ratio of BCS plus RT vs BCS alone was 0.39 (95% CI, 0.20-0.77) for InvBEs and 0.34 (95% CI, 0.22-0.52) for TotBEs. In the low-risk group, the pooled hazard ratio of BCS plus RT vs BCS alone was significant for TotBEs at 0.62 (95% CI, 0.39-0.99) but was not significant for InvBEs (hazard ratio, 0.58; 95% CI, 0.25-1.32). The researchers maintained that molecular signatures can discriminate between high- and low-risk individuals; individuals at high risk had a significant benefit with RT for the reduction of invasive and in situ local recurrence, and individuals at low risk did not have benefit for prevention of recurrence of invasive BC. While molecular signatures may be promising tools for balancing over- and undertreatment of DCIS, further understanding of the basis of invasive cancer is needed. The study is limited by the lack of data on BC-specific mortality and individual data on recurrence-free survival. Further high-quality evaluation, including studies focused on the impact on mortality, is required.

DCISionRT

Hayes (2022; updated 2025) 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 2025 update of this assessment identified six newly published studies that may meet the inclusion criteria set out in the original report and an unlikely change in the current Hayes rating of D2.

Shah et al. (2021) documented the results of the PREDICT study, which was a prospective, multi-institutional, observational registry designed to evaluate the clinical utility of testing with DCISionRT on clinical recommendations regarding RT for participants who had undergone BCS for a diagnosis of DCIS. The study included 539 women who were over the age of 25 years and 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 who had previously received a recommendation for RT had a change in recommendation to not receive RT. Conversely, among 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 the RT treatment plan was made for 42% of women in the study, with a net reduction in overall RT recommendations of 20%. The elevated decision score had the strongest association with an RT recommendation [odds ratio (OR), 43.4] compared with 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 that it was the factor most strongly associated with RT recommendations compared with traditional decision-making. The authors also noted limitations to the study. One such limitation is the lack of participant- or physician-reported outcomes regarding satisfaction or quality (pending at the time of publication). In addition, data on recommendations for RT were only based on two points in time: prior to 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 is included in the Molecular Test Assessment by Hayes, 2022, updated 2025.)

Oncotype DX Breast DCIS Score

A Hayes (2024; updated 2026) Molecular Test Assessment addressed the Oncotype DX Breast DCIS Score. The assessment revealed an overall low-quality body of evidence addressing the use of the DCIS score test in individuals recently diagnosed with DCIS to evaluate 10-year recurrence risk and assist with treatment decision-making. Although there is limited evidence suggesting that this test may be correlated with reduced RT in those individuals with low DCIS scores, the clinical outcomes of DCIS score–based treatment decisions are unknown. No studies were identified that assessed the performance or clinical utility of the current version of the test, and no studies directly comparing the test with other clinical tools for predicting recurrence risk and informing treatment were identified. The 2026 update of this assessment identified one newly published clinical utility study that may meet the Hayes inclusion criteria set out in the original report, but it is unlikely to change the current Hayes rating.

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Clinical Practice Guidelines

American Society of Clinical Oncology (ASCO)

Andre et al. (2022) updated the 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 patients or patients 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 patients with node-negative, ER+, HER2- BC.
- For premenopausal patients, Oncotype DX may be used with node-negative, ER+, HER2- BC.
- BCI may be offered to patients with zero to three positive nodes who have undergone 5 years of ET, with no evidence of recurrence, to aid with decision-making regarding EET.
- No assays are recommended for patients with HER2+ or triple-negative BC.

Evidence from ASCO's review indicates that premenopausal women with one to three positive nodes will benefit from chemotherapy regardless of genomic assay results. No data supporting the use of genomic tests for informing adjuvant chemotherapy in patients with four or more positive nodes were identified. ASCO further recommends the incorporation of factors such as disease stage, comorbidities, and patient preference into decision-making.

Hassett et al. (2020) published recommendations for managing male BC. These recommendations were the result of a review of 26 reports/observational studies by an ASCO expert panel. The panel found that several of the management methods used for men with BC are predominantly the same as those used for women. The panel recommended GEP to guide adjuvant treatment decision-making in male BC.

European Society for Medical Oncology (ESMO)

In the 2024 ESMO clinical practice guideline addressing the diagnosis, treatment, and follow-up of early BC, Loibl et al. makes the following recommendations regarding the use of GEP tests:

- When uncertainty about indications for adjuvant chemotherapy exists after consideration of all clinical and pathological factors, GEP testing and endocrine response assessment in the preoperative setting can be used (level of evidence II; grade of recommendation B).
- The treatment strategy for each patient should be based on their own risk-benefit analysis, considering many factors, including tumor burden and biology (including biomarkers and gene expression) as well as age, menopausal status, general health, and individual preference (level of evidence I; grade of recommendation A).

(Level of evidence and grades of recommendation are adapted from the Infectious Diseases Society of America-United States Public Health Service Grading System.)

National Comprehensive Cancer Network® (NCCN®)

The NCCN's guidance indicates that multigene panel testing using a tumor tissue or plasma-based ctDNA assay is recommended as an option to identify candidates for targeted therapies in recurrent/stage IV (M1) disease; if one specimen is negative for actionable biomarkers, testing on the alternative specimen can be considered (category 2A recommendation). The NCCN BC guidelines also indicate that the use of GEAs is not required for staging. The 21-gene assay (Oncotype DX) is preferred by the NCCN Breast Cancer Panel for the prognosis and prediction of chemotherapy benefit. Other prognostic GEAs can provide prognostic information, but the ability to predict chemotherapy benefit is unknown. The NCCN notes that the BCI test is predictive of benefit of extended adjuvant ET (NCCN Breast Cancer, v2.2026).

The NCCN categorizes the primary GEAs for consideration of adjuvant systemic therapy in patients with invasive BC as follows:

Assay	Predictive	Prognostic	NCCN Category of Preference	NCCN Category of Evidence and Consensus
21-Gene 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
21-Gene Oncotype DX for pN1 (one to three positive nodes)	Yes	Yes	Postmenopausal: Preferred	1
			Premenopausal: Other recommended	2A
70-Gene MammaPrint for pN0 and pN1 (one to three positive nodes)	Not determined	Yes	Other recommended	1
50-Gene Prosigna for pN0 and pN1 (one to three positive nodes)	Not determined	Yes	Other recommended	2A
12-Gene EndoPredict for pN0 and pN1 (one to three positive nodes)	Not determined	Yes	Other recommended	2A
BCI	Predictive of benefit of extended adjuvant ET	Yes	Other recommended	2A

NCCN Categories of Preference:

- Preferred: Interventions that are based on superior efficacy, safety, and evidence; and, when appropriate, affordability.
- Other recommended: Other interventions that may be somewhat less efficacious, more toxic, or based on less mature data; or significantly less affordable for similar outcomes.

National Institute for Health and Care Excellence (NICE)

The 2024 NICE guideline addressing tumor profiling tests for informing adjuvant chemotherapy decisions in early BC makes the following recommendations regarding gene expression tests:

- EndoPredict, Oncotype DX, or Prosigna may be used as options, along with consideration of clinical risk factors, to guide adjuvant chemotherapy decisions for the treatment of ER- or PR-positive, HER2-negative, early BC with one to three positive LNs in women who have been through menopause; men; and/or transgender, nonbinary, or intersex people, depending on their hormonal profile. Clinical judgment should be used to determine if testing is appropriate for men and transgender, nonbinary, or intersex patients.
- EndoPredict, Oncotype DX, or Prosigna should not be used to guide adjuvant chemotherapy decisions for ER- or PR-positive, HER2-negative, early BC with one to three positive LNs in women who have not been through menopause.
- MammaPrint should not be used to guide adjuvant chemotherapy decisions for patients with ER- or PR-positive, HER2-negative, early BC with one to three positive LNs.
- MammaPrint and IHC4 plus clinical factors should not be used to guide adjuvant chemotherapy decisions for people with ER- or PR-positive, HER2-negative, and LN-negative early BC.
- EndoPredict, Oncotype DX, or Prosigna may be used to guide adjuvant chemotherapy decisions for ER- or PR-positive, HER2-negative, early BC if:
  - The patient undergoing testing will use the results to help in the decision, along with their health care professional, whether to have adjuvant chemotherapy.
  - The tests are used for their intended purpose:
    - EndoPredict (ER positive or both ER and PR positive).
    - Oncotype DX (ER or PR positive or both).
    - Prosigna (ER or PR positive or both; only for women who have been through menopause).
- The test and results should be used alongside NICE's guideline on shared decision-making. An oncologist should explain to the patient what their tumor profiling test results mean and the risks and benefits of treatment options, based on all available risk factors.

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Lung Cancer

Wang et al. (2020) conducted a cohort study using a multiplexed polymerase chain reaction–based panel to simultaneously test 118 hotspot mutations and fusions in nine driver genes capable of comprehensively determining individual genotypes as tumor predictive biomarkers. Surgically resected samples from 214 participants with non-small cell lung cancer (NSCLC; 168 with adenocarcinomas and 46 with squamous cell cancers) were included in this cohort study. Genetic alterations were detected in 143 participants (66.8%). The three most common alterations identified were *EGFR* mutations (50.9%), *KRAS* mutations (8.4%), and *ALK* fusions (4.7%). Eight participants (3.7%) harbored concurrent mutations, and the most common partners were *EGFR* mutations, which were observed in the eight participants. No associations between survival and *EGFR, KRAS*, and *ALK* status were observed. Participants with two or more alterations had shorter DFS than those with single mutations ($p = 0.032$) but without a difference in OS ($p = 0.245$). However, TNM stage was an independent predictor of OS (hazard ratio, 2.905; $p < 0.001$) as well as DFS (hazard ratio, 2.114; $p < 0.001$) in this cohort in a multivariate analysis. Participants with the L858R mutation in *EGFR* had longer DFS ($p = 0.014$) compared with 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 suggests that 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 with 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 individuals with lung adenocarcinoma, along with a ≤ 15 pack-year smoking history, whose tumors previously tested negative for alterations in 11 genes (mutations in *EGFR, ERBB2, KRAS, NRAS, BRAF, MAP2K1, PIK3CA*, and *AKT1* and fusions involving *ALK, ROS1*, and *RET*) via multiple non-NGS methods. A broad, hybrid capture–based NGS assay (FoundationOne) evaluated 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 NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) for NSCLC was found in 26% of individuals (n = 8). The drivers identified in tumors from these eight individuals were *EGFR* G719A*, BRAF* V600E*, SOCS5-ALK, HIP1-ALK, CD74-ROS1, KIF5B-RET* (n = 2), and *CCDC6-RET*. Six of the individuals went on to receive targeted therapy. The authors noted that the reasons for nondetection of these genomic alterations via non-NGS testing can be varied, such as lower sensitivity, complex rearrangements undetectable by standard FISH, and, possibly, heterogeneity between different tumor biopsies or sites. They concluded that broad, hybrid capture–based NGS assays have the potential to uncover clinically actionable genomic alterations in never-smokers or smokers with ≤ 15 pack-years whose lung adenocarcinomas do not harbor a potential driver via non-NGS testing.

Percepta Genomic Sequencing Classifier

A 2023 (updated 2024) Hayes Molecular Test Assessment evaluated the use of the Percepta Genomic Sequencing Classifier (GSC; Veracyte, Inc.) to stratify the risk of primary lung cancer to guide the management of current or former smokers with inconclusive bronchoscopy results. One poor-quality study that assessed clinical validity reported high NPVs for down-classifying risk of malignancy (ROM) in individuals at low and intermediate risk, mixed PPVs for up-classifying ROM in individuals at intermediate and high risk, and unknown clinical performance in individuals with unchanged ROM after Percepta GSC testing. Regarding clinical utility, one poor-quality study reported statistically insignificant reductions in invasive procedure plans in individuals whose ROMs were down-classified (74% reduction) and unchanged (50% reduction). This low-quality body of evidence is insufficient to fully assess the clinical benefits of the Percepta GSC. The 2024 update to this Hayes assessment revealed no newly published studies that met the inclusion criteria for evaluation of this test.

Clinical Practice Guidelines

American Society of Clinical Oncology (ASCO)

ASCO (Reuss et al., 2025) recommends that biomarker testing with a tissue- and/or blood-based broad multigene panel should be universally accessible for all patients diagnosed with NSCLC (evidence quality: high; strength of recommendation: strong). They advised that while the false-negative rate of liquid biopsy should be considered, the combination of blood and tissue testing may maximize the chance of detecting molecular alterations. Tissue testing should be attempted when feasible to facilitate histological assessment. To inform second-line and subsequent treatment options based on driver alterations, the ASCO guideline includes a note that advises that every effort should be made to assess for the presence of new mutations by tissue and/or blood testing by NGS due to the development of potentially targetable resistance mechanisms.

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ASCO (Kalemkerian et al., 2018) endorsed the 2018 clinical practice guideline update jointly issued by the College of American Pathologists (CAP), International Association for the Study of Lung Cancer, and Association for Molecular Pathology (AMP), with minor modifications. The guidelines supported by ASCO include the following relevant points:

- Physicians may use molecular biomarker testing:
  - In tumors with an adenocarcinoma component;
  - In tumors with nonsquamous, non–small cell histology; or
  - When clinical features indicate a higher probability of an oncogenic driver [e.g., young patient age (< 50 years) and light or absent tobacco exposure] (expert consensus opinion).
- It is appropriate to include *ERBB2*, *RET*, *KRAS*, and/or *MET* molecular testing as part of larger testing panels performed either initially or when routine *EGFR, ALK, BRAF*, and *ROS1* testing is negative (expert consensus opinion).
- Multiplexed genetic sequencing panels are preferred where available over multiple single-gene tests to identify other treatment options beyond *EGFR, ALK, BRAF*, and *ROS1* (expert consensus opinion).
- There is currently insufficient evidence to support the use of circulating cell-free plasma DNA molecular methods for the diagnosis of primary lung adenocarcinoma (no recommendation).

National Comprehensive Cancer Network (NCCN)

Non-Small Cell Lung Cancer

The NCCN Guidelines® for NSCLC (v3.2026) 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 that is unlikely to provide clinical benefit. The NCCN recommends that when feasible, testing should be performed via a broad, panel-based approach, most often using NGS, acknowledging that many of the marketed NGS-based assays that are used to fully genotype NSCLC are larger than 50 genes. Use of these larger panels may be practical to achieve full genotyping information. In addition, the guidelines include a discussion of the role of plasma cell-free/ctDNA testing, stating that ctDNA testing should not be used in lieu of a tissue diagnosis and is generally not recommended in settings other than advanced/metastatic disease. However, the NCCN also suggests the use of ctDNA testing (using clinically validated tests) in certain clinical circumstances, including the following:

- The patient is medically unfit for invasive tissue sampling.
- There is insufficient tumor tissue available for molecular analysis; however, ctDNA should be followed by tissue-based analysis when an oncogenic driver is not identified.
- Consideration of ctDNA and tissue-based testing in parallel to identify resistance mechanisms is recommended as an option at the time of progression.
- RNA-based NGS is recommended as an option if no identifiable driver oncogenes are identified with DNA-based molecular profiling.

In addition, peripheral blood testing (most commonly using plasma-based testing of ctDNA) can be performed in conjunction with tissue-based testing to accomplish the necessary genotyping for recommended biomarkers. Concurrent testing can improve turnaround time for results and should be considered in appropriate clinical circumstances.

Small Cell Lung Cancer

The NCCN Guidelines for small cell lung cancer (v1.2026) state that consideration of comprehensive molecular profiling via blood, tissue, or both is recommended as an option in rare cases, particularly for patients with extensive-stage/relapsed disease who do not smoke tobacco, who lightly smoke, who have remote smoking history, who have a diagnostic or therapeutic dilemma, or at the time of relapse.

Prostate Cancer

The Tabriz et al. (2025) systematic review evaluated the impact of three tissue-based genomic classifiers (GCs) [Decipher (Veracyte, Inc.), Genomic Prostate Score (GPS; mdxhealth Inc.), and Prolaris (Myriad Genetics Laboratories, Inc.)] on risk stratification and treatment decisions among individuals with localized prostate cancer (PCa) who were considering first-line therapy. Overall, 19 studies were included, comprising one RCT (ENACT) and 18 observational cohorts. Most studies were conducted in the United States between 2010 and 2024. The risk of bias was low in seven studies, moderate in nine, and high in three. In low-risk individuals, observational studies showed that GC testing rarely led to reclassification to higher risk categories (GPS: 0%-11.9%; Decipher: 12.8%-17.1%; Prolaris: 21.8%-23.1%). However, the ENACT trial found that GPS testing reclassified 34.5% of very low-risk and 29.4% of low-risk individuals to higher risk categories. Among intermediate-risk individuals, observational studies reported minimal upward reclassification (0%-1.7%) and more frequent downward shifts (3.8%-28.8%), while the ENACT trial reported that 69.2% reclassified to higher risk and 15.4% reclassified to lower risk. Reclassification patterns varied by test type and baseline risk classification system. Regarding treatment decisions, 12 observational studies indicated that GC testing was associated with increased recommendations

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for active surveillance, with relative increases ranging from 7.5% to 61.8%. In contrast, two analyses from the ENACT trial found that GPS testing modestly decreased active surveillance selection and increased preferences for prostatectomy or radiation (OR for active treatment preference, 2.55; 95% CI, 1.25-5.43). The Decipher and Prolaris tests were also associated with shifts toward conservative management, although Prolaris showed mixed effects, depending on baseline risk. Limitations include the heterogeneity in clinical practices, risk classification systems, and test interpretation as well as a moderate to high risk of bias in most treatment-related studies. Many studies were supported by test developers. Overall, while GC tests may influence risk stratification and treatment decisions, findings were inconsistent across study designs and test types, underscoring the need for well-designed trials to clarify their clinical utility. (Morris et al., 2021, previously cited in this policy, is included in this systematic review.)

Boyer et al. (2025) conducted a systematic review to evaluate the prognostic capability of three GCs (Decipher, GPS, and Prolaris) for biochemical recurrence (BCR), development of metastases, and PCa-specific mortality in individuals with localized PCa. All individuals had been treated with definitive surgery and/or radiation. The prognostic ability of the three GCs' outcomes was compared with standard clinical risk stratification models. Overall, 39 studies, comprising over 10,000 individuals, were included. The results of the review revealed that each of the three GCs showed a slightly improved prognostic ability for BCR, development of metastatic disease, and PCa-specific mortality compared with commonly used risk classification methods. However, the certainty of evidence was low to very low; this was predominantly due to bias related to the retrospective nature of 37 of the 39 studies, heterogeneity in the treatments received, and time period of the treatments (prior to 2000; detection and management of PCa has advanced significantly since this time). In addition, the risk classification models used as comparators to the GC results were not consistent across the studies reviewed. Nevertheless, the authors concluded that based on the overall results, GCs do provide a small but consistent improvement in the predictive ability of standard risk stratification models, which could influence treatment decisions when there is uncertainty regarding the best options for treatment. They recommended further analysis, with more up-to-date data. Of note, multiple studies included in the systematic review were sponsored or coauthored by companies with rights to the GC tests. Publications by Vince et al. (2022), Brooks et al. (2021), Feng et al. (2021), Kornberg et al. (2019), Berlin et al. (2019), Klein et al. (2016), Glass et al. (2016), and Cuzick et al. (2015), previously discussed in this policy, were included in this systematic review.

The Spohn et al. (2023) systematic review explored the evidence on the use of GCs in individuals treated with RT; the authors conducted a survey among 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 the 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 five studies were identified and sent to the reviewers, for a total of 31. Ongoing clinical trials were also screened, and nine studies on GC 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 the 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 PCa (30%), in the postoperative setting (27%), and in newly diagnosed PCa (23%). Of the experts surveyed, 47% did 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 feel that GCs have potential for use in guiding treatment decisions for RT-field definition and intensification/deintensification in various stages of disease. The study authors postulated that the outcome of this study confirms (1) the value of GCs and (2) the promising evidence that is emerging regarding the utility of GCs with respect to RT. The authors recommended ongoing study of GCs as prognostic biomarkers and of the predictive ability of GCs for optimization of RT and/or systemic therapy; they are awaiting 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), Feng et al. (2021), Marascio et al. (2020), and Berlin et al. (2019), previously discussed in this policy, were included in the Spohn et al. systematic review.

Hu et al. (2018) conducted an observational study to evaluate the current utility of gene expression classifiers (GECs) related to the management of newly diagnosed, localized PCa. 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 GECs, and the effect of GECs' results on the management of PCa. Using the Michigan Urological Surgery Improvement Collaborative registry, the researchers determined that 18.8% of 3,966 individuals who were newly diagnosed with PCa underwent testing with a GEC. The rate of use of the GEC varied in individual practice settings from 0% to 93%, and individuals who had GEC testing were more likely to have a lower prostate-specific antigen (PSA) level, lower Gleason score, lower clinical T stage, and fewer positive cores (all $p < 0.05$). In those individuals with clinically favorable cancer risk, the rate of active surveillance was significantly different among those with GEC results above the

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threshold (46.2%), those with GEC results below the threshold (75.9%), and those who did not have GEC testing (57.9%). Based on these results, the authors estimated that for every nine individuals with favorable cancer risk who participate in GEC testing, one additional individual may be managed with active surveillance. Individuals with favorable-risk PCa 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 (OR, 1.84; p = 0.006). The researchers concluded that there are currently high levels of variability among practices regarding 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 GEC testing should be included in the initial care of individuals with PCa to improve clinical outcomes is encouraged. (This study is included in the Molecular Test Assessment by Hayes, 2024, updated 2025.)

Genomic Prostate Score

The Cui et al. (2024) meta-analysis evaluated the prognostic value of the 17-gene GPS test in 1,962 individuals with clinically localized PCa. Eight articles, based on seven cohort studies, were included, and all were deemed high quality by the reviewers. Follow-up periods ranged from 20 months to 15.5 years. Five studies reported on the association between GPS scores and distant metastases; the pooled hazard ratio of distant metastases was 5.22 for the high-GPS group vs the low-GPS group, with no evidence of significant heterogeneity among the studies. Further analysis of these five studies revealed that each 20-unit increase in GPS was significantly associated with distant metastases (hazard ratio, 2.99; 95% CI, 1.97-4.53). Six studies reported on the relationship between GPS and BCR. The pooled hazard ratio of BCR was 4.41 (95% CI, 2.29-8.49) for the high-GPS group vs the low-GPS group, with significant heterogeneity between studies (I^2 = 78.4%; p = 0.010). In this analysis, a 20-unit increase in GPS had a significant association with BCR (hazard ratio, 2.18; 95% CI, 1.64-2.89). A subgroup analysis showed improved prognostic value per 10-point GPS increase for studies that reported more than 5 years of follow-up. Four studies reported results on the relationship between GPS and PCa-specific mortality; these showed a pooled hazard ratio of 3.81 (95% CI, 1.74-8.33) for the high-GPS vs the low-GPS group, with no evidence of significant heterogeneity between studies. For this analysis, there was, again, a significant association between a 20-unit increase in GPS and PCa-specific mortality (hazard ratio, 3.14; 95% CI, 1.86-5.30). Based on these results, the researchers asserted that a higher GPS predicts distant metastases, BCR, and PCa-specific mortality in individuals with clinically localized PCa. They suggested that the GPS test may improve the accuracy of risk stratification and assist with clinical decision-making in affected individuals and encouraged further large-scale, prospective studies. Studies by Janes et al. (2023), Helfand et al. (2022), Brooks et al. (2021), and Kornberg et al. (2019), previously discussed in this policy, were included in this meta-analysis. (This study is included in the Molecular Test Assessment by Hayes, 2024, updated 2025.)

In a 2024 Molecular Test Assessment (updated 2025), Hayes evaluated the use of the GPS assay to inform choice between active surveillance or more aggressive treatments in individuals with untreated, localized PCa who met NCCN very low-risk, low-risk, or favorable intermediate-risk criteria. Hayes concluded that the overall body of evidence is of very low quality and insufficient to draw conclusions regarding this use of the GPS assay. Overall, the evidence suggests that the GPS assay may be a useful tool for informing management decisions, but evidence for its accuracy in lower-risk populations is limited, and it is not clear whether GPS-influenced management decisions resulted in more favorable outcomes in these individuals. Limited evidence suggests that the GPS assay can predict adverse pathology at radical prostatectomy (RP) and can help direct decisions in terms of treatment intensity and active surveillance. Evidence comparing GPS with other risk stratification tools is also limited; the additive use of GPS may improve the CAPRA (Cancer of the Prostate Risk Assessment) risk stratification tool but not NCCN risk group stratification for predicting adverse pathology. Finally, evidence is conflicting regarding whether GPS testing generally leads to increased or decreased treatment intensity and whether any treatment decisions based on GPS testing are appropriate and beneficial for the individual.

In another 2024 Molecular Test Assessment (updated 2025), Hayes evaluated the use of the GPS assay to inform treatment intensity in individuals with untreated, localized PCa who met NCCN unfavorable intermediate-risk or high-risk criteria. Hayes identified an overall low-quality body of evidence, which provided insufficient data from which to draw conclusions for this population. While some evidence suggests that the GPS assay may predict the risk of PCa recurrence, metastasis, and death in individuals with higher-risk, localized disease and potentially inform treatment decisions, the overall body of evidence is small, with limited comparisons to other risk prediction models. No studies evaluated the impact of the testing on health outcomes in affected individuals.

Covas Moschovas et al. (2022) performed a retrospective analysis in 749 patients who had undergone GPS testing to evaluate the association between GPS and final pathology [including extraprostatic extension (EPE), positive surgical margin, and seminal vesicle invasion (SVI)]. After testing, the patients had robotic RP performed by the same surgeon. In an OR 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, the percentage of cases with EPE and SVI increased with GPS when they were grouped by quartile. Based on these results, the authors asserted that GPS is

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significantly associated with adverse pathology after RP and noted that the risk of EPE and SVI will increase with GPS. They suggested that the use of GPS may help providers improve preoperative counseling and implement surgical plans for individuals with a greater risk of EPE or other adverse pathology. This study was included in the Hayes Molecular Test Assessment of GPS for lower-risk localized PCas (2024; updated 2025).

Eggener et al. (2019) performed a multicenter study to validate the GPS assay on biopsy tissue to predict adverse pathology in a group of 1,200 prospectively enrolled participants with very low-, low-, and favorable intermediate-risk PCa. A prespecified subanalysis of GPS from biopsy and its relationship with adverse pathology found on RP was performed in the group of participants who immediately proceeded to RP. A total of 114 participants underwent RP, and of them, 40 had adverse pathology. In this study, GPS results were shown to be a significant predictor of adverse pathology, based on results of a univariable analysis [OR per 20 GPS units (OR/20 units): 2.2; 95% CI, 1.2-4.1; p = 0.008]. 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 = 0.04), or NCCN risk group (OR/20 units: 2.0; 95% CI, 1.1-3.7; p = 0.02). The researchers also evaluated the impact of GPS scores on physician and participant attitudes about decision-making related to their management; Decisional Conflict Scale scores improved significantly (from 27 to 14) after GPS testing was performed. Based on the overall results, the authors concluded that the GPS assay is an independent predictor of adverse pathology at surgery and reduces decision-making conflict. This study was included in the Hayes Molecular Test Assessment of GPS for lower-risk localized PCas (2024; updated 2025).

Prolaris Biopsy Test

In a multicenter, retrospective, observational study, Kaul et al. (2019) evaluated the selection of active surveillance, along with the safety and durability of Prolaris. The study population was patients with low-risk PCa [according to both Prolaris score (disease-specific mortality ≤ 3.2%) and NCCN Guidelines] who had previously undergone Prolaris testing during their care. Initial treatment selection (active surveillance vs treatment) and duration of active surveillance were evaluated. The adverse events measured were BCR and metastasis of disease. Of 664 patients with low-risk disease (per Prolaris score and NCCN Guidelines), 82.4% (n = 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 patients who chose active surveillance experienced an adverse event, and two-thirds of the patients remained on active surveillance for more than 3 years. Markers of tumor aggressiveness showed the only significant difference between the two groups; patients who underwent definitive treatment within 6 months of diagnosis had more aggressive pathological features than those who chose active surveillance. The authors determined that the use of Prolaris in evaluating PCa risk can safely increase selection of active surveillance compared with the use of only clinical/pathological criteria and potentially allow more individuals to avoid unnecessary treatment of PCa and treatment-related side effects. Limitations include the lack of a control group to assess active surveillance selection and durability in patients who did not receive a Prolaris score; a relatively short median follow-up time; and inclusion of only patients with low-risk PCa. In addition, several study authors disclosed employment or association with the manufacturer of Prolaris, creating the potential for bias. This study was included in the Hayes Molecular Test Assessment of the Prolaris Biopsy Test (2019; updated 2022).

Hayes assessed the use of the Prolaris Biopsy Test using FFPE prostatic adenocarcinoma specimens for determination of the 10-year risk of both metastatic disease after definitive therapy and disease-specific mortality if conservatively managed in 2019 (updated 2022). Hayes found insufficient evidence to support the analytical and clinical validity of this test to aid in the prediction of PCa-specific mortality and metastasis, and studies supporting clinical utility were limited as well.

Hayes assessed the use of the Prolaris Post-Prostatectomy Test for determination of BCR risk within 10 years of prostatectomy in 2019 (updated 2022). Hayes found minimal evidence of analytical validity and preliminary evidence for clinical validity but no studies that provided evidence for the clinical utility of Prolaris for postprostatectomy use.

Decipher Prostate

The Phillips et al. (2025) retrospective biomarker analysis used pretreatment biopsy specimens from 283 patients with high-risk, localized prostate adenocarcinoma who were enrolled in the NRG/RTOG 0521 phase 3 trial between 2005 and 2009. Eligible patients had a Gleason score of 9 to 10, Gleason score of 7 to 8 with a PSA of ≥ 20 ng/mL, or Gleason score of 8 with a PSA of < 20 ng/mL and T2 stage; those with a PSA of > 150 ng/mL were excluded. All patients received definitive RT and androgen suppression and were randomized to receive either no additional therapy or adjuvant docetaxel-based chemotherapy. Transcriptomic profiling was performed using the Decipher GC and a research-use-only prostate subtyping classifier (PSC) to assess prognostic value and predict treatment benefit. Of the 283 samples, 183 passed quality control and were analyzed. The primary outcome was metastasis-free survival (MFS), with secondary outcomes including distant metastasis, OS, and disease-specific mortality. Over a median follow-up of 9.9 years, 67 MFS events and 34 distant metastasis events occurred. A multivariable analysis showed that the Decipher score was

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independently associated with MFS (hazard ratio, 1.20; 95% CI, 1.03-1.41; p = 0.02) and distant metastasis (subdistribution hazard ratio, 1.45; 95% CI, 1.11-1.90; p = 0.007). Patients with very high GC scores (> 0.85) had significantly worse MFS (hazard ratio, 2.28; 95% CI, 1.07-4.85; p = 0.03) and distant metastasis (subdistribution hazard ratio, 3.47; 95% CI, 1.24-9.72; p = 0.02) than those with low or intermediate scores. No significant biomarker-by-treatment interaction was found between Decipher score and chemotherapy. However, PSC subtyping identified a luminal proliferating subgroup (28.4% of tumors) that derived greater benefit from chemotherapy. In luminal proliferating tumors, the addition of chemotherapy improved 10-year OS restricted mean survival time by 13.7 months (95% CI, -0.2 to 27.5 months; p = 0.05), while no significant restricted mean survival time differences were observed in non–luminal proliferating tumors. Limitations include the retrospective design, modest sample size, and incomplete specimen availability. These results suggest that transcriptional profiling using the Decipher GC and PSC may improve risk stratification and guide chemotherapy use in individuals with high-risk, localized PCa. Author disclosures include employment by Veracyte, Inc., and advisory roles with pharmaceutical companies.

Dal Pra et al. (2025) assessed the predictive value of the Post-Operative Radiation Therapy Outcomes Score (PORTOS), a genomic biomarker derived from the same clinical-grade microarray platform as the Decipher GC, for identifying individuals with PCa who benefit from radiation dose escalation. PORTOS was evaluated in two randomized phase 3 trials: SAKK 09/10, which enrolled 226 individuals with biochemically recurrent PCa randomized to salvage RT at 64 Gy vs 70 Gy, and NRG/RTOG 0126, which included 215 individuals with intermediate-risk localized PCa randomized to definitive RT at 70.2 Gy vs 79.2 Gy. Genomic signatures were generated using the Decipher assay, and PORTOS was calculated using previously published methods. The primary outcomes were clinical PFS in SAKK 09/10 and biochemical failure by Phoenix and American Society for Radiation Oncology (ASTRO) criteria in NRG/RTOG 0126. The secondary outcomes included distant metastasis and receipt of salvage therapy. In SAKK 09/10, individuals with higher PORTOS scores had a statistically significant benefit from dose escalation, with a hazard ratio for clinical PFS of 0.19 (95% CI, 0.05-0.70; p = 0.01), while those with lower PORTOS had worse outcomes with dose escalation (hazard ratio, 1.78; 95% CI, 1.02-3.11; p = 0.04). The interaction between PORTOS and treatment arm was significant (p = 0.003). In NRG/RTOG 0126, dose escalation conferred no benefit in the lower PORTOS tertile (Phoenix biochemical failure subdistribution hazard ratio, 1.03; 95% CI, 0.45-2.36; p = 0.94) but was beneficial in the average (subdistribution hazard ratio, 0.45; 95% CI, 0.22-0.90; p = 0.02) and higher tertiles (subdistribution hazard ratio, 0.30; 95% CI, 0.12-0.75; p = 0.009). Interaction testing confirmed a significant difference in dose escalation benefit between higher and lower PORTOS groups (p = 0.048). PORTOS was not prognostic overall and showed no consistent association with clinicopathologic variables or the Decipher GC score in either trial or in a real-world dataset of 42,407 prostatectomy and 31,107 biopsy samples. In the latter, PORTOS was modestly associated with hypoxia signatures and strongly associated with immune signatures and the basal-immune subtype. Limitations include the retrospective nature of biomarker analysis. Unlike the Decipher GC, which was previously shown to be prognostic but not predictive for a dose escalation benefit in SAKK 09/10, PORTOS demonstrated predictive utility in both salvage and definitive RT settings. These findings support PORTOS as a potential tool for guiding personalized radiation dose decisions in PCa.

The Michael et al. (2025) retrospective cohort study examined the association between genomic biomarkers and adverse pathological features (APFs) in patients eligible for active surveillance who proceeded to RP at a single tertiary care center between February 2012 and September 2024. Among 184 patients classified as NCCN low or favorable intermediate risk, 22.3% (n = 41) had APF at surgery, defined as a grade group of ≥ 3, pathological stage of ≥ T3b, or nodal involvement. The Decipher GC was used to assess GEPs. Patients with APF had significantly higher Decipher scores than those without (0.55 vs 0.41; p = 0.004). In a multivariable logistic regression adjusted for log-transformed PSA, the Decipher score remained significantly associated with APF (OR, 1.61; 95% CI, 1.11-2.32; p = 0.01), along with *PTEN* loss, activated CD4 expression, and *ERG* positivity. These findings suggest that higher Decipher scores are associated with an increased risk of adverse pathology in active surveillance–eligible individuals. Limitations include the use of both biopsy and prostatectomy specimens; potential selection bias due to inclusion of prostatectomy specimens, which may be enriching for APFs; and modest sample size. The authors reported high concordance between biopsy and RP Decipher scores and consistent effect sizes across specimen types. Disclosures include affiliations with Veracyte, Inc., for three authors. These results may inform future research on integrating GCs into risk stratification strategies for individuals considering active surveillance.

Morgan et al. (2025) published results from a prospective, randomized controlled, cluster-crossover trial (G-MINOR) that evaluated the impact of Decipher GC results on adjuvant treatment after RP compared with usual care. The study enrolled 175 participants who underwent testing with Decipher and 163 participants who received usual care. Eligible participants had undergone RP within 9 months of study enrollment, had pT3 to 4 disease and/or positive surgical margins, and had PSA levels of < 0.1 ng/mL. On average, participants in the Decipher arm received adjuvant treatment 9.7% of the time compared with 8.7% of the time in participants in the usual-care arm at 18 months after RP (mean difference, 0.99%; 95% CI, -7.6% to 9.6%; p = 0.8). Higher Decipher scores were associated with an increased likelihood of adjuvant treatment, but this was not statistically significant (OR, 1.35 per 0.1 increase in GC score; 95% CI, 0.98-1.85; p = 0.066). Using

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Decipher risk groups, a high risk was associated with significantly greater odds of receiving adjuvant treatment compared with a low risk score (OR, 6.9; 95% CI, 1.8-26; p = 0.005, adjusted for CAPRA postsurgical score). Other symptoms, such as participant-reported urinary and sexual function, did not differ between the two groups. The authors concluded that Decipher testing impacted adjuvant therapy administration when considered with risk categories, but the study results did not provide sufficient evidence to conclusively support Decipher testing in the adjuvant treatment setting. In addition, long-term results were not measured in this dataset, as oncological outcomes were immature. The researchers indicated that long-term follow-up from this and other studies will address remaining uncertainties regarding whether genomic testing provides meaningful benefit and improved outcomes in individuals with PCa.

Leapman et al. (2025) performed a retrospective cohort study using a newly developed linkage of transcriptomic data from Decipher GC and clinical data obtained from insurance claims, pharmacy data, and electronic health records across various payers and sites of care to measure the associations between Decipher GC testing results and the risk of metastasis and BCR after prostate biopsy and RP in a real-world setting. Overall, 58,935 patients who had undergone Decipher GC testing were included; 33,379 individual samples were from biopsies, and 25,556 samples were from RP. The median GC score was 0.43 in the biopsy group and 0.54 in the RP group. The researchers found that the Decipher GC was independently associated with the risk of metastasis in both groups after adjusting for baseline clinical and pathological risk factors. Decipher GC was also associated with the risk of BCR in the RP group in models that were adjusted for age and CAPRA scores. Based on these findings, the authors concluded that their results support the prognostic validity of Decipher GC across varying clinical populations and settings. Noted limitations include the retrospective study design, use of real-world data, lack of control and comparator groups, and limited follow-up time periods. There was also a potential for bias, as several study authors had affiliations with the test manufacturer.

The Ross et al. (2024) preplanned transcriptomic analysis was conducted in the phase 2 ENACT RCT, which evaluated enzalutamide monotherapy vs continued active surveillance in participants with clinically localized low- or intermediate-risk PCa. Of the 227 participants randomized, 95 had evaluable screening biopsy samples and were included in the analytic cohort (enzalutamide, n = 49; active surveillance, n = 46). An expanded cohort incorporated 26 additional participants from the active surveillance arm, with evaluable samples collected during surveillance. Biopsies were obtained at screening, year 1, and year 2 and analyzed using a clinical-grade transcriptome assay. The Decipher GC was one of three prespecified gene expression signatures evaluated. The primary end point was time to pathological or therapeutic disease progression, with secondary end points that included PSA progression and incidence of negative biopsy. Higher Decipher scores were significantly associated with an increased risk of disease progression in the expanded cohort (hazard ratio per 0.1-unit increase, 1.23; 95% CI, 1.05-1.44; p = 0.01) and in the active surveillance arm of the expanded cohort (hazard ratio, 1.17; 95% CI, 1.01-1.35; p = 0.04). Decipher score also predicted therapeutic disease progression in both the analytic cohort (hazard ratio, 1.51; 95% CI, 1.07-2.12; p = 0.02) and expanded cohort (hazard ratio, 1.46; 95% CI, 1.18 -1.80; p < 0.001). Among participants treated with enzalutamide, higher Decipher scores were associated with greater benefit, as measured by a delayed secondary PSA rise of > 25% above baseline (interaction p = 0.03). The study's limitations include the small sample size, limited representation of intermediate/high Decipher scores, and incomplete transcriptomic data due to sample depletion. The authors disclosed financial relationships with Astellas Pharma Inc and Pfizer Inc, which supported the study. These findings support the use of the Decipher GC as a prognostic biomarker for disease progression and a potential tool for identifying individuals undergoing active surveillance who may benefit from enzalutamide therapy.

Hayes addressed the Decipher Prostate Biopsy GC in a 2024 Molecular Test Assessment (updated 2025). Hayes identified a very low-quality body of evidence, limiting the ability to draw conclusions regarding the use of this test to determine the risk of adverse outcomes and inform treatment decisions in individuals with localized PCa undergoing prostate biopsy. Although evidence has shown that a higher Decipher score is associated with a greater risk of metastatic disease in individuals with primarily intermediate- to high-risk cancer, as classified per the NCCN Guideline, questions remain about overall test performance and the impact on clinical outcomes. Additional research in NCCN lower-risk groups is needed; it is not clear if this testing changes clinical management or clinical outcomes in these groups. There is little evidence for superior performance of the Decipher Biopsy GC compared with standard nongenomic methods of risk stratification, such as NCCN risk groups.

Hayes evaluated the Decipher Prostate RP GC in a 2024 Molecular Test Assessment for the prediction of the risk of metastasis or PCa-specific mortality in individuals with PCa that has been treated with RP. Hayes found some low-quality evidence supporting the usefulness of this test to assist with decision-making for post-RP adjuvant treatments, but the evidence was insufficient to ascertain whether the testing improves long-term outcomes. Evidence comparing the performance of the Decipher RP with other risk-calculating tools as well as evidence addressing optimal selection of individuals for testing was found to be inadequate, especially in individuals who have undergone RP but have no preoperative high-risk features.

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Spratt et al. (2023) enrolled participants in the NRG Oncology/RTOG 0126 randomized phase 3 trial to investigate the performance of the 22-gene Decipher GC in intermediate-risk PCa. This study was the first validation of a biopsy-based GEC to evaluate both the prognostic and predictive value using data from a randomized phase 3 clinical trial in participants with intermediate-risk PCa. The NRG Oncology/RTOG 0126 trial randomized these participants to 70.2 Gy vs 79.2 Gy of RT, with no androgen deprivation therapy. With the National Cancer Institute's approval, biopsy slides from NRG Oncology/RTOG 0126 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 of this ancillary study was disease progression, using a composite of biochemical failure, local failure, distant metastases, PCa-specific mortality, and use of salvage therapy. Using multivariable analysis, Decipher was independently prognostic for disease progression (subdistribution hazard ratio, 1.12; 95% CI, 1.00-1.26; p = 0.04), biochemical failure (subdistribution hazard ratio, 1.22; 95% CI, 1.10-1.37; p < 0.001), distant metastasis (subdistribution hazard ratio, 1.28; 95% CI, 1.06-1.55; p = 0.01), and PCa-specific mortality (subdistribution hazard ratio, 1.45; 95% CI, 1.20-1.76; p < 0.001). In participants with Decipher low-risk results, 10-year distant metastasis was 4% compared with 16% in Decipher high-risk results. The authors contended that the 22-gene Decipher GC improves risk stratification and can help inform treatment decisions in individuals with intermediate-risk disease. A limitation of this study is the limited availability of sufficient-quality tissue samples, which impacted the power of the study and prohibited well-powered subset analyses. (This study is included in the Molecular Test Assessment by Hayes, 2024, updated 2025.)

The Jairath et al. (2021) systematic review evaluated the available evidence supporting the clinical utility of the Decipher GC. Overall, 144 studies were identified, and of those, 42 studies, which included 30,407 individuals, met the inclusion criteria for this review, with GC performance data available for localized, postprostatectomy, nonmetastatic, castration-resistant, and metastatic hormone-sensitive PCa. Individuals were part of retrospective studies (n = 12,141), prospective registries (n = 17,053), and prospective and post hoc randomized trial analyses (n = 1,213). On multivariate analysis, 32 studies showed that Decipher was independently prognostic for study end points, including biochemical failure, metastasis, adverse pathology, and both cancer-specific survival and OS. In 24 studies, GC improved discrimination over standard of care, and in five 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 grades A and B by American Urological Association (AUA) criteria, depending on the state of disease. Based on this review, the authors asserted that consistent data have emerged from diverse levels of evidence, and when evaluated overall, the 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.

Other Prostate Cancer Assays

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

Hayes (2025) published a Precision Medicine Research Brief describing the published literature related to the use of the MyProstateScore 2.0 (MPS2) test to predict the likelihood of clinically significant PCa detection on biopsy in the setting of elevated PSA or abnormal digital rectal examination. Hayes did not identify any abstracts addressing the test's clinical utility and only identified two cohort studies evaluating test performance. While Hayes concluded that there is weak support for MPS2 in clinical practice guidelines and position statements, the lack of published, peer-reviewed literature precluded further evidence evaluation.

Hayes (2025) published a Precision Medicine Research Brief describing the published literature related to the use of the OncoAssure Prostate test for assessing the probability of aggressive disease in the setting of recently diagnosed, early-stage PCa and assessing the risk of BCR within 5 years post RP. Hayes did not identify any abstracts addressing the test's clinical utility and only identified two cohort studies evaluating test performance. Hayes concluded that there is no/unclear support for the test in clinical practice guidelines and position statements. The lack of published, peer-reviewed literature precluded further evidence evaluation.

The Plas et al. (2025) systematic review evaluated urine-based biomarkers for PCa detection. The search identified 286 studies, and 66 met the inclusion criteria, focusing on individuals with suspected PCa but without a prior diagnosis. Five commercially available tests were analyzed: ExoDx Prostate, MyProstateScore (MPS), Select mdx, Protexam urinary protein profiling, and the Progensa PCA3 RNA quantification assay. Most studies enrolled biopsy-naive individuals or those undergoing repeat biopsy, typically with PSA levels between 4 and 10 ng/mL. The sample sizes ranged from 27 to 3,073, and the primary end points varied, with newer tests targeting clinically significant cancer, which was defined as an International Society of Urological Pathology grade group of ≥ 2. Across studies, the area under the curve (AUC) for

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detecting clinically significant disease ranged from 0.55 to 0.86. ExoDx Prostate demonstrated a sensitivity around 92% to 97% and a specificity between 26% and 34% in prospective multicenter trials, with AUC values of up to 0.76. MPS achieved sensitivities of 94% to 100% and specificities from 15% to 42%, with AUCs near 0.77; newer versions (MPS2.0 and MPS2+) improved specificity modestly. Protexam's proteomic panels showed AUCs near 0.8, with sensitivity of up to 93% and specificity as high as 69% in validation cohorts. Select mdx initially reported AUCs of 0.86, but recent prospective data showed lower performance (AUC, 0.63), while Progensa PCA3 exhibited wide variability, with sensitivity ranging from 42% to 96% and specificity from 18% to 91%. The review highlights substantial heterogeneity in study design, thresholds, and populations, precluding meta-analysis and limiting generalizability. Most studies relied on systematic biopsy as the reference standard, which may underestimate clinically significant disease. Selection bias is possible, as only PubMed was searched. Conflicts of interest were disclosed for two authors employed by diagnostic companies. Overall, while ExoDx Prostate and MPS appear most promising for risk stratification, the evidence remains insufficient for routine clinical implementation. Prospective, standardized, multicenter trials that use consistent inclusion criteria are needed to confirm diagnostic accuracy and cost-effectiveness before these biomarkers can be integrated into practice. Studies by Tosoian et al. (2021), McKiernan et al. (2018), and McKiernan et al. (2016), previously discussed in this policy, were included in this systematic review.

The Sequeira et al. (2024) systematic review evaluated liquid biopsy–based biomarkers for pretreatment risk stratification in PCa, aiming to overcome limitations of PSA and Gleason-based tools that require tissue biopsies. The authors searched PubMed, Scopus, and MEDLINE up to February 2023, including only original studies that analyzed biomarkers prior to treatment. Studies that focused on posttreatment risk or metastatic castration-resistant disease were excluded. Overall, 24 studies met the eligibility criteria, encompassing urine, plasma, serum, whole blood, and semen samples, with biomarker quantification primarily by quantitative polymerase chain reaction (41.7%) and, less frequently, by digital polymerase chain reaction or NanoString. Risk stratification methods varied, most commonly using International Society of Urological Pathology grading, with some incorporating PSA or clinical stage. Quality assessment using QUADAS-2 (Quality Assessment of Diagnostic Accuracy Studies, version 2) revealed a high risk of bias in the selection of individuals for nearly half of the studies, while index test and reference standard biases were generally low. Among messenger RNA (mRNA)–based strategies, urine panels ranging from 25 to 167 genes achieved AUCs of 0.72 to 0.93. One DNA methylation study was included in the analysis, yielding an AUC of 0.89. Across studies, there was limited reporting of CIs and p values. The authors noted substantial heterogeneity in biomarkers, technologies, and clinical stratification criteria as well as small sample sizes and a lack of multicenter validation, which threaten external validity. The standardization of risk categories and assay protocols, along with robust validation in larger cohorts, is essential before these strategies can be integrated into routine practice.

The Kawada et al. (2024) systematic review and diagnostic meta-analysis evaluated the accuracy of commercially available liquid biomarkers for detecting clinically significant PCa. Histopathology from systematic or image-targeted biopsy served as the reference standard. The authors included 49 prospective and retrospective studies, comprising 29,525 individuals undergoing initial or repeat biopsy. Nine studies evaluated Select mdx in 2,609 individuals, reporting a pooled sensitivity of 0.82 (95% CI, 0.69-0.91) and a specificity of 0.56 (95% CI, 0.41-0.70), with a diagnostic OR (DOR) of 6.16 (95% CI, 2.62-14.49). Two studies assessed ExoDx in 732 individuals, yielding a sensitivity of 0.85 (95% CI, 0.71-0.93), specificity of 0.54 (95% CI, 0.27-0.79), and DOR of 6.07 (95% CI, 3.84-9.61). MPS was examined in two studies, with 1,793 individuals, showing a sensitivity of 0.82 (95% CI, 0.52-0.95), specificity of 0.59 (95% CI, 0.36-0.79), and DOR of 7.00 (95% CI, 4.20-11.69). NPVs for all three biomarkers exceeded 0.88, indicating a strong ability to rule out clinically significant PCa. Limitations, including heterogeneity in biopsy settings, data scarcity (particularly for ExoDx and MPS), and reliance on biopsy as the reference standard, may have missed some clinically significant PCa cases. These findings suggest that Select mdx, ExoDx, and MPS may offer meaningful improvements over PSA alone for detecting clinically significant PCa, particularly for reducing unnecessary biopsies, although integration with imaging remains essential for optimal decision-making. One investigator reported conflicts of interest related to industry honoraria and advisory roles. Studies by Tosoian et al. (2021) and McKiernan et al. (2018), previously discussed in this policy, were included in this systematic review.

Confirm mdx

In a Molecular Test Assessment [Confirm mdx (mdxhealth Inc.), 2024; updated 2025], Hayes found insufficient evidence to support the use of Confirm mdx for ruling whether repeat biopsy is needed in individuals with prior negative biopsy. Although Confirm mdx appeared to be a significant predictor of PCa on repeat biopsy while other factors were not, no direct comparisons were performed; thus, no conclusions regarding comparative performance can be reached. No identified studies assessed clinical outcomes associated with the use of Confirm mdx. Additional studies are required to evaluate whether Confirm mdx results in improved outcomes in individuals with PCa.

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ExoDx Prostate Test

The Hamid et al. (2025) systematic review evaluated 137 case-control and cohort studies published between 2009 and 2024, encompassing 17,419 individuals with PCa and control groups of healthy individuals and individuals with benign prostatic hyperplasia. The review focused on exosomal biomolecules obtained from liquid biopsy samples (primarily blood and urine) as potential diagnostic, prognostic, and treatment response markers compared with conventional methods such as PSA testing and tissue biopsy. Multiple prospective and multicenter cohort studies were included that validated the diagnostic and prognostic performance of ExoDx Prostate in individuals undergoing an initial or repeat biopsy, who typically had PSA levels between 2 and 10 ng/mL. Across large cohorts, the ExoDx Prostate score consistently demonstrated superior accuracy compared with PSA and other single biomarkers. These results were robust across diverse populations and independent of age, race, and PSA level. Prognostically, higher ExoDx Prostate scores correlated with adverse pathology, upgrading, and BCR risk after prostatectomy, supporting its role in risk stratification and active surveillance decision-making. The review noted substantial heterogeneity in the study design, sample sources, and analytic methods, with a predominance of retrospective studies and limited prospective validation. The lack of standardized protocols for exosome isolation and biomarker detection, small sample sizes in some reports, and variable control groups were identified as threats to validity and reliability. Despite these limitations, the findings suggest that ExoDx may offer a noninvasive alternative to tissue biopsy that improves the early detection of aggressive disease. Multicenter prospective trials and standardized analytic frameworks are needed to confirm clinical utility and enable integration into routine practice. Studies by McKiernan et al. (2018) and McKiernan et al. (2016), previously discussed in this policy, were included in this systematic review.

In a Molecular Test Assessment, Hayes found a low-quality body of evidence that addresses the clinical benefit of the ExoDx Prostate Test, which is proposed for use in individuals ≥ 50 years of age with PSA levels of 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 the test were reviewed, the evidence indicates low to acceptable ability to detect clinically significant PCa. No studies were found that compared ExoDx Prostate's clinical performance with other PSA derivatives, magnetic resonance imaging (MRI), or other commercially available similar tests. Evidence for clinical utility was insufficient [Hayes, ExoDx Prostate Test (Exosome Diagnostics Inc.), 2023; updated 2025].

The Tutrone et al. (2020) prospective, randomized, blinded, two-armed clinical utility study evaluated the impact of the ExoDx Prostate Test on the decision whether to perform a biopsy in a real-world clinical setting. The ExoDx Prostate Test is designed to assess the risk for high-grade PCa. The study enrolled 1,094 participants from 24 urology practices and a total of 72 urologists. All participants underwent ExoDx Prostate testing but were randomized into ExoDx Prostate vs control. Only the ExoDx Prostate arm received results for the biopsy. In the ExoDx Prostate group, 458 of the participants received negative ExoDx Prostate scores. Of them, 63% were recommended to defer biopsy, and 74% of those did indeed defer the biopsy. Of those with positive ExoDx Prostate scores, 87% were recommended by a urologist to proceed with biopsy, and 72% of participants adhered to that recommendation. Ultimately, this led to detection of 305 more cases of high-grade PCa compared with the control group, and the researchers estimated that 49% fewer high-grade cancers were missed due to deferred biopsy compared with standard of care. Overall, 68% of participating urologists indicated that the ExoDx Prostate influenced their decision regarding biopsy recommendation. The authors stated that this was the first report on a PCa biomarker utility study with a blinded control group. The authors feel that the study showed that the ExoDx Prostate Test influenced decision-making regarding prostate biopsy and participant stratification. Despite these positive outcomes, there were limitations. In the ExoDx Prostate group, there was a 5.7% assay failure, and in the entire group of participants, there was a failure rate of 7.1%. Data are lacking regarding long-term outcomes in the participants who deferred biopsy after using ExoDx Prostate, and the large number of testing sites and urologists involved required the use of streamlined questionnaires, which limited feedback. Lastly, a small number of participants (< 5%) had undergone prebiopsy MRI, which can help refine biopsy accuracy and provide additional information related to ExoDx Prostate Test performance. The researchers suggested that future studies could include a larger percentage of individuals with MRI data available.

Select mdx

Another Molecular Test Assessment produced by Hayes (2024; updated 2025) focused on Select mdx. This gene expression test evaluates *HOXC6* and *DLX1*, along with the reference gene *KLK3* via a urine sample. This result, combined with clinical risk factors such as age, PSA, digital rectal examination result, and prostate volume, leads to a test outcome indicating either an increased risk or a very low risk of clinically significant PCa on biopsy. Hayes identified an overall very low-quality body of evidence, which was inadequate to reach conclusions regarding the effectiveness of Select mdx testing for the prediction of clinically significant PCa risk and for informing clinical decision-making regarding biopsy. Some evidence suggests that Select mdx could lead to unnecessary biopsies in lower-risk individuals, and comparative evidence was inconsistent and insufficient in quantity. Further study is required to clarify test accuracy and substantiate improvement in clinical outcomes using this test [Hayes, Select mdx (mdxhealth Inc.), 2024; updated 2025].

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Clinical Practice Guidelines

American Association of Clinical Urology (AACU)

In a 2018 position statement endorsed by the Large Urology Group Practice Association, the AACU states their support of the use of tissue-based molecular testing as a component of risk stratification in PCa treatment decision-making as well as their support of ongoing research to further refine the prognostic power of these tests. The AACU recommends that tissue-based molecular tests are incorporated with other measures of PCa risk, including PSA, Gleason grade, and clinical stage.

American Society of Clinical Oncology (ASCO)

ASCO guidelines issued in 2025 (Yu et al.) provide recommendations for genomic testing in metastatic PCa, including the following:

- Patients with metastatic PCa (both castration-sensitive PCa and castration-resistant PCa) who are being considered for biomarker-directed systemic treatment should undergo somatic testing with NGS technologies. While there are no current U.S. Food and Drug Administration (FDA)–approved biomarker-directed treatments following somatic testing for metastatic, castration-sensitive PCa, somatic testing may be warranted in the presence of high-volume disease or where there is a high likelihood that the patient's disease will progress to castration-resistant PCa, at which point the patient is a candidate for future treatment with a biomarker-directed therapy [poly (ADP-ribose) polymerase (PARP) inhibitor or checkpoint inhibitor] (evidence quality: high; strength of recommendation: strong).
- Sequential somatic testing may be offered when there has been a meaningful change in the patient's status or treatment plan, especially in cases in which prior tests were negative or uninformative (i.e., insufficient or low tumor content) (evidence quality: moderate; strength of recommendation: weak).
- Archival tissue samples are preferred in initial testing. ctDNA is preferred when there is no accessible metastatic site to biopsy or for sequential testing. In the setting of minimal disease burden associated with low ctDNA fraction, metastatic biopsy is preferred (evidence quality: low; strength of recommendation: weak).
- Patients with pathogenic germline variants or somatic alterations in *BRCA1* and *BRCA2* have poorer outcomes but are candidates for treatment with PARP inhibitor monotherapy, PARP inhibitor with androgen receptor pathway inhibitor combination therapy, and platinum-based agents (evidence quality: high; strength of recommendation: strong).
- Treatment recommendations should not be made based on prognostic-only biomarkers. However, they may be considered for directing patients to clinical trials (evidence quality: high; strength of recommendation: strong).

ASCO issued updated guidelines in 2025 (Garje et al.) for systemic therapy in patients with metastatic, castration-resistant PCa. As a principle of practice, the panel recommends both germline and somatic testing for patients with metastatic PCa at the earliest available opportunity. The panel recommends enrollment in clinical trials after progression on platinum-based chemotherapy for eligible patients, noting that broad NGS testing at the time of disease progression may aid in assessment of clinical trial eligibility (evidence quality: NA; strength of recommendation: strong).

ASCO published the following guidance on molecular biomarkers in localized PCa in 2020 (Eggener et al.):

- Molecular PCa biomarkers with which to identify patients who are most likely to benefit from active surveillance:
  - Summary: The current commercially available, biopsy-based multigene expression classifiers (e.g., Decipher, Oncotype DX Prostate, Prolaris) and one protein-based biomarker (ProMark) each seem to independently improve the prognostic accuracy of clinical multivariable models for identifying men with biologically significant disease. The clinical benefit of integrating these classifiers in selecting patients for surveillance has not been prospectively demonstrated. There are no comparative data that indicate that one may be more accurate than another. These may be considered, for instance, in select men with NCCN low- or favorable intermediate-risk PCa who might benefit from refined risk classification (e.g., high-volume grade group 1; grade group 1 with abnormal digital rectal examination or high PSA density; low-volume grade group 2) when considering active surveillance.
  - Recommendation: Commercially available molecular biomarker tests (e.g., Oncotype DX Prostate, Prolaris, Decipher, 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).
  - Recommendation: 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).
- Molecular biomarkers with which to diagnose clinically significant PCa:
  - Summary: There are commercially available biopsy-based multigene expression classifiers (e.g., Decipher, Oncotype DX Prostate, Prolaris) and a protein-based biomarker (ProMark). While these assays may also inform patients considering active surveillance, additional prognostic value may contribute to risk stratification and patient

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counseling when added to standard clinical parameters. The ability of these tests to improve outcomes (QOL and risk of metastasis or death) has not been prospectively evaluated. Comparative studies between tests have not been reported. These may be considered, for instance, in select unfavorable intermediate-risk patients when deciding whether to add androgen deprivation therapy to RT.
    - Recommendation: Commercially available molecular biomarkers (e.g., Oncotype DX Prostate, Prolaris, Decipher, 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).
    - Recommendation: 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).
- Molecular biomarkers to guide the decision of postprostatectomy adjuvant vs salvage radiation:
    - Summary: If an RP exhibits APFs (≥ T3a, node positive) and the PSA is undetectable, the Decipher GC may help risk stratify men and identify those who are most likely to benefit from postoperative adjuvant vs early salvage RT. These retrospective studies currently lack prospective validation and long-term follow-up. For instance, for a man with adverse pathology at prostatectomy (e.g., grade group 3-5, T3a, margin positive), an undetectable PSA, and early postoperative continence, the Decipher GC may inform the decision of adjuvant radiation vs observation. If radiation is chosen, it may also inform whether to include concomitant androgen deprivation.
    - Recommendation: Consideration of a commercially available molecular biomarker test (e.g., Decipher GC) 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 vs salvage radiation or to initiate systemic therapies should not be offered (type: evidence based; evidence quality: intermediate; strength of recommendation: moderate).
    - Recommendation: 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).
- The comparative strengths and weakness of genomics vs MRI in identifying clinically significant PCa:
    - Summary: Both MRI and genomics may help identify clinically significant PCa. There have been few studies directly comparing genomics with MRI. Two used multiparametric MRI with the 17-gene GPS (Oncotype DX), and one compared multiparametric MRI with a GC (Decipher). The data suggest that MRI and genomics can each provide clinically relevant information regarding the likelihood of upgrading on subsequent biopsy or at prostatectomy. Furthermore, there are patients for whom MRI and genomics can provide independent and actionable information. For instance, if there are concerns of unsampled high-grade cancers in the prostate, MRI would be favored to guide targeted biopsy. To optimize the understanding of the natural history of a biopsy-detected intermediate-risk cancer (e.g., grade group 2-3), genomics would be favored.
    - Recommendation: In men with newly diagnosed PCa who are eligible for active surveillance, both MRI 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, and 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 an updated guideline that addresses risk assessment, staging, and risk-based management of clinically localized PCa (Eastham et al., 2022a). This guideline was endorsed by the Society of Urologic Oncology (SUO) and provides the following recommendations regarding the use of genomic testing in risk assessment:

- Clinicians may selectively use tissue-based genomic biomarkers when added risk stratification may alter clinical decision-making (expert opinion).
- Clinicians should not routinely use tissue-based genomic biomarkers for risk stratification or clinical decision-making (moderate recommendation; evidence level: grade B).
- Clinicians should perform an assessment of patient and tumor risk factors to guide the decision to offer germline testing that includes mutations known to be associated with aggressive PCa and/or known to have implications for treatment (expert opinion).

The AUA and ASTRO (Eastham et al., 2022b) noted, in an updated guideline addressing principles of radiation and future directions in clinically localized PCa, that the ability for commercially available GCs to improve outcomes in patients with clinically localized PCa has not been validated in prospective clinical trials to date. Prospective validation of the predictive capacity of GCs will be important to support widespread use for treatment selection.

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

AUA/SUO guidelines for the early detection of PCa (Wei et al., 2023a; Wei et al., 2023b) include the following recommendations:

- When screening for PCa, clinicians should use PSA as the first screening test (strong recommendation; evidence level: grade A).
- For people with a newly elevated PSA, clinicians should repeat the PSA prior to a secondary biomarker, imaging, or biopsy (expert opinion).
- 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).
- After a negative initial biopsy in patients with a low probability for harboring grade group 2 or higher PCa, clinicians should not reflexively perform biomarker testing (clinical principle).
- After a negative biopsy, clinicians may use blood-, urine-, or tissue-based biomarkers selectively for further risk stratification if results are likely to influence the decision regarding repeat biopsy or otherwise substantively change the patient's management (conditional recommendation; evidence level: grade C).

National Comprehensive Cancer Network (NCCN)

The NCCN Guidelines for PCa (NCCN Prostate Cancer, v4.2026) state that the Decipher test is recommended as an option 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 RP (category 2B evidence). The NCCN has deemed the Decipher 22-gene GC an advanced risk stratification tool, with a high level of evidence for use as a GEP test for risk stratification in patients with intermediate-, high-, or very high-risk PCa and for use post RP. Advanced risk stratification tools should only be considered as an option when they have the potential ability to change disease management. The NCCN also lists the following options for the molecular evaluation of PCa:

Considerations prior to molecular/biomarker analysis:

- Tumor molecular and biomarker analysis is recommended as an option for patients with metastatic disease for treatment decision-making.
- Consideration of reevaluation of tumor molecular profiles for change with subsequent treatment is recommended as an option at the time of cancer progression for treatment decision-making.

Testing considerations for molecular/biomarker analysis:

- Multigene tumor testing for alterations in homologous recombination repair genes, including but not limited to *BRCA1, BRCA2, ATM, PALB2, FANCA, RAD51D, CHEK2,* and *CDK12*, is recommended as an option for patients with metastatic PCa.
- Consideration of multigene tumor testing for alterations in homologous recombination repair genes, including but not limited to *BRCA1, BRCA2, ATM, PALB2, FANCA, RAD51D, CHEK2,* and *CDK12*, is recommended for patients with regional PCa.
- Tumor testing for microsatellite instability (MSI)–high or deficient DNA mismatch repair (dMMR) is recommended as an option for patients with metastatic, castration-resistant PCa.
- Consideration of tumor testing for MSI-high or dMMR is recommended as an option for patients with regional or metastatic, castration-sensitive PCa.
- Tumor mutation burden (TMB) testing is recommended as an option for patients with metastatic, castration-resistant PCa.

Tumor specimen and assay considerations for molecular/biomarker analysis:

- A metastatic biopsy, which could include LN biopsy for patients with N1 disease, is strongly recommended as an option for histological and molecular evaluation.
- Plasma ctDNA is recommended as an option when metastatic biopsy is unsafe or unfeasible.
- Collection of plasma ctDNA is not recommended as an option when PSA is undetectable.

In its 2026 version 1 guideline addressing PCa early detection, the NCCN panel discusses principles of biomarker testing, with the following recommendations:

- Consideration of certain biomarker tests before proceeding to immediate biopsy is recommended as a category 2B option if multiparametric MRI is not available, contraindicated, or of low quality or if the patient and physician wish to more precisely estimate the risk of high-grade (grade group ≥ 2) PCa.
- Consideration of the use of biomarkers for patients with a negative biopsy is recommended as a category 2B option if there remains suspicion that clinically significant PCa may be present and if a high-quality multiparametric MRI has already been obtained.

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- Use of biomarker testing is not recommended as an option for use in place of multiparametric MRI.

The NCCN states that biomarker tests must meet validity and clinical utility criteria that are listed in the guideline, including validation in a patient cohort that is representative of the intended-use population, ideally in a multisite, prospective, external validation study or multiple single-site studies. The panel provides lists of molecular tools that meet most or all of the NCCN validity and utility criteria. Tools listed for use prior to initial biopsy include Select mdx and the ExoDx Prostate Test. The list of tools for use after an initial negative biopsy includes Confirm mdx and the ExoDx Prostate Test.

Thyroid Cancer/Thyroid Nodules

The Chowdhury et al. (2025) systematic review and meta-analysis evaluated the impact of molecular testing on surgical decision-making for indeterminate thyroid nodules classified as Bethesda III or IV. The authors searched eight databases for studies published between 2019 and 2024 and included 31 studies, encompassing 4,464 nodules. The eligible studies enrolled adults with indeterminate cytology and reported surgical avoidance rates after molecular testing, excluding non-English publications and studies lacking surgical outcome data. The review assessed five platforms: the Afirma GEC, Afirma GSC, ThyroSeq V2, ThyroSeq V3, and ThyGeNEXT/ThyraMIR. A quality appraisal using the Newcastle-Ottawa Scale and Cochrane tools indicated that most studies were of moderate to high quality, although limitations include incomplete outcome validation and heterogeneity in controlling confounders. Across platforms, the pooled surgical avoidance rates varied: ThyroSeq V2 achieved 50.3% (95% CI, 20.8%-79.6%), ThyroSeq V3 achieved 62.5% (95% CI, 54.8%-70.0%), Afirma GEC achieved 58.8% (95% CI, 43.6%-73.1%), Afirma GSC achieved 50.6% (95% CI, 34.3%-66.8%), and ThyGeNEXT/ThyraMIR achieved 68.6% (95% CI, 63.1%-73.9%). ThyGeNEXT/ThyraMIR demonstrated the highest avoidance rate and lowest heterogeneity (I^2 = 51.2%), while ThyroSeq showed improvement from V2 to V3. However, substantial heterogeneity persisted for most platforms (I^2 up to 98%), reflecting variability in institutional practices and case selection. Limitations include the predominance of observational designs, variable follow-up (6-84 months), and geographic concentration in North America, which may affect generalizability. These findings support molecular testing as an adjunct to reduce unnecessary thyroid surgeries, particularly with platforms such as ThyroSeq V3 and ThyGeNEXT/ThyraMIR, although integration with clinical and imaging assessment remains essential. The observed heterogeneity and limited representation of some platforms underscore the need for standardized protocols and prospective comparative studies to confirm long-term outcomes and cost-effectiveness. (Kim et al., 2023, and Livhits et al., 2021, previously cited in this policy, are included in this systematic review. This systematic review is included in the 2026 Hayes Precision Medicine Research Brief.)

The Liao et al. (2025) systematic review and meta-analysis evaluated the diagnostic accuracy of molecular genetic testing on fine-needle aspiration (FNA) samples from indeterminate thyroid nodules, using postoperative histopathology as the reference standard. Eligible studies included individuals who were aged over 14 years with Bethesda III, IV, or V cytology classifications and were required to report true-positive, false-positive, true-negative, and false-negative counts, along with molecular test results. Studies published between January 2018 and October 2024 were considered, while case reports, small case series, and studies lacking surgical confirmation were excluded. Overall, 68 studies from 16 countries met these criteria, encompassing seven molecular panels. Data synthesis used a bivariate random-effects model to calculate sensitivity, specificity, DOR, likelihood ratios, and AUC, with heterogeneity assessed via I^2 statistics and publication bias evaluated using Deek funnel plots. Across panels, the Afirma GEC and GSC demonstrated high sensitivity (0.94 for both) but low specificity (0.21 and 0.36, respectively), yielding DORs of 4 and 8. ThyroSeq V2 and V3 achieved sensitivities of 0.91 each, with specificities of 0.52 and 0.42 and DORs of 10 and 8. NGS showed balanced performance, with a sensitivity of 0.75, specificity of 0.72, and DOR of 8, while the ThyGeNEXT/ThyraMIR multigene point-of-care test exhibited the strongest discriminatory ability (DOR, 18) and the lowest negative likelihood ratio (0.12), indicating superior capacity to rule out malignancy. Limitations include the heterogeneity in specificity across panels, incomplete individual-level data, and potential inflation of sensitivity due to inclusion of surgically confirmed cases. These findings suggest that molecular testing can improve preoperative risk stratification and reduce unnecessary thyroidectomy, with ThyGeNEXT/ThyraMIR showing the greatest promise for excluding malignancy. However, variability among studies and the lack of standardized reporting underscore the need for further validation and long-term outcome studies to confirm reliability and optimize clinical application. (Livhits et al., 2021, Endo et al., 2019, and Steward et al., 2019, previously cited in this policy, are included in this systematic review.)

Vardarli et al. (2024) conducted a meta-analysis of the performance of commercial molecular tests for thyroid nodules with indeterminate cytology. An electronic search was conducted using PubMed/MEDLINE, Embase, and the Cochrane Library; studies that assessed the diagnostic accuracy of the Afirma GEC, Afirma GSC, ThyroSeq V2, or ThyroSeq V3 in individuals with indeterminate cytology (Bethesda category III or IV) were selected. Statistical analyses were performed using Stata. The meta-analysis included 53 studies, with 6,490 FNAs, and showed pooled estimates of sensitivity at 0.95 and specificity at 0.35. ThyroSeq V3 demonstrated the best overall performance, followed by ThyroSeq v2, GSC, and GEC. GSC had the best rule-out performance, while ThyroSeq V2 was superior for rule in. The meta-regression analysis

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identified study design, Bethesda category, and type of molecular test as independent factors. The findings suggest that ThyroSeq V3 has superior molecular diagnostic performance for indeterminate cytology, with GSC and ThyroSeq V2 excelling in specific diagnostic aspects. A limitation of the analysis is the retrospective design of many of the included studies. Additionally, not all indeterminate cytology with benign or negative molecular tests underwent surgery, thus the true false-negative rate is unknown. Studies did not include head-to-head comparisons of the tests using the same cytological sample. Conducting additional studies with a prospective design, blinding, and surgical intervention (including histopathologic diagnosis of indeterminate cytology) in all individuals undergoing molecular diagnostics would be beneficial to ascertain true false-negative results.

Lee et al. (2022) conducted a systematic review and meta-analysis to appraise the diagnostic performance of second-generation molecular tests in diagnosing thyroid nodules with indeterminate FNA biopsy results. Included in the evaluation were 15 studies: seven Afirma 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% CI, 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 the 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 in the meta-analysis, absence of long-term analysis of the utility of the tests, and inclusion of only two studies that evaluated ThyGeNEXT. The authors concluded that high sensitivity and NPV in GSC and ThyroSeq V3 may help rule out malignancy of thyroid nodules with indeterminate cytology results. There was no difference in diagnostic performances between the two molecular tests, suggesting that either test is suitable for this purpose. (Livhits et al., 2021, Endo et al., 2019, and Steward et al., 2019, previously cited in this policy, are included in this systematic review.)

Afirma Genomic Sequencing Classifier

A Hayes Molecular Test Assessment found limited but positive evidence supporting the Afirma GSC assay for identification of benign thyroid nodules in results that are deemed indeterminate by cytopathology, which could help individuals avoid unnecessary surgical intervention. The evidence shows that 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 (GEC); 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 in individuals with Afirma benign outcomes, specifically around missed malignancies, to support test performance. An updated review states that the current Hayes rating is unlikely to change from the previous annual rating [Hayes, Afirma GSC (Veracyte, Inc.), 2021; updated 2024]. 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].

ThyGeNEXT/ThyraMIR

Hayes assessed the use of the ThyGeNEXT and ThyraMIR (V1) tests in a Molecular Test Assessment (2021; updated 2022). The assessment uncovered inadequate evidence to support the use of the ThyGeNEXT and ThyraMIR tests to assist in reclassifying thyroid nodules with indeterminate cytology. In follow-up, Hayes published a Precision Medicine Research Brief (2026) describing the evidence related to ThyGeNEXT and ThyraMIR V2 for the management of indeterminate thyroid nodules. Hayes identified three cohort studies evaluating these assays' performances in the detection of thyroid carcinoma, one single-institution clinical utility study, and a systematic review, evaluating their relationships to surgical rate reduction. Hayes stated that their review of position statements and clinical practice guidelines further found weak support for the use of these tests. However, Hayes did not offer a full appraisal of the evidence in this research brief.

ThyroSeq V3

A Hayes Molecular Test Assessment addressing the ThyroSeq V3 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 a 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 the use of the ThyroSeq V3 GC in the preoperative assessment of indeterminate thyroid nodules to measure cancer probability or

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provide prognostic data for clinical management [Hayes, ThyroSeq V3 GC (University of Pittsburgh Medical Center and Sonic Healthcare USA), 2023; updated 2025].

Other Thyroid Cancer/Thyroid Nodule Assays

The Liang et al. (2025) retrospective study evaluated the mutational landscape of thyroid neoplasms using targeted NGS on FNA cytology (FNAC) samples. The analysis included 952 patients who underwent thyroid surgery and cervical LN dissection at a single tertiary care center between January 2021 and December 2023. Eligibility required preoperative ultrasound, FNAC, and NGS testing, while exclusions included inadequate FNAC samples, failed biopsies, absence of LN dissection, LN tuberculosis or other primary LN diseases, metastases from nonthyroid tumors, and prior neck surgery or RT. DNA from FNAC samples was sequenced using three validated panels covering 17, 18, or 88 thyroid carcinoma–related genes, and associations between genetic alterations and clinicopathologic features were analyzed with multivariable logistic regression. Among the cohort, papillary thyroid carcinoma (PTC) predominated (95.27%), with smaller proportions of benign tumors, low-risk neoplasms, follicular thyroid carcinoma, poorly differentiated/anaplastic carcinoma, and medullary thyroid carcinoma. Mutations were detected in 924 patients, most commonly *BRAF* V600E (84.45%), followed by *RET* (6.41%), *BRCA1/2* (4.41%), and the RAS gene family (*HRAS*. *KRAS*, and *NRAS*; 4.41%). Molecular subgroups included *BRAF*-like (n = 830), RAS-like (n = 36), high risk (e.g., *BRAF* mutations in combination with other gene mutations such as *TERT*, *PIK3CA*, or *TP53*; n = 25), and other mutations (n = 28). High-risk mutations were strongly associated with older age (≥ 55 years: 52.00% vs 12.65% in *BRAF*-like; OR, 7.50; 95% CI, 3.20-17.60; p < 0.001), male sex (48.00% vs 21.45%; OR, 3.25; 95% CI, 1.42-7.45; p = 0.008), larger tumors (> 4 cm: 19.05% vs 1.33%; OR, 17.45; 95% CI, 4.35-70.00; p < 0.001), and multifocality (96.00% vs 43.26%; OR, 30.67; 95% CI, 4.05-232.00; p < 0.001). *BRAF*-like tumors exhibited a higher LN metastasis rate than RAS-like tumors (58.77% vs 33.33%; p < 0.001). Comutations involving *TERT* promoter and *BRAF* or a RAS gene were linked to aggressive features, with *BRAF* + *TERT* cases showing 60% nodal metastasis and RAS + *TERT* cases demonstrating larger mean tumor size (56 mm). Limitations include the predominance of PTC in this cohort, variability in panel coverage, and retrospective design, which may affect generalizability and mutation detection consistency. These findings underscore the potential utility of NGS-based molecular profiling of FNAC samples for preoperative risk stratification and personalized surgical planning, particularly in identifying high-risk genotypes that warrant more aggressive management.

The Ramone et al. (2025) prospective study evaluated whether molecular profiling could predict progression in participants with low-risk PTC managed under active surveillance at a single center. Overall, 95 participants who had tumors of ≤ 1.3 cm were enrolled between November 2014 and December 2022; they underwent FNAC for molecular analysis and were followed up with neck ultrasound and blood tests every 6 months for 2 years and then annually. Eligibility required low-risk PTC without evidence of aggressive features; progression was defined as ≥ 3 mm of growth in each diameter, confirmed twice, or development of LN metastasis. The study also included a control group of 10 participants with low-risk PTC and LN metastases at diagnosis who underwent surgery. NGS assessed somatic mutations and gene fusions, and *TERT* promoter status was analyzed by Sanger sequencing or droplet digital polymerase chain reaction. In total, 11 samples had to be excluded from *TERT* promotor mutation analysis due to insufficient material. A molecular analysis revealed *BRAF* p.V600E in 66.3% of cases (63 of 95), a RAS mutation in 3.2% (three of 95; i.e., two *NRAS* and one *KRAS*), and gene fusions in 3.2% (three of 95), while no *TERT* promoter mutations were detected in any of the 84 analyzed samples. After a median follow-up of 38 months, eight of 95 participants (8.4%) experienced progression, primarily due to LN metastases (six of eight). Comparison of stable vs progressive cases showed no significant association between mutation profile and outcome (p = 0.6); *BRAF* prevalence was similar in both groups (66.7% vs 62.5%; p = 0.8). Among progressive cases, four of five *BRAF*-positive tumors developed LN metastases, and one *NRAS*-positive progressive case demonstrated nodule enlargement. Five of eight progressive cases required adjuvant radioiodine therapy post surgery, with all achieving excellent response. Limitations of this study include the small number of progression events and single-center design, which constrain statistical power. The authors concluded that common driver mutations and gene fusions do not predict progression in low-risk PTC under active surveillance, and molecular profiling should not influence enrollment decisions. These findings reinforce that molecular testing at diagnosis does not aid in stratifying progression risk for low-risk PTC managed conservatively.

The Potonnier et al. (2025) retrospective study evaluated the diagnostic accuracy of an NGS panel on thyroid nodules with indeterminate cytology (Bethesda III, IV, or V). The study included 121 patients with cytologically indeterminate thyroid nodules whose FNA samples were analyzed using the AmpliSeq cancer NGS panel. Results were then compared against final histological diagnoses. The panel's performance for Bethesda III and IV nodules revealed a sensitivity of 55.0%, specificity of 76.9%, PPV of 37.9%, and NPV of 87.0%. Although the results were promising, the authors indicated that the NPV was not sufficiently high to eliminate the need for diagnostic surgery in cases of indeterminate thyroid nodules.

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Afirma Xpression Atlas

Babazadeh et al. (2022) reported on the clinical utility of Afirma Xpression Atlas (XA) testing during 2 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). Overall, 43 of those had positive Afirma XA results, 83.7% of which were follicular cell–derived thyroid cancer on surgical histopathology. The overall PPV among Afirma GSC–suspicious indeterminate nodules during the same time frame 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 at which the study took place. It is still uncertain whether Afirma XA results significantly increase the preoperative ROM 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.

Clinical Practice Guidelines

American Thyroid Association (ATA)

The 2025 ATA guidelines (Ringel et al.) for the management of adults with differentiated thyroid cancer (DTC) include the following:

- Genomic evaluation of confirmed DTC prior to surgery is not recommended routinely (conditional recommendation; low-quality evidence).
- Molecular profiling of histological specimens post operation is not recommended routinely (conditional recommendation; low-quality evidence).
- Tissue-based biomarker testing to identify actionable oncogenic driver alterations in radioactive iodine–resistant DTC should be performed prior to initiating systemic therapy for progressive disease (strong recommendation; moderate-quality evidence).
- Whenever feasible, surgical or core tumor biopsy to allow for NGS testing to identify potential molecular mechanisms of acquired resistance should be performed (Good Practice statement).
- Surgical or core biopsy is preferred over ctDNA analysis, which may be considered for patients in whom tumor biopsy is not possible (conditional recommendation; low-quality evidence).

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.

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

- Nondiagnostic cytology: Some studies suggest that the use of a thyroid CNB with *BRAF* testing, gene panel, or 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: 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 multigene panels of *BRAF, NRAS, HRAS*, and *KRAS* point mutations as well as *RET/PTC1* and *RET/PTC3*, with or without *PAX8/PPARγ* rearrangements, and an mRNA expression profile of 167 genes. The authors concluded that more data were needed to fully understand how such tests can impact clinical management. They concluded 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 to 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 genes, *RET/PTC*, and *PAX8/PPARγ)* may be considered in nodules with cytology that is suspicious for malignancy if such data would be expected to alter surgical decision-making. Molecular testing using the 167 GEC has a PPV that is similar to that of cytology alone (76%) and an NPV of 85%; therefore, it is not indicated in patients with this cytological diagnosis.

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- Malignant cytology: While studies have been presented in the literature that suggest that *BRAF* and other multigene 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 the clinical management of patients with primary thyroid medullary cancer.
- Postoperative radioiodine therapy: Molecular testing to guide postoperative radioiodine 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 cytological features, molecular testing may be considered as a diagnostic adjunct for cytologically indeterminate nodules (strong recommendation; moderate-quality evidence).
- The 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 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 patients with nodules cytologically categorized as Bethesda IV (strong recommendation; moderate-quality evidence).

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

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

National Comprehensive Cancer Network (NCCN)

The 2025 NCCN Guidelines (NCCN Thyroid Carcinoma, v1.2025) for thyroid carcinoma indicate that molecular diagnostics may be helpful to reclassify follicular lesions as more/less likely to be benign or malignant, based on genetic profile. In addition, molecular testing may be useful for the 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 GCs are promising regarding these specimens. A requirement for the diagnosis of oncocytic carcinoma and follicular carcinomas is evidence of either vascular or capsular invasion, which FNA 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 cytological 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 the use of drug therapy or clinical trial participation. Some mutations may also have prognostic importance. Molecular testing of single genes or a GEC panel test may be considered and should be selected by the clinician based on the specific clinical question being asked. Following an FNA or core biopsy finding of anaplastic thyroid carcinoma, molecular testing for actionable mutations is recommended as an option. For unresectable or borderline resectable stage IVA or IVB anaplastic thyroid carcinoma, consideration of targeted neoadjuvant therapy is recommended as an option when it is safe to do so. Molecular testing for actionable mutations, if not previously done, is recommended as an option in aggressive therapy for stage IVC anaplastic thyroid carcinoma. Molecular testing should include *BRAF*, *NTRK*, *ALK*, *RET*, MSI, dMMR, and TMB.

Melanoma

Cutaneous Melanoma

Several molecular tests designed to assess the 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 that support the accuracy and clinical utility of these tests are needed.

Guenther et al. (2025a) published outcomes related to disease recurrence from the DECIDE (DecisionDx-Melanoma Impact on Sentinel Lymph Node Biopsy Decisions and Clinical Outcomes) study, a prospective multicenter investigation

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that evaluated the impact of results of the 31-GEP on sentinel LN biopsy (SLNB) decisions for participants with T1 to T2 cutaneous melanoma tumors. This evaluation included 130 participants diagnosed with cutaneous melanoma who received a class 1A 31-GEP result. Of them, 63 underwent SLNB, with two participants having a positive result (3.2%). The median follow-up period was 2 years. At the conclusion of the follow-up period, none of the 130 participants with class 1A results experienced cutaneous melanoma recurrence, regardless of SLN status. Based on these data, the authors suggested that individuals with class 1A 31-GEP results may be able to safely avoid SLNB. However, the study is limited by the small sample size and the low number of participants with SLNB results available. Another limitation is the 2-year median follow-up time, although prior studies suggest that the median time to recurrence in early-stage melanoma is typically less than 2 years. Lastly, the study was funded by Castle Biosciences, Inc., the manufacturer of the 31-GEP; several authors are also affiliated with this manufacturer. Although the results are promising, additional high-quality studies, with larger populations and more robust data, are necessary to validate these findings.

Historically, individuals diagnosed with cutaneous melanoma have been evaluated for SLNB using the American Joint Committee on Cancer (AJCC) staging criteria. The development of GEP tests has introduced a molecular approach to risk stratification in cutaneous melanoma. Prieto et al. (2025) compared two GEP tests, clinicopathologic factors and a GEP test (CP-GEP) and 31-GEP/i31-SLNB, with the NCCN standard for SLNB, which requires a true negative to false negative ratio of 19:1 (equivalent to a 5% false omission rate) to safely omit the procedure. Analysis from five studies of CP-GEP showed a true negative to false negative ratio of 15:1, with a 6.2% false omission rate, falling short of the NCCN standard. Four studies of 31-GEP/i31-SLNB revealed a true negative to false negative ratio of 34:1 and a 2.8% false omission rate, exceeding the NCCN standard. One of these studies, Yamamoto et al. (2023), is discussed below. The authors compared the true negative to false negative ratio and false omission rates in individuals considered low risk by the CP-GEP or 31-GEP/i31-SLNB tests to the same data based on staging. A false omission rate of 5% or higher (ratio of less than 19:1) indicated inferior test performance compared with current staging, while a false omission rate below 5% (ratio of greater than 19:1) signifies a superior method for identifying individuals who could safely avoid SLNB. A post hoc analysis found that the 31-GEP/i31-SLNB test was superior to AJCC staging and CP-GEP for identifying individuals with T1 to T2 melanoma who had less than a 5% risk of a positive SLNB. The findings are constrained by the post hoc nature of the analysis. Additional limitations identified by the authors include small sample sizes and challenges comparing results across different studies. Finally, the authors' interpretation of the findings may have been biased due to financial conflicts of interest. This study was funded by Castle Biosciences, Inc., which is the manufacturer of 31-GEP/i31-SLNB, and several authors had affiliations with this manufacturer as well.

The Hieken et al. (2025) double-blinded, multicenter, prospective, prognostic study investigated whether CP-GEP could accurately predict sentinel node status and thereby identify participants with biopsy-proven stage T1 to T3 cutaneous melanoma tumors and clinically negative regional LNs who could safely forego SLNB. The study included 1,761 participants who underwent SLNB and successful CP-GEP testing (using the Merlin assay, SkylineDx) at nine academic medical facilities. A total of 651 participants (37%) had outcomes indicating low risk per CP-GEP. Of the low-risk cases, 46 (7.1%) were found to be SLN positive, yielding an NPV of 92.9% (95% CI, 90.7%-94.8%). Of cases that were found to be high risk (n = 1,110), the researchers observed a 23.8% rate (n = 264) of SLN positivity. The likelihood of SLN metastasis was found to increase with higher clinical stage and T category/subcategory; likewise, the rate of cases with low-risk CP-GEP results decreased as the T category increased [T1 = 346 of 507 (68.2%); T2 = 295 of 897 (32.9%); T3 = 10 of 357 (2.8%)]. Based on these results, the authors asserted that CP-GEP test results may be beneficial to individuals and their surgeons during shared decision-making discussions regarding the use of SLNB in their melanoma treatment plan. Additional high-quality, prospective studies are needed to confirm the clinical utility of GEP and other predictive genomic assays in the setting of cutaneous melanoma. The authors discussed limitations of the study as well. The absence of a requirement to enroll every participant with a T1b to T3b tumor for whom SLNB was being considered may have impacted the outcome; instead, the participants were selected for participation based on the judgment of the surgeon and the participant’s willingness to participate in the study. Additionally, SkylineDx, the manufacturer of the Merlin CP-GEP assay, funded the study, and several of the authors had affiliations that potentially could have contributed to bias.

The Krebs et al. (2025) population-based, quasiexperimental, retrospective target trial emulation used linked administrative and cancer registry data to compare multigene panel sequencing (capable of identifying variants in 54 genes) with single-gene *BRAF* testing among adults with clinically determined advanced or metastatic melanoma tested between September 2016 and December 2018. Patients were assigned to groups based on the test they received and were matched 1:1 using genetic algorithm–based methods on preselected baseline covariates to emulate randomization; 147 of 205 panel-tested patients were matched to 147 of 158 single-gene–tested controls with good covariate balance and up to 3 years of follow-up. The study was multicenter within a single health system, unblinded, and nonrandomized. The outcomes prioritized clinical effectiveness measured as OS, analyzed primarily as an intention-to-treat contrast with inverse probability of censoring weights, complemented by a per-protocol analysis that additionally applied stabilized inverse probability of treatment weights and artificial censoring 90 days after results for patients who had not initiated

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systemic therapy. Test turnaround was longer for panels than single-gene tests, and treatment patterns after matching were similar; roughly two-thirds in each arm received systemic therapy, with comparable proportions receiving programmed cell death 1 protein inhibitors and, among those with *BRAF* mutations, targeted therapy. In intention-to-treat analyses, the study failed to demonstrate group differences. In per-protocol analyses, OS differences favored panels, indicating improved survival when analyses were conditioned on treatment initiation. Life-years gained mirrored the survival findings, with intention-to-treat incremental life-years of 0.22 (95% CI, -0.05 to 0.49) and per-protocol incremental life-years of 0.39 (95% CI, 0.02-0.85). The authors concluded that broad implementation of multigene panel sequencing alone did not yield statistically significant survival differences at the population level, whereas analyses accounting for treatment initiation suggested improved OS associated with panel testing. The findings are limited by the observational design and lack of randomization.

The Guenther et al. (2025b) prospective multicenter study evaluated the accuracy of DecisionDx-Melanoma integrated with Breslow thickness, mitotic rate, age, and ulceration (i31-GEP) for SLNB in predicting positive SLN results in 322 participants with T1 or T2 tumors. This study expanded on the original DECIDE study results (Yamamoto et al., 2023, discussed below). To ascertain whether incorporation of the i31-GEP into treatment decision-making led to fewer SLNBs, the researchers used propensity score matching from a matched, nonoverlapping cohort (n = 322) in which the i31-GEP was not used for decision-making regarding SLNB. The study results revealed that no participants with less than 5% i31-GEP–predicted risk had a positive SLNB (zero of 35). Use of propensity matching showed an 18.5% reduction in SLNBs performed (43.7% vs 62.2%; $p < 0.001$). The researchers calculated that the use of the i31-GEP could have reduced the volume of unneeded biopsies in 35 of 140 participants (25%); they asserted that the performance and clinical utility of the i31-GEP for predicting positivity in SLNs was confirmed by this study and suggested that incorporation of this test into clinical decision-making could reduce the rate of SLNB and improve risk-related care in individuals with T1 to T2 cutaneous melanoma. Noted limitations include the number of participants with SLNB results available; there was not a separate cohort for comparing SLNB procedure rates. Participants and physicians were also allowed to choose whether SLNB was performed, with the participant's preference serving as the greatest influence, which may have introduced variability into clinical decision-making. Finally, the manufacturer of the GEP test under evaluation provided funding for the study, and several authors had affiliations with the manufacturer as well, which presents a potential risk of bias.

Hayes (2025; updated 2026) published a Molecular Test Assessment on the MyPath Melanoma test in 2025, evaluating use of the test to aid in diagnosis for individuals with ambiguous melanocytic lesions. The assessment included seven studies; four focused on clinical validity, and three evaluated clinical utility. All seven studies were determined to be low or very low quality. Based on their review, Hayes concluded that the existing evidence was insufficient to adequately assess the use of the MyPath Melanoma test as a diagnostic adjunct tool or its impact on clinical outcomes. Hayes' 2026 annual review identified no newly published studies that met inclusion criteria for an updated report, and no abstracts were reviewed.

Durgham et al. (2024) conducted a systematic review and meta-analysis that compiled data from 13 studies, which involved a total of 14,760 individuals diagnosed with cutaneous melanoma. The included studies reported survival outcomes stratified by the 31-GEP test and used either prospective institutional data, retrospective data, or national registry databases (SEER 2009-2018). The primary goal was to assess the prognostic accuracy and clinical utility of the 31-GEP test in stratifying risk and predicting survival outcomes in individuals with cutaneous melanoma. The studies were selected based on their reporting of survival outcomes by 31-GEP risk class. Random-effects meta-analytic models were used to generate pooled survival estimates. Analyses included meta-analyses of proportions (for sex, tumor characteristics, and survival outcomes) and single means (for age and Breslow thickness), with each measure weighted according to the number of individuals affected. Statistical analyses were conducted using R version 4.4.0. The 31-GEP test consistently categorized individuals into distinct risk groups, with significantly different survival outcomes. The 5-year melanoma-specific survival (MSS) rates were 99.8% (95% CI, 98%-100%) for class 1A (lowest risk), 97.6% (95% CI, 92.4%-99.3%) for class 1B/2A (intermediate risk), and 83.4% (95% CI, 66.5%-92.7%) for class 2B (highest risk). The recurrence-free survival and DMFS groups were observed to have similar patterns. According to the study, the 31-GEP test was found to enhance current staging systems and provide valuable information for personalized management strategies in melanoma care. While the study is encouraging for 31-GEP for future use, there were limitations that need careful consideration, according to the authors. The number of studies available for each GEP risk class and survival outcome was limited, reducing the precision and reliability of pooled estimates. Substantial heterogeneity was observed across studies (I^2 up to 99.4% for 5-year survival), introducing uncertainty and limiting the generalizability of findings despite the use of random-effects models. Few studies detailed adjuvant treatments beyond primary surgery, even though the introduction of immunotherapies during the study period may have independently influenced outcomes. The categorical nature of the GEP risk stratification contrasts with recommendations from the Melanoma Prevention Working Group to report GEP results as a continuous variable, potentially reducing the granularity and precision of risk assessment. The analysis primarily focused on comparing class 2B (highest risk) and class 1A (lowest risk), providing less insight into intermediate-risk groups (class 1B and 2A). The authors concluded that the 31-GEP test offers significant

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prognostic value by enabling more accurate risk stratification for recurrence and metastasis in individuals with cutaneous melanoma. This improved risk assessment has the potential to optimize personalized treatment approaches and balance treatment efficacy with QOL. However, the 31-GEP should be considered a complementary tool rather than a replacement for standard clinicopathologic assessments. Additional research is required to clarify its optimal application, including identification of which individuals benefit most and how best to incorporate test results into clinical decision-making algorithms. Publications by Bailey et al. (2023) and Zager et al. (2018), previously discussed in this policy, were included in the Durgham et al. systematic review and meta-analysis.

Yamamoto et al. (2023) performed a prospective multicenter study (DECIDE) to quantify reductions in SLNB related to the use of the 31-GEP and monitor 5-year clinical outcomes in each 31-GEP subclass. The study enrolled 193 participants with T1 to T2 cutaneous melanoma tumors who had been deemed eligible for SLNB by expert physicians. Prior to performance of SLNB but after receipt of 31-GEP results, treating providers were queried regarding the clinical and pathological factors that influenced their decision regarding SLNB (n = 191). SLNB procedure rates from this study were compared with baseline SLNB rates from a contemporary study (Whitman et al., 2021) using the exact binomial test. Logistic regression modeling was then used to pinpoint features associated with the rates of SLNB procedures. Results of the analyses showed that 52.4% (100 of 191) of clinical decisions to abstain from SLNB were impacted by 31-GEP test results, and in 70% of these instances (70 of 100), providers did not move forward with SLNB. Of the 30 of 100 SLNBs that were performed in this group, all were negative. In addition, the 31-GEP contributed to 63 clinical decisions (33%) to perform SLNB; of these, 58 of 63 (92.1%) were performed. These findings represented a 29.4% reduction in SLNBs completed in participants with class 1A results compared with the baseline rate of 78% ($p < 0.01$). In total, clinical decisions related to SLNB were impacted by 31-GEP results in 85.3% of cases. The results described led the authors to conclude that the 31-GEP test may provide clinically relevant information regarding the decision to forego or proceed with SLNB in individuals with T1 to T2 tumors, which could, in turn, significantly decrease the rate of SLNB. However, the study has limitations; the researchers did not include tumor location as a question with respect to its influence on SLNB performance decisions (previous studies have shown that tumors of the head/neck have lower rates of SLNB), which may have been a confounding factor in the analysis. In addition, this assessment did not include outcome data, which were still accruing at the time of publication. The collection of various clinical and pathological data likely varied by site and could have contributed to bias. Lastly, the study was funded by Castle Biosciences, Inc., which was the test manufacturer, and several authors had affiliations with this manufacturer.

The Thomsen et al. (2023) systematic review assessed the diagnostic accuracy of tape stripping for detecting cutaneous melanoma in suspicious pigmented skin lesions. Ten studies were included. Sensitivity ranged from 68.8% (95% 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 (preferentially expressed antigen in melanoma) 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 has several limitations, including a lack of information related to the characteristics of the study population, lack of histological examination for tape-stripping lesions, potential risk of overlap of individuals, and lack of RCTs that would determine the difference between tape stripping and no tape stripping in terms of the impact to prognosis. The authors indicated that in the studies evaluated, tape stripping 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 pigmented lesion assay (PLA) testing in cutaneous melanoma. Publications by Ferris et al. (2017) and Ferris et al. (2018), previously discussed in this policy, were included in this systematic review and meta-analysis.

A Molecular Test Assessment published by Hayes (2023; updated 2025) focused on the clinical validity and clinical utility of the Merlin test (SkylineDx) in individuals diagnosed with primary cutaneous melanoma who were eligible for SLNB. Hayes identified three poor- to fair-quality clinical validity studies suggesting that the Merlin test exhibits a high NPV as well as high sensitivity for detecting nodal metastasis, signifying that false negatives are rare and individuals with low-risk results are indeed unlikely to have nodal involvement. However, Merlin was also found to have low specificity and a low PPV, suggesting that false positives are not uncommon; this may lead to unnecessary SLNB procedures. Hayes did not identify any studies addressing the clinical utility of the Merlin test. While Hayes identified additional studies of the Merlin test's clinical validity in both their 2024 and 2025 annual reviews of this topic, no evidence was identified that would be likely to change their overall assessment. Hayes has indicated that there is considerable uncertainty stemming from the limited number and quality of clinical validity studies, lack of direct comparisons with alternative methods for assessing metastatic risk, and general absence of research that evaluates the clinical utility of the Merlin test.

In their Molecular Test Assessment on the DecisionDx-Melanoma gene expression test, Hayes reviewed 10 studies 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

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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 SLNs. They did not identify any studies in peer-reviewed literature that met the criteria and addressed the clinical utility of the test to improve clinical decision-making and individuals' 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 SLN positivity in individuals with AJCC stage I, II, or III cutaneous melanoma (Hayes, DecisionDx-Melanoma, 2022; updated 2024).

Ludzik et al. (2022) conducted a retrospective case-control study that evaluated the use of the PLA. The PLA is used to noninvasively detect the presence of three genes associated with melanoma (*LINC00518, PRAME*, and *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 authors' aim in this study was to identify possible barriers that reduce the clinical utility of PLA testing by dermatologists. Data were collected from April 2021 to April 2022 from an academic tertiary-level center, and 472 lesions were evaluated. 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 to 25 minutes. The authors noted that this novel, noninvasive PLA test for melanoma, using an adhesive tape-stripping technique and GEP, may be a promising technique to reduce unnecessary biopsies and optimize the triage of pigmented lesions. However, 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 nonactionable 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) evaluated the diagnostic accuracy, clinical utility, and budget impact of PLAs for people with suspected melanoma skin lesions. The systematic review included seven studies, which consisted of six cohort studies and one survey that were conducted in dermatology offices, examining adults (> 18 years old) with suspected melanoma lesions using the DermTech PLA. 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 include the potential bias from the industry-sponsored studies and overestimation of the diagnostic accuracy of PLA; additionally, the diagnostic accuracy of visual assessment may have been underestimated compared with published literature, and many parameters and assumptions used by the economic analysis were not reported in the study, which, the authors stated, had potentially serious limitations. They concluded that there is no evidence demonstrating the impact of PLA on individuals' outcomes and that the low-quality evidence for the diagnostic accuracy of PLA remains uncertain compared with 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 that of the standard care pathway is also uncertain. Publications by Ferris et al. (2017), Ferris et al. (2018), and Ferris et al. (2019), previously discussed in this policy, were included in this Health Technology Assessment.

Marchetti et al. (2020) completed a systematic review and meta-analysis to assess the performance of prognostic GEP tests in individuals with AJCC stage I or stage II cutaneous melanoma. The review included seven studies, with a total of 1,450 individuals. 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 individuals with stage I disease and 212 with stage II disease who were tested with DecisionDx-Melanoma. The authors found that DecisionDx-Melanoma correctly classified recurrence in 29% of the individuals 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 individuals without recurrence. When they reviewed the data for MelaGenix, which included 88 individuals 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 individuals 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, lack of availability of individual-level data, short follow-up, significant censoring, variability in the definitions used for melanoma recurrence, risk of bias, and quality of the evidence. The authors concluded that the prognostic ability of DecisionDx-Melanoma and MelaGenix to predict recurrence among individuals with localized melanoma varied by AJCC stage and appeared to be poor in individuals with stage I disease. They recommended that more rigorously structured studies be performed to better quantify the association of GEP tests with melanoma outcomes and to demonstrate clinical utility.

The Greenhaw et al. (2020) meta-analysis reported on the strength of the prognostic value of the 31-GEP for cutaneous melanoma. To perform the assessment, a meta-analysis was performed on three studies that met the inclusion criteria. The clinical outcomes for the 31-GEP test were compared with the AJCC on cancer staging. The 31-GEP was able to identify the AJCC stages 1 to 3 categories with a high likelihood for distant metastases and recurrence. When the GEP and SLNB were evaluated in conjunction, sensitivity and the NPV related to DMFS both improved. The authors concluded that the 31-gene test accurately and consistently identified individuals with melanoma who were at an increased risk of

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metastasis, functioned independently of other clinicopathologic factors, and improved accuracy of current risk stratification. However, several limitations were noted. There is a possibility that unpublished, negative-result studies exist that were not considered in this analysis. The studies that were included had different designs, which could impact the strength of the effect of GEP due to evolving treatments and population differences. Follow-up time also varied across the studies, which is a consideration when interpreting OS estimates. Further studies are needed to evaluate the most appropriate follow-up and treatment in 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 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 aged 18 years or older. However, published studies do not address full follow-up in individuals with negative results, and most studies used a 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].

Uveal Melanoma

The Suwajanakorn et al. (2025) single-center retrospective cohort study assessed whether DecisionDx-UM GEP and PRAME status have clinical utility for anticipating long-term tumor thickness regression and metastatic risk after proton beam irradiation in uveal melanoma (UM). Patients with choroidal or ciliochoroidal melanoma treated between 2013 and 2021 were eligible if they had a DecisionDx-UM result and at least three postradiation ultrasound measurements; seven with local recurrence at baseline or during follow-up were excluded, and PRAME results were available only from 2016 onward. The cohort included 106 patients, with DecisionDx-UM distribution of class 1A of 46 of 106 (43.4%); class 1B of 27 of 106 (25.5%); and class 2 of 33 of 106 (31.1%). The primary end point was percentage change in ultrasound-measured tumor thickness from pretreatment over time; secondary analyses examined factors influencing regression and whether fast vs slow regression (dichotomized at the cohort's 18-month median of 27.1%) was associated with metastasis. Overall, mean thickness reductions were 20.9% at 1 year, 35.1% at 2 years, 51.4% at 4 years, and 59.3% at 6 years. Time (95% CI, -1.86 to -1.37; $p < 0.001$) and greater baseline thickness (95% CI, -1.91 to -0.37; $p = 0.004$) predicted greater percentage reduction. Predicted regression for class 1A at 6, 12, 24, 48, and 72 months was 10.2%, 18.2%, 31.2%, 47.5%, and 54.3%; for class 1B was 14.9%, 25.5%, 40.8%, 56.5%, and 67.4%; and for class 2 was 11.0%, 20.6%, 35.6%, 52.6%, and 59.2%, with no significant between-class differences through 72 months. Subgroup analyses by baseline thickness (≤ 3 mm; > 3 to ≤ 8 mm; > 8 mm) showed no class-based differences, and among 70 patients with PRAME data, there were no significant differences in regression between class 1/PRAME negative, class 1/PRAME positive, and class 2 through 72 months. Metastasis occurred in three of 46 class 1A (6.5%), one of 27 class 1B (3.7%), and 17 of 33 class 2 (51.5%) ($p < 0.01$) and all-cause mortality in two of 46 (4.4%), two of 27 (7.4%), and 10 of 33 (30.3%) ($p < 0.01$). When regression speed was compared with metastasis, fast vs slow regression did not differ in risk (95% CI, 0.54-3.01; $p = 0.62$); in multivariable analysis, class 2 status and larger baseline largest basal diameter were significant risk factors for metastasis. These data indicate that DecisionDx-UM class and PRAME status do not predict the kinetics of postirradiation tumor thickness regression and that faster regression should not be used as a surrogate for molecular metastatic risk; DecisionDx-UM class 2 remains associated with higher observed metastasis rates. Important limitations of this study include the retrospective design, reliance on a single dimension (thickness) rather than cross-sectional area, absence of intra-/interobserver reproducibility assessment, limited PRAME availability and sample size, and potential confounding between tumor size and selected radiation dose (dose did not improve model fit, but size and dose were correlated). Clinically, the findings may support using DecisionDx-UM and baseline dimensions to guide surveillance intensity and systemic risk discussions, without inferring metastatic risk from the speed of post–proton beam irradiation thinning. Author disclosures include conflict of interest statements related to funding and consultancies.

The Harbour et al. (2024) prospective multicenter cohort study enrolled 1,687 participants with UM of the choroid, ciliary body, and/or iris across 26 North American ocular oncology centers between January 2017 and April 2020; 101 with primary iris melanoma and nine with metastatic disease at baseline were excluded, yielding 1,577 participants analyzed (median follow-up, 43.6 months), with the primary end point of MFS and MSS as a secondary outcome. Eligibility required an age of ≥ 18 years and a diagnosis of UM. Exclusion criteria included prior RT, while prior photodynamic therapy or transpupillary thermotherapy was permitted if there was tumor regrowth. All tumors underwent DecisionDx-UM 15-GEP (DecisionDx-UM; classes 1A/1B or 2) and PRAME RNA expression testing (negative or positive); 369 of 1,577 (22%) had residual clinical samples collected from 2014 to 2016, with subsequent prospective follow-up. The cohort distribution was DecisionDx-UM class 1 in 1,082 of 1,577 (68.6%) and class 2 in 495 of 1,577 (31.4%); PRAME was negative in 1,106 of 1,577 (70.1%) and positive in 471 of 1,577 (29.9%). Five-year MFS was 92.3% (95% CI, 90.2%-94.4%) for class 1 vs 52.1% (95% CI, 47.0%-57.8%) for class 2; 5-year MSS was 97.4% (95% CI, 96.2%-98.7%; $p < 0.0001$) vs 68.8% (95% CI, 63.6%-74.5%; $p < 0.0001$), respectively. By PRAME status, 5-year MFS was 86.6% (95% CI, 84.2%-89.1%) for

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PRAME negative vs 63.7% (95% CI, 58.5%-69.3%) for PRAME positive, and 5-year MSS was 93.1% (95% CI, 91.2%-95.0%; p < 0.0001) vs 78.5% (95% CI, 73.9%-83.3%; p < 0.0001). The integrated four-group classifier further separated risk: 5-year MFS was 95.6% (95% CI, 93.9%-97.4%) for class 1/PRAME negative, 80.6% (95% CI, 73.9%-87.9%) for class 1/PRAME positive, 58.3% (95% CI, 51.1%-66.4%) for class 2/PRAME negative, and 44.8% (95% CI, 37.9%-52.8%) for class 2/PRAME positive. Median time to metastasis was 31.3 months (class 1/PRAME negative), 34.9 months (class 1/PRAME positive), 24.7 months (class 2/PRAME negative), and 16.5 months (class 2/PRAME positive). Class 2 (hazard ratio, 9.77; 95% CI, 7.36-12.95; p < 0.001) and PRAME positivity (hazard ratio, 3.31; 95% CI, 2.60-4.20; p < 0.001) were associated with higher metastatic risk; in multivariable analysis, DecisionDx-UM (hazard ratio, 5.95; 95% CI, 4.43-7.99; p < 0.001) and PRAME (hazard ratio, 1.82; 95% CI, 1.42-2.33; p < 0.001) remained independent predictors. The integrated classifier identified substantial within-stage heterogeneity; for example, in T1 tumors, 5-year MFS remained high overall (94.4%; 95% CI, 92.1%-96.8%) yet fell to 77.6% for class 2/PRAME negative and 69.5% for class 2/PRAME positive, whereas in T4 tumors, 5-year MFS was 92.9% (95% CI, 80.3%-100%) for class 1/PRAME negative vs 28.1% (95% CI, 16.0%-49.4%) for class 2/PRAME positive. This study's findings suggest that integrating PRAME with DecisionDx-UM yields more precise risk stratification than DecisionDx-UM or staging alone, with numerically large and statistically significant separation in 5-year MFS and MSS across groups, supporting more tailored surveillance intensity and a framework for adjuvant trial stratification based on clearly quantified metastatic risk. Several authors reported relationships with Castle Biosciences, Inc. (including honoraria, consulting, funding, and royalties related to licensed intellectual property), and testing was performed by Castle's laboratory; these disclosures represent potential conflicts. (This study is included in the Molecular Test Assessment by Hayes, 2020, updated 2025.)

Miguez et al. (2023) conducted a retrospective analysis to assess and validate the prognostic value of GEP testing in patients with UM. To date, no studies predicting metastasis by including tumor size have been performed. In this study, the researchers sought to determine the prognostic value of combining tumor size with the GEP classification to predict metastases. The results included 337 patients from three different institutions, with 87 demonstrated metastases. The mean follow-up time was 37.2 (SD, 40.2) months in patients with metastases and 55.0 (SD, 49.3) months in those without metastases. Tumors of larger thickness and a GEP class of 2 (vs class 1) were associated significantly with an 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 an increased risk of metastasis compared with tumors with a thickness of < 7.0 mm (hazard ratio, 3.23; 95% CI, 1.61-6.51), whereas class 1 tumors with a thickness of 9.0 mm or more were associated with an increased risk of metastasis compared with tumors with a thickness of < 9.0 mm (hazard ratio, 2.07; 95% CI, 0.86-4.99). No difference in MFS was found between patients with class 1A tumors compared with those with class 1B tumors (p = 0.8). Patients with class 2 tumors had an observed 5-year MFS rate of 47.5% (95% CI, 36.0%-62.8%). The study limitations include the retrospective design, inclusion of patients from three different institutions, and likely variation in tumor size and biopsy techniques among providers. Despite the limitations, the authors indicated that tumor size was the most significant predictor of metastasis; it provided additional prognostic value that was independent of GEP classification. (This study is included in the Molecular Test Assessment by Hayes, 2020, updated 2026.)

Singh et al. (2022) conducted a retrospective 10-year cohort study to assess the accuracy of the predicted MFS rate by a GEP test in patients with UM by comparing the patient's GEP test results with what was found in the clinics. The authors reported that the test predicted worse outcomes in patients with UM than what occurred. The study included a retrospective record review in 352 consecutive patients from two clinics, with a mean age at diagnosis of 59.4 years (+13.0 years), who were followed up for a median interval of 38.0 months (19.0-57.0 months). All patients had undergone an FNA 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, five who had class 1A tumors, and three who had 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 rate was 87% in patients with class 1 tumors and 47% in those with class 2 tumors. Limitations of this cohort study include 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 appeared 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 (2020; updated 2026) completed a Molecular Test Assessment of the DecisionDx-UM test, finding that it has potential but unproven benefit when used to predict the likelihood of metastasis within 5 years in individuals with UM. The evidence that Hayes evaluated in their 2025 updated assessment was found to have overall low quality. It consisted of four clinical validity cohort studies and five clinical utility cohort studies. The five clinical utility studies (three very poor-quality and two poor-quality) assessed the impact of DecisionDx-UM on surveillance decisions and/or individual outcomes. Across studies, results consistently indicated that DecisionDx-UM may influence surveillance

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recommendations, including the intensity, frequency, and modality of testing, as well as referrals to medical oncology. Hayes concluded that additional controlled studies are needed to determine the test's impact on individuals' health outcomes. Overall, Hayes found that the evidence reviewed consistently suggests that DecisionDx-UM is predictive of metastatic risk and may impact surveillance and decisions in individuals with UM. Although this reflects an improvement in Hayes' overall rating of the DecisionDx-UM test compared with their 2020 assessment, Hayes noted that substantial uncertainty remains in the overall body of evidence for DecisionDx-UM due to individual study limitations, limited direct comparisons with other methods for predicting risk, and limited evidence for the impact of testing on individuals' health outcomes. Hayes' 2026 annual review identified no newly published studies that met the inclusion criteria for an updated report, and no abstracts were reviewed.

The Aaberg et al. (2020) 5-year clinical outcome report from a prospective registry of participants tested with a prognostic 15-GEP test for UM and a meta-analysis with published cohorts found that testing with the 15-GEP test guided the management of participants with UM. UM, a rare intraocular cancer, has a 30% to 50% risk of metastasis within 5 years of diagnosis. The prognostic 15-GEP was designed to predict the 5-year metastatic risk using three risk categories, indicating low-, intermediate-, and high-risk groups. In this study, 89 participants who had undergone 15-GEP testing were prospectively enrolled at four separate locations. The clinical outcomes and management plans were tracked every 6 months. Overall, 80% of class 1 (low risk) participants received low-intensity management, and all class 2 (high risk) participants received high-intensity management ($p < 0.0001$). The 5-year melanoma survival rates were 94% for class 1 and 63% for class 2. The 5-year MFS rates were 90% for class 1 and 41% for class 2. A meta-analysis performed on several prior studies to evaluate clinical outcomes in participants tested with 15-GEP showed that class 2 was associated with an increased risk of both metastasis and mortality and was also the only independent predictor of metastasis. (This study is included in the Molecular Test Assessment by Hayes, 2020, updated 2025.)

Klufas et al. (2017) retrospectively reviewed the role of GEP analysis vs chromosome 3–specific analysis. The records of consecutive patients diagnosed with posterior UM, who underwent intraoperative FNA 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; multiplex ligation-dependent probe amplification results were obtained. Discordance between GEP and chromosome 3 status by FISH and multiplex ligation-dependent probe amplification occurred in the series at a rate of 15.9% and 16.3%, respectively. The authors concluded that caution must be advised when counseling an individual with a good prognosis GEP class 1 result that the uveal tumor may harbor monosomy 3, which is associated with a poor prognosis for metastasis in nearly 20% of individuals.

Plasseraud et al. (2016) (included in the original 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.). Overall, 70 participants were enrolled to document the management differences and clinical outcomes associated with low-risk class 1 and high-risk class 2 results indicated by DecisionDx-UM testing. In total, 37 participants in the prospective study were class 1, and 33 were class 2. Class 1 participants had 100% 3-year MFS compared with 63% for class 2 (log-rank test $p = 0.003$), with 27.3 median follow-up months in this interim analysis. Class 2 participants received significantly higher-intensity monitoring and more oncology/clinical trial referrals than class 1 (Fisher exact test $p = 2.1 \times 10^{-13}$ and $p = 0.04$, respectively). In the authors' opinion, the results of this study provide additional, prospective evidence through an independent cohort of participants, in which class 1 and class 2 participants were 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 include recommendations for molecular testing of primary cutaneous melanoma (Swetter et al., 2019).

- Ancillary diagnostic molecular techniques (e.g., comparative genomic hybridization, FISH, GEP testing) 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., SLN 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.

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National Comprehensive Cancer Network (NCCN)

NCCN Guidelines (NCCN Cutaneous Melanoma, v1.2026) include the following recommendations for cutaneous melanoma [including but not limited to vulvar and vulvovaginal melanoma (NCCN Vulvar Cancer, v2.2026)]:

- Diagnostic testing using ancillary tests, such as GEP and NGS, as adjuncts to clinical and expert dermatopathologic examination is recommended as an option for indeterminate melanocytic neoplasms following histopathology (category 2A).
- Predictive GEP testing to differentiate melanomas at low vs high risk for nodal metastasis, as a replacement for surgical oncology discussion of pathological staging procedures, is not recommended as an option outside the context of a clinical study or trial (category 2A).
- Strong consideration of larger NGS panels to identify other potential genetic targets is recommended as an option if *BRAF* single-gene testing was the initial test performed and is negative (category 2A).
- NGS testing is recommended as an option for resected stage I to II cutaneous melanoma if it will inform clinical trial participation (category 2A).
- Consideration of broader genomic profiling is recommended as an option for patients with stage III melanoma if the test results might guide further treatment decisions or eligibility for participation in a clinical trial for stage III clinically node positive, sentinel node positive, clinical satellite/in-transit, or stage IV metastatic cutaneous melanoma (category 2A).
- Multigene panel testing for initial presentation with stage IV disease or clinical recurrence is recommended as an option, if feasible, especially if the test results might guide future treatment decisions or eligibility for participation in a clinical trial (category 2A).

The guideline states the following regarding prognostic/predictive testing:

- Despite commercially available GEP tests being marketed to risk stratify cutaneous melanomas, current GEP platforms do not provide clinically actionable prognostic information when combined or compared with known clinicopathologic factors (e.g., sex, age, primary tumor location, thickness, ulceration, mitotic rate, lymphovascular invasion, microsatellites, and/or SLNB status). Furthermore, the clinical utility of these tests to inform treatment recommendations and improve health outcomes by prompting an intervention has not been established.
- Various studies of prognostic GEP tests suggest their role as an independent predictor of worse outcome. However, GEP studies to date have not demonstrated added benefit beyond comprehensive clinicopathologic variables, and it remains unclear whether available GEP tests are reliably predictive of outcome across the risk spectrum of cutaneous melanoma. Validation studies in prospectively collected, independent cohorts (similar to those performed in BC) are necessary to define the clinical utility of molecular prognostic GEP as an adjunct to AJCC staging and other known prognostically significant clinicopathologic variables or as part of the multidisciplinary decision-making process to guide surveillance imaging, SLNB, and adjuvant therapy.
- Existing and emerging GEP tests and other molecular techniques (e.g., ctDNA tests) should be prospectively compared to determine their clinical utility, including with no-cost, contemporary models that incorporate readily available clinicopathologic variables. Prospective study of the utility of predictive GEP for SLNB risk, in conjunction with well-established clinicopathologic factors, is ongoing.

The guideline states the following regarding biomarkers with potential utility for immunotherapy:

- The use of mutation burden to guide treatment decisions remains investigational at this time.

The guideline states the following regarding retesting metastatic tissue:

- Repeat molecular testing on recurrence or metastasis is likely to be of low yield, unless new or more comprehensive testing methods are used or a larger, more representative sample is available if there is concern for sampling error.
- Repeat testing following progression on targeted therapy does not appear to have clinical utility, since the mechanisms of resistance are diverse and do not have prognostic or therapeutic relevance.

The guideline states the following regarding follow-up recommendations:

- For melanocytic neoplasms that are clinically/dermoscopically suspicious for melanoma, prediagnostic patch testing may also be helpful to guide biopsy decisions.

The NCCN uveal melanoma guidelines (v2.2026) address the staging and management of UM, stating that a biopsy is not usually necessary for the initial diagnosis of UM 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 a biopsy for prognostic purposes should be carefully considered and discussed at length. The NCCN recommendations include the following:

- Molecular/chromosomal testing for prognostication is a preferred option over cytology alone if biopsy is performed (category 2A).

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- Consideration of genetic testing is recommended for extraocular recurrence or metastasis if it might affect treatment options (category 2A).

Society for Immunotherapy of Cancer (SITC)

In 2023, the SITC (Pavlick et al.) issued the following evidence- and consensus-based recommendations for immunotherapy for the treatment of melanoma:

- For unresectable/metastatic cutaneous melanoma, NGS is recommended if feasible.
- UM:
  - For rare melanoma subtypes (e.g., uveal, mucosal), molecular mutation testing is recommended. The discovery of an actionable mutation offers the opportunity for targeted therapy or enrollment in molecularly directed clinical trials.
  - GEP may be useful for predicting recurrence risk and has been prospectively validated for UM.
  - Systemic treatment options for metastatic UM are limited, and clinical trial enrollment is always preferred when possible. GEP can be helpful to determine prognosis and eligibility for clinical trial enrollment.

Society of Surgical Oncology (SSO)

In 2025, the SSO (Bartlett et al.) issued a consensus statement on the clinical utility of GEP testing in primary cutaneous melanoma based on findings from a systematic literature review. The panel published the following recommendations for the use of GEP testing in three clinical scenarios: patient selection for LN biopsy, guidance on surveillance, and adjuvant therapy decisions.

- GEP testing is not currently recommended for routine use in predicting SLN status.
- GEP testing is not currently recommended to guide a specific surveillance or follow-up approach in melanoma care.
- GEP testing is not currently recommended to replace SLN biopsy for prognostication or staging or to guide surveillance and adjuvant treatment approaches in patients (AJCC pT1b-pT4b) who are otherwise recommended for the procedure.
- There is currently a lack of evidence supporting the use of GEP testing to inform treatment decisions for the use or the utility of adjuvant therapy.

Bladder Cancer

There is currently insufficient evidence to support the use of molecular testing for the management of bladder cancer. Additional large, high-quality studies are required to evaluate the clinical utility of this technology.

The Heard and Mitra (2024) systematic review examined the performance of urine-based biomarker assays for bladder cancer detection and surveillance. The Cxbladder Triage was evaluated in five studies for hematuria workup and two studies for non–muscle-invasive bladder cancer (NMIBC) surveillance. In the hematuria setting, the Cxbladder Triage demonstrated a median sensitivity of 93.0% (range, 82.0%-95.5%) and specificity of 62.5% (34.2%-85.0%), with an exceptionally high NPV of 98.3%. Sensitivity for high-grade tumors reached 97%, suggesting a strong discriminatory ability for clinically significant disease. In surveillance, the Cxbladder Triage achieved a sensitivity of 92.0% (91.0%-93.0%) and NPV of 96.5%, again indicating robust performance in ruling out recurrence. Specificity and the PPV were not consistently reported, limiting full interpretation of its diagnostic balance. Bladder EpiCheck, assessed in four surveillance studies, had a median sensitivity of 65.6% (62.3%-68.2%) and specificity of 87.2% (82.1%-88.0%), with an NPV of 91.7% and PPV of 48.1%. A subgroup analysis revealed a sensitivity of 86.1% for high-grade lesions but only 43.1% for low-grade disease, highlighting the strength of Bladder EpiCheck in detecting aggressive tumors while underscoring limitations for low-grade recurrence. Both assays outperformed older tests in sensitivity and NPV, particularly in surveillance contexts, but neither demonstrated sufficient accuracy to replace cystoscopy. The variability in study design, incomplete reporting of the PPV for the Cxbladder Triage, and heterogeneity in the study population risk profiles remain key limitations. These findings suggest that the Cxbladder Triage and Bladder EpiCheck may serve as adjuncts for reducing unnecessary cystoscopies, especially when a high NPV is clinically desirable, but confirmatory cystoscopy remains essential until prospective, standardized trials validate their reliability.

Bladder EpiCheck

The Fleshner et al. (2025) prospective, blinded, multicenter cohort study enrolled 674 participants undergoing cystoscopic surveillance for NMIBC across 11 North American centers between August 2016 and March 2019; 449 participants met the eligibility criteria, which included an age of ≥ 22 years and surveillance within 12 months of the last transurethral resection of bladder tumor. Exclusions were planned cystectomy, chemoradiation, or inability to provide a 45-mL voided urine sample. Voided urine was collected prior to cystoscopy for Bladder EpiCheck testing, cytology, and UroVysion at baseline, with follow-up visits up to three cycles. Bladder EpiCheck testing was performed centrally, and clinicians were blinded to the results. The gold standard was defined as a positive cystoscopy confirmed by a biopsy or cytology

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indicating or suspicious for high-grade urothelial carcinoma (HGUC). The median age was 71 years (range, 37-93 years); 81% of participants were male, and 64% had a history of high-grade disease. Among 449 evaluable participants, 138 (31%) experienced recurrence, including 64 high-grade events. Bladder EpiCheck demonstrated an overall sensitivity of 67% (95% CI, 58%-74%), specificity of 84% (80%-88%), PPV of 65% (57%-73%), and NPV of 85% (81%-89%). For high-grade recurrence, the sensitivity was 77% (65%-85%), and the NPV was 95% (92%-97%). In participants with a negative cystoscopy and cytology at baseline, a positive Bladder EpiCheck result was associated with a 5.3-fold higher ROR within 12 months (hazard ratio, 5.3; 95% CI, 2.7-10.3; $p < 0.0001$). In cases of equivocal cytology, Bladder EpiCheck was positive in 75% to 89% of those who later developed high-grade disease, with the PPV ranging from 42% (15%-72%) to 63% (38%-84%). These findings were contextualized by a meta-analysis of seven previously published studies (n = 1,564) that found an overall published pooled sensitivity of 82% (66%-92%), high-grade sensitivity of 91% (82%-95%), specificity of 85% (80%-88%), PPV of 60% (55%-64%), and high-grade NPV of 98% (97%-99%). This study's strengths include a blinded design, centralized testing, and broad eligibility criteria, enhancing generalizability. Limitations include the absence of randomized comparisons. These results suggest that Bladder EpiCheck may offer a high NPV for high-grade disease and potential utility in adjudicating equivocal cytology or anticipating recurrence; however, randomized trials are needed to define its role in surveillance strategies. Funding was provided by Nucleix, and several authors disclosed advisory roles in the industry.

Hayes (2024; updated 2025) published a Molecular Test Assessment evaluating the use of the Bladder EpiCheck test for detection of tumor recurrence in individuals with NMIBC. Hayes found a very low-quality body of evidence and concluded that the test had acceptable to excellent performance for detecting recurrence, but uncertainty remains regarding the impact of false-positive and false-negative test results. The comparative performance of the test vs that of alternative surveillance methods is largely unknown. The 2025 update of this assessment identified two newly published clinical validity studies that may meet the Hayes inclusion criteria set out in the original report but are unlikely to change the current Hayes rating of D2.

The Yao et al. (2024) systematic review and meta-analysis evaluated the diagnostic accuracy of urine methylation testing compared with that of urine cytology for the surveillance of individuals with NMIBC after initial treatment. The review included six studies published between 2018 and 2022, encompassing 1,676 individuals who were aged 65.1 to 74 years, with male representation ranging from 63.2% to 82.86%. All studies required individuals to have a confirmed NMIBC diagnosis and prior initial treatment, and individuals had to undergo both urine cytology and urine methylation testing; studies were excluded if they were case reports, animal studies, or reviews or if they lacked sufficient diagnostic data. Urine methylation testing was performed using polymerase chain reaction–based assays generating an EpiScore (positive ≥ 60), while cytology was interpreted using the Paris System. Across studies, the pooled sensitivity for urine methylation testing was 0.69 (95% CI, 0.59-0.77), with a specificity of 0.87 (95% CI, 0.84-0.90), compared with a cytology sensitivity of 0.52 (95% CI, 0.35-0.68) and specificity of 0.93 (95% CI, 0.64-0.99). The area under the receiver operating characteristic curve was higher for methylation testing (0.89; 95% CI, 0.85-0.91) than for cytology (0.71; 95% CI, 0.67-0.75). Subgroup analyses indicated greater sensitivity of methylation testing for high-grade NMIBC in several studies. All pooled estimates were derived using random-effects models due to heterogeneity (I^2 up to 94%). A methodological quality assessment revealed risks of bias in the selection of individuals and reference standards in some studies, and the high heterogeneity limited precision. While urine methylation testing demonstrated superior sensitivity and overall diagnostic accuracy compared with cytology, its lower specificity may have led to more false positives. These findings suggest that methylation testing could complement existing surveillance strategies, but the results should be interpreted cautiously, given variability in study design and incomplete data. Larger, multicenter trials are needed to confirm the reliability and generalizability.

A study by Pierconti et al. (2023) was a single-center retrospective analysis that evaluated the Bladder EpiCheck DNA methylation test as a predictor of HGUC recurrence in patients with NMIBC. From March to December 2019, 290 patients (205 men, 85 women; mean age, 72.5 years; range, 47-89 years) with histologically confirmed NMIBC [classified as high-grade papillary carcinoma (n = 143), moderately high-grade papillary carcinoma (n = 105), or carcinoma in situ (n = 42)] were treated with intravesical bacille Calmette-Guérin (n = 216) or mitomycin (n = 74) and followed up for 1 year. Eligibility included clinically high-grade tumors per the European Association of Urology guidelines. No upper tract neoplasms were detected during follow-up. Surveillance consisted of urine cytology, white-light cystoscopy, and Bladder EpiCheck testing on bladder washing specimens, with EpiScores of ≥ 60 indicating a high risk for HGUC. Patients with negative cystoscopy underwent random biopsies, while those with visible lesions had targeted biopsies. Cytology and histology were reviewed by two uropathologists, with consensus for equivocal cases. Among 175 patients with cytology positive or suspicious for HGUC, histology confirmed recurrence in 127 (72.6%). All patients had EpiScores of ≥ 60, distributed as 60 to 69 in 20 cases, 70 to 79 in 36, 80 to 89 in 56, and > 90 in 63. Histological confirmation rates rose with EpiScore: 25% for 60 to 69, 64% for 70 to 79, 75% for 80 to 89, and 90% for > 90. Stratification at ≥ 80 yielded a strong association with recurrence ($p < 0.0001$; OR, 10.45; 95% CI, 4.99-21.87). Diagnostic performance for EpiScore of ≥ 60 showed an AUC of 0.811 ($p < 0.001$; 95% CI, 0.745-0.866; sensitivity, 83.6%; specificity, 72.9%), which outperformed the ≥ 80 cutoff (AUC, 0.686; $p = 0.0074$). In 48 patients with positive cytology, an EpiScore of ≥ 60, and negative histology, 20 (42%) developed HGUC

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within 6 to 12 months; recurrence occurred in 61% of those with EpiScores of > 70, including all with scores of > 90 by 6 months. No recurrences were observed in patients with EpiScores of < 60 or in those cytologically negative for HGUC. Limitations include the retrospective design, single-center setting, reliance on bladder washings rather than voided urine, and specimen splitting for cytology and methylation analysis. These findings suggest that Bladder EpiCheck, combined with cytology, may enhance risk stratification and early detection of HGUC recurrence, although further validation in larger, prospective cohorts is needed. (This study is included in the Molecular Test Assessment by Hayes, 2024, updated 2025.)

Cxbladder Monitor

Hayes (2023; updated 2025) published a Molecular Test Assessment that evaluated the use of the Cxbladder Monitor test to help rule out recurrence and reduce cystoscopy in individuals with previously diagnosed urothelial carcinoma who were undergoing surveillance. Hayes found a very low-quality body of evidence and concluded that evidence for clinical validity comprised only two studies of poor or fair quality and that the evidence of clinical utility comprised only two very poor-quality studies that lacked statistical analyses and individuals' survival data. The 2025 update of this assessment identified one newly published clinical validity study that may meet the Hayes inclusion criteria set out in the original report, but Hayes was unlikely to change the current rating of D2.

Cxbladder Triage

The Lotan et al. (2024) multicenter prospective RCT evaluated whether Cxbladder Triage, a urinary genomic test, could reduce cystoscopy use in adults referred for microhematuria, without compromising urothelial cancer detection. Conducted across 12 sites, the study enrolled 390 participants who were aged 18 to 94 years (median, 62 years; 54% male) between March 2019 and May 2024. Participants were stratified into lower risk (lower risk: 3-29 red blood cells/high-power field and smoking history < 10 pack-years) and not lower risk (not lower risk: > 29 red blood cells/high-power field and/or smoking history > 10 pack-years). Lower-risk participants were randomized 2:1 to a marker-informed arm or standard-of-care control, while not-lower-risk participants underwent standard of care regardless of Cxbladder Triage results. The exclusion criteria included prior urological malignancy or pelvic RT. The primary outcome was reduction in cystoscopy rates; the secondary outcomes included test performance metrics. Among lower-risk participants (n = 135), cystoscopy was performed in 67% of the standard-of-care vs 27% of the marker arm, which represented a statistically significant 59% relative reduction (relative risk, 0.41; 95% CI, 0.27-0.61). In the marker arm, 87.7% had a negative Cxbladder Triage result, and only 19.7% of them chose cystoscopy compared with 80% of participants with a positive Cxbladder Triage result. Across 270 participants with both Cxbladder Triage and cystoscopy results, the sensitivity for urothelial cancer was 90% (95% CI, 70%-99%), specificity was 56% (49%-62%), and NPV was 99% (95%-100%); for high-grade disease, the sensitivity was 100% (78%-100%). Limitations include the incomplete follow-up, risk stratification misalignment with current AUA guidelines, and potential bias from providing additional Cxbladder Detect results to some positive cases. Funding was provided by Pacific Edge Ltd, and several authors disclosed financial relationships with diagnostic and pharmaceutical companies. While these findings suggest that Cxbladder Triage can safely reduce unnecessary cystoscopies in lower-risk microhematuria, improving individuals' comfort and resource utilization, along with long-term outcomes and applicability under the current guidelines, requires further study.

Hayes (2023; updated 2025) published a Molecular Test Assessment that evaluated the use of Cxbladder Triage to help rule out bladder cancer in low-risk individuals with hematuria. Hayes found a very low-quality body of evidence and concluded that the test's clinical performance and impact on individual outcomes remain largely unknown. The 2024 and 2025 updates to this assessment identified a total of four newly published studies that may meet the Hayes inclusion criteria set out in the original report; however, they were unlikely to change the current Hayes rating of D2.

Decipher Bladder Genomic Classifier

The de Jong et al. (2025b) follow-up to the de Jong et al. (2025a) multicenter retrospective study discussed below evaluated the Decipher Bladder genomic subtyping classifier in predicting pathological upstaging and survival in patients with clinically organ-confined HGUC (cTa-T2N0M0) who underwent radical cystectomy (RC) without NCT. The cohort included 226 patients treated between 2003 and 2020 across eight U.S. tertiary centers; 134 had clinical NMIBC (cNMIBC; cTa/Tis/T1), and 92 had cT2 disease. Eligibility required FFPE tumor specimens from transurethral resection, absence of neoadjuvant systemic therapy, no variant histology other than nonpredominant mixed urothelial with squamous or glandular differentiation, and no tumor in a bladder diverticulum. All patients had negative imaging for extravesical disease and hydronephrosis prior to surgery. A molecular analysis classified tumors as luminal [n = 138 (61%)] or nonluminal [n = 88 (39%)]. The primary end point was upstaging to non–organ-confined disease (pT3+ and/or pN+) at cystectomy; the secondary end points included upstaging to muscle-invasive disease [muscle-invasive bladder cancer (MIBC)+, pT2+, and/or pN+] in cNMIBC cases and OS. Pathological upstaging to non–organ-confined disease occurred in 33% overall, including 19% of cNMIBC and 53% of cT2 cases. Rates were 28% in luminal vs 41% in nonluminal tumors (univariable OR, 1.82; 95% CI, 1.03-3.20; p = 0.04), but this association was not significant after

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adjustment (OR, 1.12; 95% CI, 0.59-2.11; p = 0.74). Among patients with cNMIBC, upstaging to MIBC+ was 32% for luminal and 51% for nonluminal tumors; the nonluminal subtype remained significant on multivariable analysis (adjusted OR, 2.49; 95% CI, 1.09-5.72; p = 0.03). For OS, nonluminal tumors were associated with worse outcomes (adjusted hazard ratio, 1.67; 95% CI, 1.01-2.78; p = 0.05), with a more pronounced difference in the cT2 subgroup (adjusted hazard ratio, 2.15; 95% CI, 1.00-4.59; p = 0.05). The findings suggest that luminal tumors are less likely to harbor advanced disease and have more favorable survival after cystectomy, whereas nonluminal tumors carry a higher risk of upstaging and poorer prognosis, which may inform discussions on neoadjuvant therapy candidacy. Limitations include the retrospective design, potential referral and selection bias, incomplete data on restaging and intravesical therapies, and limited power for interaction analyses. Financial disclosures note multiple author consultancies and industry affiliations, and the study was supported by Veracyte, Inc.

The de Jong et al. (2025a) multi-institutional retrospective study evaluated a previously developed long noncoding RNA–based GC in 226 patients with clinically organ-confined HGUC (cTa-T2N0M0) who underwent RC without neoadjuvant therapy. Eligibility required a diagnosis within 4 months of RC, absence of variant histology other than mixed urothelial with squamous or glandular differentiation, and no evidence of extravesical disease on imaging or bimanual examination. Patients with cancer in a diverticulum or prior systemic therapy were excluded. Tumor samples from transurethral resection were profiled using Decipher Bladder, and luminal favorable status was determined by the GC. The cohort was predominantly male (81%), with a median age of 71 years and a median follow-up of 33 months; 59% had cNMIBC, and 41% had cT2 disease. The GC classified 60 tumors (27%) as luminal favorable, which were associated with significantly lower odds of pathological upstaging compared with nonfavorable tumors: non–organ-confined disease (OR, 0.43; 95% CI, 0.19-0.96; p = 0.04), pT3+ stage (OR, 0.32; 95% CI, 0.12-0.82; p = 0.02), any upstaging (OR, 0.41; 95% CI, 0.20-0.83; p = 0.01), and any upstaging and/or nodal involvement (OR, 0.50; 95% CI, 0.25-1.00; p = 0.05). At RC, 17% of luminal favorable tumors were upstaged to non–organ-confined disease vs 39% of others. A survival analysis showed that luminal favorable status was strongly associated with improved OS (hazard ratio, 0.33; 95% CI, 0.15-0.74; p = 0.007), with only two cancer-specific mortality events in this group within 36 months. These findings may support the GC's validity for identifying luminal favorable tumors with less aggressive biology and suggest clinical utility for risk stratification in RC candidates. Limitations include the retrospective design, potential selection bias, and lack of detailed perioperative therapy data. Funding was provided by Veracyte, Inc., and multiple authors disclosed consultancy or employment relationships with industry sponsors.

The Reike et al. (2024) retrospective study examined how molecular subtypes, assigned using multiple classifiers that included the Decipher Bladder GC, relate to OS in MIBC treated with RC, with or without cisplatin-based NCT. The analysis used the NACmeta cohort of 601 patients and combined four previously published datasets; 247 received NCT followed by RC, and 354 underwent RC alone. Eligibility included clinical stages T2-4aN0-3M0 for the NCT cohorts and cT1-4N0-1M0 for the non-NCT cohorts, with all tumors confirmed as MIBC at RC. Patients with variant histology or neuroendocrine-like profiles were excluded, as were those treated with non-cisplatin chemotherapy or without RC. Molecular subtyping was performed using the Decipher Bladder clinical-grade whole-transcriptome genomic subtyping classifier, alongside Consensus, The Cancer Genome Atlas, and Lund classifiers. The Decipher Bladder assay classified tumors into the following genomic subtyping classifier subtypes: luminal, basal, claudin low, and infiltrated luminal. Luminal-like tumors identified by Decipher showed high concordance with luminal categories in other classifiers but demonstrated no OS benefit from NCT. The 3-year OS for genomic subtyping classifier-Luminal was 63% with NCT vs 65% without (p = 0.7), and even among locally advanced luminal cases (≥ cT3), NCT did not improve survival (p = 0.93). In contrast, nonluminal genomic subtyping classifier subtypes derived significant benefit: 71% OS with NCT vs 61% without (p = 0.02). Similar patterns were observed across Consensus, The Cancer Genome Atlas, and Lund classifiers, and a multivariate analysis confirmed improved survival in nonluminal groups (hazard ratio, 1.52; 95% CI, 1.04-2.21; p = 0.03). These findings may underscore Decipher Bladder's role in stratifying MIBC by molecular subtype and highlight its potential utility in guiding NCT decisions. The results suggest that luminal-like MIBC identified by Decipher may not benefit from current NCT regimens, supporting consideration of treatment de-escalation, while nonluminal subtypes appear to gain a meaningful survival advantage. Limitations of this study include the retrospective design, pooled cohorts, possible batch effects, and lack of adjuvant therapy data. Conflicts of interest were disclosed, including consultancy roles and employment with Veracyte, Inc., which is the developer of Decipher.

Clinical Practice Guidelines

American Urological Association (AUA)/Society of Urodynamics, Female Pelvic Medicine & Urogenital Reconstruction (SUFU)

The AUA/SUFU (Barocas et al.) issued updated guidelines in 2025 to provide a clinical framework for the diagnosis, evaluation, and follow-up of microhematuria, which includes the following statements pertaining to molecular urine-based tumor marker (UBTM) assays:

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- In appropriately counseled, intermediate-risk patients who want to avoid cystoscopy and accept the risk of forgoing direct visual inspection of the bladder urothelium, clinicians may offer urine cytology or validated UBTMs to facilitate the decision regarding the utility of cystoscopy. Renal and bladder ultrasound should still be performed in these cases (conditional recommendation; evidence level: grade C).
- Clinicians should not routinely use urine cytology or UBTMs to decide whether to perform cystoscopy in the initial evaluation of low-/negligible- or high-risk patients with microhematuria (strong recommendation; evidence level: grade C).
- Clinicians should not routinely use cytology or UBTMs as adjunctive tests in the setting of a normal cystoscopy (strong recommendation; evidence level: grade C).

The AUA panel determined that the strength of evidence regarding different urine markers and cytology is highly variable. In their assessment of the available molecular UBTM assays, the panel's principal outcome of interest was NPV, given the theoretical intent of identifying patients who can safely avoid cystoscopy with a lower risk of missing cancer. Cxbladder Triage was found to have the highest level of evidence. Cxbladder Resolve and Xpert Bladder Cancer Detection were also noted to have an NPV that satisfied the AUA panel's requirements.

National Comprehensive Cancer Network (NCCN)

Consideration of the evaluation of urinary urothelial tumor markers is recommended by the NCCN as a category 2B option during surveillance of high-risk NMIBC (NCCN Bladder Cancer, v3.2025).

Cutaneous Squamous Cell Carcinoma

There is currently insufficient evidence to support the use of molecular testing (e.g., DecisionDx-SCC test) for the management of cutaneous squamous cell carcinoma (cSCC). Additional large, high-quality studies are required to evaluate the clinical utility of this technology.

Hayes (2025) published a Molecular Test Assessment evaluating the use of the DecisionDx-SCC test to predict the risk of metastasis in ≤ 3 years to inform management decisions in cSCC with high- or very high-risk factor(s). Hayes found a very low-quality body of evidence that suggests that DecisionDx-SCC has some potential for this indication, may influence treatment recommendations, and may outperform other risk categorization systems. However, Hayes emphasized that there is substantial uncertainty due to the small literature base and individual study limitations. Hayes was unable to assess whether the test influenced actual treatment decisions or individual health outcomes.

Gopal et al. (2024), in their multidisciplinary consensus guidelines, discussed the integration of DecisionDx-SCC (Castle Biosciences, Inc.), a 40-GEP test, into clinical recommendations for ART in individuals with cSCC. These guidelines were developed by a panel of experts, including radiation oncologists and dermatologists, to enhance the precision of ART recommendations. The DecisionDx-SCC test classifies individuals into risk categories (class 1, 2A, and 2B) based on their likelihood of metastasis, providing a more accurate prognostic tool than traditional clinicopathologic factors alone. The guidelines suggest that incorporating DecisionDx-SCC testing, along with existing staging systems (e.g., *AJCC Cancer Staging Manual*. 8th ed.) and management guidelines (e.g., NCCN), can better stratify individuals' risk and inform decisions regarding ART. This approach aims to improve outcomes by identifying high-risk individuals who would benefit most from ART while avoiding unnecessary treatment in lower-risk individuals. The proper application of DecisionDx-SCC testing requires a collaborative decision-making process between the multidisciplinary team and the individual with cSCC. This approach considers various factors, including the individual's risk of metastasis and personal preferences and the characteristics of the tumor and surrounding tissues. The development of this article was funded by Castle Biosciences, Inc. The authors had affiliations with the manufacturer as well, which introduces a potential risk of bias.

Ruiz et al. (2024) conducted a retrospective validation study of the DecisionDx-SCC test for predicting the benefit of ART in patients with cSCC. The study focused on cSCC tumor tissue specimens that were received from two academic centers, examining associated 5-year MFS as well as projected time to metastasis. In random sampling of matched patient pairs (52 ART treated; 371 without ART), there was a median 50% decrease in the 5-year progression rate in ART-treated patients (vs no ART) with DecisionDx-SCC class 2B results. Class 2A results were associated with a small ART benefit; no differences were noted between ART-treated and untreated tumors in patients with class 1 results. Limitations of the study include the infrequent occurrence of class 2B results, the study's retrospective design, and inclusion of only two clinical sites, which may limit generalizability. In addition, the study was partially funded by the manufacturer of the GEP test, and several authors had affiliations with Castle Biosciences, Inc., which introduces a potential risk of bias. Despite the limitations, the researchers asserted that the study's statistical power was adequate due to the significant effect size observed. The authors concluded that in this study, the DecisionDx-SCC test effectively identified patients with cSCC who were most likely to benefit from ART as well as those who may consider deferring

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treatment. These results validate the previously reported benefits of ART for class 2B tumors and the lack of benefit for class 1 tumors.

The Zakria et al. (2024) expert panel consensus report discussed the integration of the DecisionDx-SCC test into the management of cSCC. The panel, comprising eight dermatologists with cSCC expertise, reviewed existing literature and developed guidelines for incorporation of the DecisionDx-SCC test into clinical practice. The panel unanimously voted to adopt seven consensus statements and recommendations, six of which were given a Strength of Recommendation Taxonomy Level strength of "A" and one of which was given a strength of "C." The authors concluded that the DecisionDx-SCC test provided accurate and independent prognostic information beyond standard staging systems that only incorporate pathological data and proposed that incorporation of GEP testing into national guidelines may help further stratify individuals based on risk of metastasis and thereby improve morbidity and mortality. Of note, the study was partly funded by and some of the authors have affiliations with Castle Biosciences, Inc., the test manufacturer.

Ibrahim et al. (2022) investigated the use of the DecisionDx-SCC test to improve metastatic risk assessment in cSCC in a multicenter study (33 sites). The researchers collected data from 420 primary tumors with known clinical outcomes, and the DecisionDx-SCC test was used, along with clinicopathologic factors, to stratify metastatic risk. The findings demonstrated that combining molecular profiling with traditional risk assessments significantly improved prognostic accuracy. Specifically, the DecisionDx-SCC test helped to better stratify participants into risk categories, with class 1 results showing metastasis rates similar to those in the general cSCC population and class 2B results showing metastasis rates of ≥ 50%. The authors acknowledged potential limitations, such as the archival nature of samples and possible underreporting of high-risk factors. However, this cohort represented a high-risk cSCC population, with a 15% metastasis rate, and reflects current clinical pathology practices. Measures such as comprehensive monitoring and independent dermatopathologist review were implemented to mitigate potential underreporting. Identifying the primary lesion among multiple cutaneous lesions was another limitation, but strict inclusion/exclusion criteria were used to address this. Lastly, a potential for bias exists related to study funding by Castle Biosciences, Inc., and the affiliation of some study authors with Castle Biosciences, Inc. The researchers suggested that ongoing research will explore tissue testing from recurrent tumors and the use of the DecisionDx-SCC test to predict risk of local recurrence as well. They proposed that further study, with a focus on the incorporation of molecular profiling with standard clinicopathologic assessments, will ultimately enhance individual risk assessment, improve clinical decision-making, and lead to better health outcomes and resource use in individuals with cSCC. (This study is included in the Molecular Test Assessment by Hayes, 2025.)

Clinical Practice Guidelines

Society for Immunotherapy of Cancer (SITC)

A 2022 clinical practice guideline (Silk et al.) issued by the SITC notes that there are no validated biomarkers that predict the benefit of immune checkpoint inhibitor (ICI) therapy in cSCC, and GEP for predictive or prognostic signatures is an ongoing area of research. The SITC recommends against using the DecisionDx-SCC test due to the lack of prospective studies of its clinical utility.

Cancers of Unknown Primary

Molecular tests [e.g., comprehensive genomic profiling (CGP), GEP, and NGS assays] that are intended to infer the primary site (the tissue of origin) and/or to identify molecularly guided treatments in individuals with metastatic cancer of unknown primary (CUP; also called occult primary tumors) have been developed. To date, peer-reviewed evidence that supports the use of these tests is insufficient. More high-quality studies that address the accuracy of these tests and demonstrate outcomes are required.

Krämer et al. (2024) reported on the CUPISCO phase 2, prospective, randomized, open-label, active-controlled, multicenter trial, which compared molecularly guided therapy (MGT) vs continuation of platinum-based chemotherapy after initial disease control in unfavorable, nonsquamous CUP across 159 sites in 34 countries. Participants were eligible if they were aged ≥ 18 years with centrally confirmed, nonsquamous CUP per ESMO 2015/updated 2023 algorithms; had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1 (two ECOG 2 protocol deviations); had a life expectancy of ≥ 12 weeks; had eligibility for platinum chemotherapy; had at least one RECIST (Response Evaluation Criteria in Solid Tumors) 1.1–measurable lesion; and had available tissue for CGP. Key exclusions included squamous histology, favorable-prognosis CUP subsets, central nervous system metastases, and leptomeningeal disease. Screening occurred from July 10, 2018, to December 9, 2022; 1,505 participants were screened, and 636 were enrolled. After three cycles of induction platinum chemotherapy, 438 achieved disease control, and 436 were randomized 3:1 to MGT (n = 326) or chemotherapy continuation (n = 110). MGT was selected by a molecular tumor board based on CGP; participants without an actionable alteration or genomic signature (TMB high or MSI high) received atezolizumab plus chemotherapy, whereas those with actionable profiles received targeted therapy or atezolizumab monotherapy. The primary end point was investigator-assessed PFS in the intention-to-treat population; secondary end points included OS, objective response

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rate, duration of response, disease control rate, and safety. The median follow-up in the treatment period was 24.1 months. MGT improved PFS: 6.1 months (95% CI, 4.7-6.5 months) vs 4.4 months (4.1-5.6 months) with chemotherapy (hazard ratio, 0.72; 95% CI, 0.56-0.92; p = 0.0079). Among participants with actionable profiles, median PFS was 8.1 months (4.6-8.7 months) with MGT vs 4.7 months (4.0-6.6 months) with chemotherapy (hazard ratio, 0.65; 95% CI, 0.42-0.99); in those without actionable profiles, median PFS was 5.5 months (4.5-6.4 months) with atezolizumab plus chemotherapy vs 4.4 months (4.2-5.6 months) with chemotherapy (hazard ratio, 0.76; 95% CI, 0.54-1.06). Interim OS medians were 14.7 months (95% CI, 13.3-17.3 months) with MGT and 11.0 months (9.7-15.4 months) with chemotherapy. The objective response rate during the treatment period was 18% (95% CI, 13.8%-22.4%) with MGT vs 8% (95% CI, 3.81%-15.0%) with chemotherapy (difference, 9.6%; 95% CI, 2.4%-16.8%). The safety rates, adjusted per 100 patient-years at risk, were generally similar or lower with MGT across most categories, with exceptions for serious adverse events leading to withdrawal and adverse events with fatal outcomes; two fatal adverse events were reported in the atezolizumab cohorts. The findings suggest support for incorporating CGP at initial diagnosis in unfavorable CUP to identify individuals who may derive greater benefit from targeted or signature-directed approaches; benefits were most pronounced in those with actionable alterations, and additional data are needed for the chemotherapy plus immunotherapy subgroup and for mature OS. Limitations of this study include the open-label conduct, investigator-assessed PFS, heterogeneous MGT regimens (including assignment to atezolizumab plus chemotherapy when no actionable target was present), and selection of participants with disease control after induction, which may introduce bias and limit generalizability. The CUPISCO study's sponsor, F. Hoffmann-La Roche, was involved in multiple trial activities, and several authors disclosed financial relationships with the sponsor and other companies, which represent potential conflicts of interest.

The Posner et al. (2023) prospective study (SUPER) assessed the diagnostic utility of RNA and DNA analysis in 215 participants with CUP in Australia. A retrospective analysis of clinicopathologic information was performed; this revealed that 166 of 215 participants (77%) with CUP did not have sufficient evidence to support a tissue-of-origin diagnosis. The remaining participants had either sufficient evidence to support a likely tissue-of-origin determination (13%) or a latent primary diagnosis (10%). A microarray analysis (CUPGuide) and/or a custom NanoString 18-class GEP test was performed on 191 CUP specimens and was found to be 91.5% accurate for high-medium confidence predictions in known metastatic cancers. In the cases in which clinicopathologic information resolved CUP, 80% had high-medium predictions, and 94% agreed with pathology results. Of note, GEP use resulted in high-medium confidence in only 56% of clinicopathologically unresolved CUPs. In 201 CUP tumors, diagnostic markers were queried based on the cancer type–specific mutations found in 22 cancer types from the American Association for Cancer Research Project GENIE database, which houses information pertaining to 77,058 tumors. Mutational signatures related to other factors, such as smoking, were also explored. For CUPs unresolved by clinicopathologic information, the assessment of mutations and mutational signatures led to added diagnostic evidence in 31% of the cases; however, GEP classification was only useful in 13% of those. Lung and biliary cancers were the most frequently identified cancers among CUPs for which genomic information assisted with identifying the tissue of origin. The researchers determined that in this study, DNA and RNA tests helped to resolve a third of CUP cases in which clinicopathologic data alone were not enough to establish the tissue of origin. While GEP is the most studied molecular diagnostic test for CUP to date, this investigation found that DNA sequencing may be of greater diagnostic value, as many tumors appear to have an atypical transcriptional profile and simultaneously maintain identifiable and important diagnostic mutational features. Although DNA mutational profiling is not currently a guideline-recommended approach, the authors proposed that the integration of this testing in cancer type assessment and for use in identifying targeted treatments has potential high clinical value.

Wang et al. (2023) evaluated the use of rapid NGS to help identify CUP and associated therapeutic biomarkers that could be used to guide site-specific therapies. Overall, 40 solid tumor samples were evaluated based on an initial diagnosis of CUP. NGS testing was performed using the Oncomine Precision Assay GX. Genomic information was used to support a site-specific cancer diagnosis in six individuals (15%). The most common genetic variations found were in *KRAS* (35%), *CDKN2A* (15%), *TP53* (15%), and *ERBB2* (12%). In total, 23 individuals had results that identified actionable molecularly targeted treatments (variations in *BRAF, CDKN2A, ERBB2, FGFR2, IDH1*, and *KRAS*). An immunotherapy-sensitizing mismatch repair (MMR) deficiency was detected in one individual. The authors asserted that this study supports the integration of rapid NGS into the care of individuals diagnosed with CUP and the viability of using genomic profiling, along with diagnostic histopathology and immunohistochemistry, in these individuals. They recommended further study that includes the incorporation of diagnostic algorithms, which include genomic profiling, to better identify CUP. This study is limited by its retrospective design, small population, and analysis that was performed in a single institution only. In addition, a relatively small testing panel was used, which may have not captured some genome-wide biomarkers, and no survival or outcome data were evaluated.

Ding et al. (2022) conducted a systematic review and meta-analysis of studies that investigated the efficacy of site-specific therapy guided by molecular profiling compared with that of empiric therapy in individuals with CUP. GEP was used to identify the tissue of origin in this study. Hazard ratios for OS and PFS were assessed to compare the efficacy of

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site-specific therapy with that of empiric therapy in individuals with CUP. In addition, subgroup analyses were conducted. Five studies, comprising 1,114 individuals, were identified; of the individuals, 454 received site-specific therapy, and 660 received empiric therapy. The meta-analysis revealed that site-specific therapy was not significantly associated with improved PFS (hazard ratio, 0.93; 95% CI, 0.74-1.17; p = 0.534) and OS (hazard ratio, 0.75; 95% CI, 0.55-1.03; p = 0.069) compared with empiric therapy. However, during a subgroup analysis, significantly improved OS was associated with site-specific therapy in the high-accuracy predictive assay subgroup (hazard ratio, 0.46; 95% CI, 0.26-0.81; p = 0.008) compared with the low-accuracy predictive assay subgroup (hazard ratio, 0.93; 95% CI, 0.75-1.15; p = 0.509). Additionally, when compared with individuals with less responsive tumor types, more survival benefit from site-specific therapy was found in individuals with more responsive tumors (hazard ratio, 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 was a nonrandomized study; its heterogeneous population is a limitation. Further investigation is needed before the clinical usefulness of this procedure is proven.

Ross et al. (2021) performed a retrospective analysis of CUP cases referred for CGP to determine how many were potentially eligible for enrollment in an experimental CUPISCO arm; CUPISCO (NCT03498521) is an ongoing randomized trial that is using CGP to assign patients with CUP to targeted or immunotherapy treatment arms. Centrally reviewed adenocarcinoma and undifferentiated CUP specimens in the FoundationCore database were analyzed using a hybrid capture–based FoundationOne assay (mean coverage, > 600×). The presence of genomic alterations, MSI, TMB, genomic loss of heterozygosity (gLOH), and programmed cell death 1 ligand 1 (PD-L1) positivity was determined. A total of 96 of 303 patients (31.7%) could be matched to an experimental CUPISCO arm. The key genomic alterations included *ERBB2* (7.3%), *PIK3CA* (6.3%), *NF1* (5.6%), *NF2* (4.6%), *BRAF* (4.3%), *IDH1* (3.3%), *PTEN* (3.6%), *FGFR2* (3.6%), *EGFR* (3.6%), *MET* (4.3%), *CDK6* (3.0%), *FBXW7* (2.3%), *CDK4* (2.3%), *IDH2* (1.0%), *RET* (1.0%), *ROS1* (1.0%), *NTRK* (1.0%), and *ALK* (0.7%). The median TMB was 3.75 mutations per megabase of DNA; 34 patients (11.6%) had a TMB of ≥ 16 mutations per megabase. Three patients (1%) had high MSI, and 42 (14%) had high PD-L1 expression (tumor proportion score of ≥ 50%). gLOH could be assessed in 199 of 303 specimens; 19.6% had a score of > 16%. The authors concluded that 32% of patients would have been eligible for targeted therapy in CUPISCO. Future studies that include additional biomarkers such as PD-L1 positivity and gLOH may identify a greater proportion of individuals who could potentially benefit from CGP-informed treatment. The findings of this retrospective analysis of CUP cases validate the experimental treatment arms being used in the CUPISCO study using CGP to assign patients with CUP to targeted or immunotherapy treatment arms, based on the presence of pathogenic genomic alterations. The authors also concluded that the findings suggest that future studies that include additional biomarkers and treatment arms, such as PD-L1 positivity and gLOH, may identify a greater proportion of individuals with CUP who could potentially benefit from CGP-informed treatment. A limitation is that this study lacks detailed clinical data for each specimen, including whether any patients received specialized therapy and subsequently experienced 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 CUP pathogenesis and focus on available data for targeted genotype–directed treatment. This systematic review consisted of studies in individuals with CUP whose tumor specimen was evaluated through NGS, according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) criteria from PubMed, the ASCO meeting library, and ClinicalTrials.gov, identifying potentially targetable alterations for which approved/off-label drugs and drugs in clinical trials are available. Case reports for individuals with CUP, who were treated with targeted therapies driven by NGS results, to explore the clinical role of NGS in this setting were identified. Overall, 15 publications were included; 11 of these studies (nine full-text articles and two abstracts) analyzed the genomic profiling of CUPs through NGS technology, with different platforms and different cohorts ranging from 16 to 1,806 individuals. Among these studies, 85% of individuals had at least one molecular alteration, with the most frequent involving *TP53* (41.88%), *KRAS* (18.81%), *CDKN2A* (8.8%), and *PIK3CA* (9.3%). A mean of 47.3% of individuals harbored a potentially targetable alteration for which approved/off-label drugs and drugs in clinical trials were available. Four case reports were identified to evaluate the clinical relevance of a specific targeted therapy identified through NGS. The authors concluded that NGS may be a tool to improve the diagnosis and treatment of CUP by identifying therapeutically actionable alterations and providing insights into tumor biology. A tissue-agnostic therapeutic approach has potential limitations; for example, extrapolating therapeutic actionability from one cancer histology to another might provide uncertain results. Therefore, for individuals with CUP, it is still important to consider putative primary sites, even when candidate actionable driver mutations are found. In addition, redundancy in the 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 of cancers in individuals with indeterminate, uncertain, or differential diagnoses. Peer-reviewed literature that supports the entire assay process as

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well as publications demonstrating that CancerTYPE ID provides accurate, clinically actionable information that results in improved outcomes are needed. A 2022 update to the original 2018 assessment found no newly published studies that met the inclusion criteria for the Hayes report [Hayes, CancerTYPE ID (Biotheranostics, Inc.), 2018; updated 2022]. A 2023 Hayes research brief identified no new relevant publications that evaluate the clinical validity or utility of the CancerTYPE ID test [Hayes, CancerTYPE ID (Biotheranostics, Inc.), 2023].

The Binder et al. (2018) systematic review evaluated the incidence and survival trends and discussed the value of CGP in individuals with CUP. Age-standardized incidence rates per 100,000 were calculated in 2,935 individuals with CUP from 1981 to 2014 using cancer registry data from 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. A literature review was conducted to assess the current use of CGP in individuals with CUP. Age-standardized incidence rates of CUP increased from 10.3 to 17.6 between 1981 and 1997 and decreased to 5.8 per 100,000 in 2014. Mean OS remained stable. Mortality was lower among individuals with squamous cell carcinoma (SCC; hazard ratio, 0.48; 95% CI, 0.41-0.57) and neuroendocrine carcinoma (0.75; 0.63-0.88) and higher for unclassified neoplasms (1.25; 1.13-1.66) compared with adenocarcinomas. The literature review identified 10 studies using CGP of CUP tissue. Clinically relevant mutations were identified in up to 85% of individuals with CUP, of whom 13% to 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 may help to identify molecular signatures in individuals with CUP and enable targeted treatment. Given poor prognosis and limited treatment options for individuals 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 the clinical usefulness of this procedure is proven.

Clinical Practice Guidelines

European Society for Medical Oncology (ESMO)

A clinical practice guideline that addresses CUP was published by Krämer et al. in 2023. The authors state that pancancer NGS can be used in CUP; however, randomized trials that assess 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 that addresses the use of GEP for site-directed therapy in CUP is provided.

National Comprehensive Cancer Network (NCCN)

In the NCCN Guidelines for occult primary (v1.2026), biomarker testing on tumor tissue and/or cell-free DNA (cfDNA) testing using NGS (or other techniques to identify gene alterations or TMB) is recommended as an option in the workup of suspected metastatic malignancy with an occult primary (category 2A recommendation).

Colorectal Cancer

There is currently insufficient evidence to support the use of molecular testing for colorectal cancer (CRC) diagnosis, prognosis, and treatment decisions. Additional large, high-quality studies are required to evaluate the clinical utility of this technology.

Blood-Based Colorectal Cancer Screening

In a commentary issued by the American Gastroenterological Association, Shaukat et al. (2025) discussed updates on the current role of blood tests for CRC screening. Key points included:

- One blood test (Guardant Shield) is FDA-approved for CRC screening in average-risk individuals every 3 years.
- Blood tests for CRC screening may improve participation in screening programs because of perceived ease of use.
- Individuals should understand that if a blood test is positive, colonoscopy would be recommended.
- Based on one-time test performance characteristics and long-term modeling projections, programmatic screening with current blood tests every 3 years is substantially better than no screening but would result in lower prevention rates for CRC or CRC death than programmatic fecal immunochemical test, Cologuard, or colonoscopy.
- The first available CRC screening blood tests are best viewed as acceptable in persons who decline the established forms of CRC screening.

A Hayes Molecular Test Assessment (2025) evaluated the effectiveness of the Shield blood-based test to detect CRC in average-risk individuals who are eligible for CRC screening. Hayes found an overall very low-quality body of evidence that comprised two studies, which were insufficient for Hayes' evaluation of the individuals' clinical impact of testing. Hayes also noted the lack of performance comparisons to alternative CRC screening methods; additionally, clinical practice guidelines appear to confer weak support against the Shield test to screen for CRC.

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The Chung et al. (2024) prospective, observational, multicenter study known as ECLIPSE (Evaluation of the ctDNA LUNAR Test in an Average Patient Screening Episode) assessed the performance of a cfDNA blood-based test (Shield, Guardant Health) to identify asymptomatic and early-stage CRC in an average-risk population. The study was sponsored by the test manufacturer, which was Guardant Health. The main outcomes evaluated were sensitivity for CRC detection and specificity for advanced neoplasia compared with colonoscopy. A secondary outcome that was assessed was sensitivity for advanced precancerous lesions. Initially, 10,258 participants were enrolled; 7,861 of them met all eligibility criteria and were included in the final analysis. The study results revealed a test sensitivity of 83.1% for detection of CRC (95% CI, 72.2%-90.3%), with 16.9% of participants who had documented CRC (detected via colonoscopy) having a negative result. For stages I to III CRC, sensitivity was calculated to be 87.5% (95% CI, 75.3%-94.1%). The sensitivity for advanced precancerous lesions was 13.2% (95% CI, 11.3%-15.3%). Almost 90% of participants who had no advanced colorectal neoplasia (either CRC or advanced precancerous lesions) detected on colonoscopy had a negative cfDNA blood test (10.4% had a positive cfDNA blood test), for an overall specificity for any advanced neoplasia of 89.6% (95% CI, 88.8%-90.3%). The specificity in participants whose colonoscopy results showed no CRC, no advanced precancerous lesions, and no nonadvanced precancerous lesions was 89.9% (95% CI, 89.0%-90.7%). Invalid cfDNA test results were received by 3.7% of participants. The authors recommended ongoing study that evaluates individuals' adherence to cfDNA blood-based testing across various care settings as well as research regarding health economic– and CRC-related outcomes. Additionally, studies that address the impact of longitudinal testing on sensitivity for advanced neoplasia are advised.

The ColonSentry test uses quantitative real-time polymerase chain reaction to measure RNA transcript expression of seven 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 that addressed this technology in 2024 and found insufficient evidence to support the use of the ColonSentry test for predicting CRC risk, citing limited studies and data and significant limitations in the existing evidence [Hayes, ColonSentry (StageZero Life Sciences), 2024]. In the 2025 update to their assessment, Hayes could not identify the laboratory website and could not confirm if the laboratory currently offers the ColonSentry test. No additional evidence was identified.

Oncotype DX Colon Recurrence Score

In a 2024 (updated 2026) Molecular Test Assessment, Hayes found insufficient evidence to support the use of the Oncotype DX Colon RS test in both of the following clinical scenarios:

- Individuals with stage II, MMR-proficient colon cancer to assess the ROR and identify those who may benefit from chemotherapy.
  - Hayes identified very limited evidence that Oncotype DX Colon RS results may be associated with ROR and impact treatment decision-making; however, substantial uncertainty remains due to the lack of evidence for individuals' clinical outcomes associated with test-informed treatment decisions in this population.
  - Hayes found only low-quality evidence that was insufficient to draw conclusions.
- Individuals with stage III A/B colon cancer to assess the ROR and identify those who are unlikely to derive additional benefit from chemotherapy.
  - Hayes identified very low-quality evidence deriving from a single study that was insufficient to draw conclusions.
  - Hayes found a lack of evidence for test clinical performance in this population and a lack of evidence for individuals' clinical outcomes associated with test-informed treatment decisions in this population.

The 2026 update of this assessment identified one newly published clinical validity study that may meet the Hayes inclusion criteria set out in the original report, but it is unlikely to change the current Hayes rating.

The Brenner et al. (2024) retrospective analysis of a prospectively designed, multicenter cohort evaluated patients with stage II, MMR-proficient colon cancer whose adjuvant treatment decisions incorporated 12-gene Oncotype DX Colon RS results between January 2011 and December 2016, with outcomes abstracted from medical records and a minimum of 3 years' follow-up. The final cohort included 938 patients (median age, 68 years; IQR, 60-76 years; 96% with T3 disease) who were stratified by Oncotype DX Colon RS into risk categories labeled as low risk (results 0-29), intermediate risk (30-40), and high risk (41-100); these risk categories comprised 64.6%, 24.0%, and 11.4% of patients, respectively. Chemotherapy use varied by risk category: 14% in low risk, 36% in intermediate risk, and 59.8% in high risk ($p < 0.001$). Chemotherapy-treated patients tended to be younger and have higher-risk clinicopathologic features. Among observation-only patients, Kaplan-Meier estimates for both 5-year RFI and colon cancer–specific survival differed significantly across risk categories ($p < 0.001$ for each), with further stratification noted with exploratory analysis in the low-risk category for Oncotype DX Colon RS results of 0 to 15; this suggests a fourth, very low-risk category. This very low-risk group had the highest 5-year RFI, with 93.8% remaining free of recurrence (95% CI, 87.6%-97.0%), compared with the low-risk category as a whole (87.0%; 95% CI, 83.8%-89.7%). The intermediate-risk category had a similar but slightly lower 5-year RFI of 84.2% (95% CI, 77.1%-89.4%). The high-risk category had the lowest 5-year RFI (69.4%; 95% CI, 53.4%-81.8%). Colon cancer–specific survival also differed significantly across these four risk categories ($p < 0.001$ for each) and favored those

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with very low-risk results (0-15; 96.6%; 95% CI, 91.2%-98.7%). The low-risk category as a whole had a survival rate of 95.3% (95% CI, 93.1%-96.9%), and the intermediate-risk category had 95.0% (95% CI, 89.8%-97.6%). The high-risk category exhibited the lowest colon cancer–specific survival rate (81.0%; 95% CI, 65.1%-90.7%). In treatment comparisons in the Oncotype DX Colon RS results strata, no differences were observed in the low- or intermediate-risk categories, whereas in the high-risk category, chemotherapy was associated with improved colon cancer–specific survival ($p = 0.035$) and a trend toward improved RFI ($p = 0.066$) despite worse baseline characteristics. Multivariable models identified higher Oncotype DX Colon RS results (per-unit hazard ratio, 1.03 for recurrence and for colon cancer death) and presence of invasion/perforation/obstruction as independent adverse prognostic factors, with male sex (recurrence) and older age (colon cancer death) also contributing. Chemotherapy was not significant in these models for the overall cohort. The 5-year RFI and colon cancer–specific survival in the entire cohort were 84.3% and 93.9%, respectively, with a median follow-up of 6.7 to 6.9 years. The authors concluded that Oncotype DX Colon RS results may be prognostic in real-world, stage II disease and that chemotherapy may confer clinical benefit in individuals in the high-risk group. Limitations of this study include the observational, nonrandomized design, with potential treatment selection bias and group imbalances; lack of masking; and absence of an intention-to-treat framework. The small subset sizes in some results strata may affect precision and generalizability, and the suggestive predictive signal for chemotherapy was not supported by a significant interaction test, warranting further comparative studies. The study received industry funding (Genomic Health/Exact Sciences) and had multiple author disclosures. (This study is included in the Molecular Test Assessment by Hayes, 2024, updated 2026.)

The Davey et al. (2023) systematic review and meta-analysis evaluated whether the Oncotype DX 12-gene expression RS assay aligns with multidisciplinary tumor board recommendations for adjuvant chemotherapy in MMR-proficient, stage II/III colon carcinoma. The authors pooled two prospective and two retrospective cohort studies, encompassing 855 individuals (mean age, 68 years; range, 25-90 years); 79.2% had stage II disease, and 20.8% had stage III disease. The assay was applied to FFPE specimens; outcomes focused on concordance vs discordance with tumor board recommendations and the direction of treatment change. Across the overall cohort, 25.8% of decisions were discordant; concordance was more likely than discordance (OR, 0.38; 95% CI, 0.25-0.56; $p < 0.001$; $I^2 = 71$). Among discordant decisions, treatment was de-escalated far more often than escalated, with chemotherapy omitted in 76.0% vs escalated in 24.0% (OR, 9.76; 95% CI, 6.72-14.18; $p < 0.001$; $I^2 = 47$). In stage II disease, 20.0% of decisions were discordant; concordance remained more likely than discordance (OR, 0.30; 95% CI, 0.17-0.53; $p < 0.001$; $I^2 = 80$), and discordant cases were over seven times more likely to lead to omission than escalation of chemotherapy (OR, 7.39; 95% CI, 4.85-11.26; $p < 0.001$; $I^2 = 0$). In stage III disease, discordance reached 44.9%, but only one study contributed data, and no meta-analysis was possible. The distribution of RS categories among evaluated individuals was 71.7% for low risk (RS < 30), 21.5% for intermediate (RS, 30-40), and 6.8% for high (RS > 40). The authors concluded that the use of the 12-gene assay refuted tumor board decisions in approximately 25% of cases, with approximately 75% of discordant instances resulting in omission of adjuvant chemotherapy, suggesting that some individuals may be overtreated when guided solely by tumor board judgment. The authors emphasized that the included evidence was observational, heterogeneity was present in several pooled analyses, and stage III estimates relied on data from a single study. Additional limitations noted were the small proportion of high-risk scores; disclosed conflicts of interest with the assay developer in the included studies; and absence of long-term oncological outcomes to verify the safety of de-escalation decisions prompted by the assay. The authors advocated for further prospective validation to determine whether assay-guided management affects outcomes. (This systematic review and meta-analysis is included in the Molecular Test Assessment by Hayes, 2024, updated 2025.)

Yothers et al. (2022) conducted an individual-specific meta-analysis of 12-gene colon cancer RS validation studies for recurrence risk assessment after surgery with or without fluorouracil (5-FU) and oxaliplatin. Three validation studies of the 12-gene colon RS assay were used (cancer and leukemia group B 9581, NSABP C-07, and the SUNRISE study); prespecified individual-specific meta-analysis methods were used to integrate the 12-gene Oncotype DX Colon RS result with the clinical and pathology risk factors stage, T stage, 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 5-FU was estimated using a meta-analysis of two studies. The effect of oxaliplatin was estimated using one of the RS assay validation studies, in which individuals were randomized to 5-FU, with or without oxaliplatin. The RS result and each of the clinicopathologic factors provided independent prognostic information for recurrence. Among stage II, T3, MMR-proficient individuals with ≥ 12 nodes examined (the most common scenario), those with an RS of ≤ 30 (approximately 48%) had an estimated 5-year recurrence risk of ≤ 10% with surgery alone. Among stage IIIA/B, T3, MMR-deficient individuals with ≥ 12 nodes examined, those with an RS of ≤ 19 (approximately 14%) had an estimated 5-year recurrence risk of ≤ 10% with surgery alone. Among stage IIIA/B, T3, MMR-proficient individuals with ≥ 12 nodes examined, those with an RS of ≤ 14 (approximately 6%) had an estimated 5-year recurrence risk of ≤ 10% with 5-FU alone. The authors concluded that the individual-specific meta-analysis integrated 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

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number of individuals with stage II and stage III cancer. Limitations include that the estimated effect of 5-FU is from a meta-analysis of a randomized study and a nonrandomized treatment comparison with a covariate adjustment to reduce bias. The SUNRISE study was a retrospective analysis that selected individuals who had not received adjuvant chemotherapy after resection for stage II or III CRC, and this may have led to the selection of individuals who clinicians had considered to be at a lower ROR. Also, the individual-specific meta-analysis risk assessment used a baseline risk assessment from the last two enrolling studies (NSABP C-07, enrolling from 2000-2002; SUNRISE, enrolling from 2000-2005). If further improvements in clinical outcomes have occurred since this time, they are not reflected in the present recurrence risk estimates. Finally, the RS result is not predictive, meaning that it is not associated with the relative treatment effect of chemotherapy with 5-FU or oxaliplatin. Further research that includes RCTs is needed to validate these findings. (This meta-analysis is included in the Molecular Test Assessment by Hayes, 2024, updated 2025.)

Yamanaka et al. (2016) evaluated the 12-gene RS assay (Oncotype DX Colon RS) for stage II and III CRC, 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 individuals with stage II to III disease who had surgery alone, 630 individuals were sampled for inclusion, with a 1:2 ratio of recurrence to nonrecurrence. Sampling was stratified by stage (II vs III). The assay was performed on FFPE primary cancer tissue. Association of the RS result with RFI was assessed by using weighted Cox proportional hazards regression. With respect to prespecified subgroups, as defined by low (< 30), intermediate (30-40), and high (≥ 41) RS risk groups, individuals with stage II disease in the high-risk group had a 5-year ROR similar to that in individuals with stage IIIA to IIIB disease in the low-risk group (19% vs 20%); participants with stage IIIA to IIIB disease in the high-risk group had a recurrence risk similar to that in those with stage IIIC disease in the low-risk group (approximately 38%). The authors concluded that this validation study of the 12-gene RS assay in stage III CRC, without chemotherapy, showed the heterogeneity of recurrence risks in stage III as well as stage II CRC.

Clinical Practice Guidelines

American College of Gastroenterology (ACG)

In their 2021 clinical guidelines for CRC screening (Shaukat et al.), the ACG recommends against blood-based screening for CRC (conditional recommendation; very low quality of evidence). Given the evidence showing low sensitivity and the lack of long-term and comparative data on test performance, the ACG states that blood-based screening is not considered an optimal screening modality.

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 that can potentially be used in treatment decision-making; however, ASCO 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, the CAP, the AMP, and ASCO convened an expert panel to create evidence-based guidelines for standard molecular biomarker testing in patients diagnosed with CRC, which included a comprehensive search of the published literature, including over 4,000 articles. Overall, 21 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 dMMR, have been shown to have clear value for prognostication, and others (*KRAS* and *NRAS*) are evidence backed for NPV for benefit to anti-*EGFR* therapies (Sepulveda et al., 2017).

National Comprehensive Cancer Network (NCCN)

The NCCN Guidelines for colon cancer (v5.2025) recommend universal MMR or MSI testing for any patient with a personal history of colon or rectal cancer to (1) identify those with Lynch syndrome, (2) assist with decision-making regarding the use of immunotherapy for patients with metastatic disease, and (3) inform clinical decisions for patients 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 that these tests add, noting insufficient data to recommend the use of multigene test panels, Immunoscore, or postsurgical ctDNA tests to either estimate the ROR or make determinations regarding adjuvant cancer therapy. The panel encourages clinical trial enrollment to generate further data on these tests. The NCCN included the following recommendations for colon cancer:

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- Testing for *KRAS*, *NRAS*, *BRAF* mutations, *HER2* amplifications, or MMR or MSI status as part of broad molecular profiling is recommended as an option in the workup of suspected or proven metastatic colon adenocarcinoma (category 2A recommendation).
- ctDNA is not recommended as an option for surveillance in stage II or III colon cancer (category 2A recommendation).
- Consideration of repeat testing is recommended as an option after targeted therapies to guide future targeted therapy decisions (category 2A recommendation).
- Repeat molecular testing is not recommended as an option after standard cytotoxic chemotherapy (category 2A recommendation).
- There is insufficient data to recommend the use of multigene assay panels to determine adjuvant therapy for pathological stage pMMR/MSS T3, N0, M0 at high ROR of T4, N0, M-0 disease.

The NCCN included the following recommendations in a separate guideline document for rectal cancer (v4.2025):
- Molecular testing conducted as part of broad molecular profiling is recommended as an option in the workup of rectal cancer with suspected or proven distant metastases (category 2A recommendation).
- In adjuvant treatment (up to 6 months perioperative) for pathological findings after transanal local excision for T1, N0 rectal cancer or after transabdominal resection for T1-2, N0 rectal cancer, there is currently insufficient evidence to recommend routine use of ctDNA assays outside of a clinical trial. De-escalation of care and treatment decision-making are not recommended based on ctDNA results (category 2A recommendation).
- ctDNA is not recommended for surveillance following operative management of stage II to IV rectal cancer (category 2A recommendation).
- Determination of tumor gene status as part of tissue- or blood-based NGS is recommended as an option in the workup of recurrence of rectal cancer with documented metachronous metastases (category 2A recommendation).
- Determination of tumor gene status as part of tissue- or blood-based NGS is recommended as an option in the treatment of unresectable, isolated pelvic/anastomotic recurrence of rectal cancer (category 2A recommendation).
- Consideration of repeat testing after targeted therapies is recommended as an option to guide future targeted therapy decisions.
- Repeat molecular testing should not be performed after standard cytotoxic chemotherapy.

The NCCN Guidelines for CRC screening (v2.2025) briefly address blood-based screening tests, noting that blood-based screening modalities should only be used to screen patients of average risk with a commitment to undergo follow-up colonoscopy for any abnormal result. The NCCN notes that blood-based screening assays are FDA-approved for use every 3 years in this population and that individualized assessment of patient risk factors should be considered when making decisions about CRC screening.

U.S. Preventive Services Task Force (USPSTF)

The 2021 USPSTF recommendation statement for CRC screening indicates that because of limited evidence, the USPSTF recommendations do not include “serum tests, urine tests, or capsule endoscopy for colorectal cancer screening.”

Ovarian Cancer

There is currently insufficient evidence to support the use of molecular testing for the management of ovarian cancer. Additional large, high-quality studies are required to evaluate the clinical utility of this technology.

Clinical Practice Guidelines

American Society of Clinical Oncology (ASCO)

An ASCO 2025 clinical practice guideline update for newly diagnosed, advanced ovarian cancer (Gaillard et al.) recommends that patients with progressive disease on NCT should have their diagnosis reconfirmed via tissue biopsy, if appropriate. Patients who have not had comprehensive genetic or molecular profiling should be offered testing as soon as possible (evidence quality: moderate; strength of recommendation: conditional).

Pancreatic Cancer

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.

Zheng et al. (2025) performed a systematic review and meta-analysis conducted under the PRISMA guidelines using PubMed, Embase, Web of Science, and the Cochrane Library. The authors analyzed 43 diagnostic accuracy studies that were published between 2014 and 2024 and included 19,326 individuals and 2,749 early-stage pancreatic cancer cases.

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The main end point was to assess how accurately novel biomarkers could identify early-stage pancreatic cancer (via assessment of sensitivity and specificity) and identify the most promising candidates for use in a clinical setting. Studies were eligible if they focused on novel biomarkers for early pancreatic cancer detection, provided diagnostic accuracy measures such as sensitivity and specificity, and demonstrated methodological quality with a QUADAS-2 rating that indicated a low risk of bias in at least three domains. Novel biomarkers, including microRNAs (miRNAs), protein markers, metabolites, and ctDNA, evaluated in combination with serum carbohydrate antigen 19-9, were explored in subgroup analyses. Using a bivariate random-effects model, the researchers calculated pooled sensitivity, specificity, DOR, and AUC. miRNAs demonstrated the highest diagnostic performance, with a sensitivity of 0.88, specificity of 0.91, and AUC of 0.95, followed by protein biomarkers and metabolites. ctDNA showed high specificity (0.94) but only moderate sensitivity (0.65), with an AUC of 0.92. Of note, sensitivity related to the use of ctDNA improved after excluding studies with small sample sizes. Subgroup analyses confirmed that combining the assessment of miRNA, protein, metabolite, and ctDNA biomarkers, with measurement of carbohydrate antigen 19-9, improved diagnostic accuracy. The authors concluded that novel biomarkers, particularly miRNAs and protein markers, showed strong diagnostic accuracy for detecting early-stage pancreatic cancer in this study and may hold promise for clinical use to improve early diagnosis and individuals' outcomes. They recommended placing focus on the standardization of identification and validation techniques in large, high-quality, prospective studies, with specific attention given to the validation of biomarker combinations instead of single markers. Noted limitations include (1) significant heterogeneity of the studies based on the study design, populations included, stages of disease, and detection methods; (2) potential publication bias, specifically notable in metabolite studies; and (3) variation in methodological quality among the included studies, which could impact pooled estimates.

The Nicolle et al. (2024) prospective multicenter study investigated the use of molecular analysis of DNA and mRNA extracted from the products of ultrasound-guided FNA biopsy of primary tumors in participants with proven pancreatic adenocarcinoma to help distinguish between metastatic tumors and other tumor types. In total, 397 participants were included. A variant allele frequency of the *KRAS* mutation was leveraged to assess tumor cellularity, which ranged from 15% to 20% in all cells regardless of the stage of the tumor. The researchers found that the molecular characteristics of metastatic primary tumors were significantly different from those of other types of tumors. In the metastatic tumors, *TP53* mutations were more common ($p = 0.0002$), and *RNF43* mutations were less common. Metastatic tumors were also found to have more basal-like tumor mRNA component ($p = 0.001$). Primary tumors in metastatic forms displayed transcriptomic characteristics that were related to worse outcomes compared with other primary tumors, suggesting that their molecular profile may be important in the spreading of cancer cells beyond the pancreas. Some molecular markers were associated with better OS rates; these included mutations in homologous recombination deficiency genes in participants who received first-line platinum-based chemotherapy ($p = 0.025$) and a wild-type *TP53* gene in participants with locally advanced tumors who received radiochemotherapy ($p = 0.01$). A specific transcriptomic profile (known as GemPred) was associated with a significantly better OS level in participants with locally advanced or metastatic pancreatic cancer who received a gemcitabine-based first line of treatment ($p = 0.019$). The authors asserted that the molecular analysis of both DNA and RNA can help to predict therapeutic response in individuals with pancreatic adenocarcinoma, which can impact OS in individuals undergoing platinum- or gemcitabine-based therapies as well as radiochemotherapy. This technology could also have potential benefit in identifying targeted treatments. Although results appear promising, the study did have limitations; 6.4% of total samples were negative for nucleic acids, even though the core biopsy was of acceptable quality. Additionally, 45% of the samples tested did not reach RNA or DNA thresholds recommended by the sequencing facility (e.g., > 150 ng/sample). Additional high-quality studies are required to confirm these results and further investigate clinical utility.

A Hayes Precision Medicine Research Brief addressed PancreaSeq, an NGS-based test that analyzes 74 genes isolated from pancreatic cyst fluid (PCF) to evaluate ROM. Hayes concluded that there is currently not enough published, peer-reviewed literature to evaluate the evidence related to PancreaSeq GC for the characterization of pancreatic cysts in a full assessment [Hayes, PancreaSeq GC (University of Pittsburgh Medical Center), 2024].

Paniccia et al. (2023) prospectively investigated the use of NGS of PCF in a real-time, multi-institutional group of participants with pancreatic cysts. Overall, 1,887 specimens from 1,832 participants were tested with the earlier, 22-gene version of the PancreaSeq NGS panel. Follow-up data were available for 66% of participants (n = 1,216). Of 251 participants (21%) with surgical pathology available, mitogen-activated protein kinase/*GNAS* mutations had a 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 an 88% sensitivity and 98% specificity for advanced neoplasia (PPV, 97%; NPV, 93%). With the inclusion of cytopathologic evaluation with PancreaSeq testing, sensitivity improved to 93%, and the 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 Gastroenterological Association and International Association of Pancreatology/Fukuoka guidelines) are used. Of 965 participants who did not undergo surgery, none developed malignancy. Postoperative testing with Oncomine found mucinous cysts with *BRAF* fusions and *ERBB2* amplification and

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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 in 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 add to the existing support for integrating targeted NGS testing into evidence-based pancreatic cyst guidelines. The identified limitations include the limited availability of surgical pathology for participants (14%), which represents surgical selection bias; testing selection bias (only PCF specimens that were satisfactory for targeted NGS testing were used); and limited follow-up period.

The Iwaya et al. (2023) prospective, single-arm pilot study analyzed the viability and potential clinical utility of NGS using liquid-based cytology (LBC) samples obtained from endoscopic ultrasound-guided fine-needle biopsy (EUS-FNB) performed in participants with pancreatic cancer. Enrolled were 33 participants with pancreatic cancer who underwent EUS-FNB; samples from 31 participants 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 the use of FFPE, LBC, or frozen samples. When results were stratified using a variant allele frequency of > 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 variant allele frequency (23.5%) for *KRAS* and *TP53* was found with LBC. In this study, a pancreatic cancer gene variant analysis via NGS was performed effectively using LBC compared with FFPE and frozen samples. The authors concluded that EUS-FNB samples provide enough 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 participants 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 endoscopic ultrasound (EUS)–guided through-the-needle biopsy (TTNB) sampling in a prospective study. In total, 101 participants with PCLs of > 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, using a 51-gene customized hotspot panel. Histological diagnosis was used to calculate sensitivity and specificity. A total of 91 participants had residual TTNB samples available for NGS after the initial microscopic analysis of the specimens had been performed. Overall, 49 of these revealed mutations, most often in *KRAS* and *GNAS*. This indicated an excess frequency of IPMNs in the study population. A 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 a 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 IPMNs. 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 cyst fluid for a combined molecular and histological diagnosis of PCLs. The study is 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 the surgical cohort was limited. Lastly, no cyst fluid was obtained for NGS analysis and comparison. Further studies that are focused on characterizing the subgroup of individuals with pancreatic cancer who would derive the greatest benefit from EUS-guided TTNB samples are recommended.

A Hayes Molecular Test Assessment (2022; updated 2025) 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 individuals with pancreatic cysts. No peer-reviewed articles were identified that assess the analytical validity, clinical validity, or clinical utility of the PancraGEN test. In the 2023 and 2024 annual reviews, Hayes did not identify any new abstracts that met the inclusion criteria set out in the 2022 report. Hayes' 2025 annual review of this technology concluded that the PancraGEN test is no longer offered in the United States, and no further evidence evaluation was documented.

Singhi et al. (2018) studied the clinical validity of using preoperative PCF for NGS of the *KRAS, GNAS, TP53, PIK3CA*, and *PTEN* genes to predict benign vs malignant lesions. PCF samples from 595 participants (626 samples) were obtained through FNA and subjected to NGS for the five genes. A different cohort of 159 PCF specimens was also evaluated for *KRAS/GNAS* mutations by Sanger sequencing. Of the 595 participants, 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 participants. For these

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102, NGS testing of PCF for *KRAS/GNAS* had a 100% sensitivity (n = 56) and 96% specificity for an IPMN. In the separate cohort of participants in whom Sanger sequencing was used, *KRAS/GNAS* mutation 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 compared with 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.

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 the following: “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).

American Society of Clinical Oncology (ASCO)

Sohal et al. (2020) published an update to the ASCO metastatic pancreatic cancer guideline, 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 a 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 (Stoffel et al., 2019), ASCO notes that despite considerable effort, no biomarkers obtained through noninvasive means (e.g., blood, stool, urine) have been proven effective for early identification of pancreatic cancer in patients with no symptoms. In addition, there is no evidence that supports the 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 patients are needed.

National Comprehensive Cancer Network (NCCN)

The NCCN pancreatic adenocarcinoma guidelines include a recommendation for genetic testing for inherited mutations in patients with confirmed pancreatic cancer. The use of molecular testing for diagnostics and risk assessment of PCF is not addressed (NCCN Pancreatic Adenocarcinoma, v2.2025). The NCCN included the following recommendations for the management of pancreatic adenocarcinoma:

- Tumor/somatic molecular profiling, preferably with an NGS assay, is recommended as an option for patients with locally advanced/metastatic disease who are candidates for anticancer therapy to identify clinically actionable and/or emerging alterations following biopsy confirmation of locally advanced, metastatic disease or recurrence after resection. This includes CGP via an FDA-approved and/or validated NGS-based assay (category 2A recommendation).
- RNA sequencing assays are recommended as a preferred option for detecting RNA fusions following biopsy confirmation of locally advanced, metastatic disease or recurrence after resection (category 2A recommendation).
- Testing on tumor tissue is recommended as a preferred option following biopsy confirmation of locally advanced, metastatic disease or recurrence after resection; however, consideration of cfDNA testing is recommended as an option if tumor tissue testing is not feasible (category 2A recommendation).

Other Molecular Oncology Testing for Solid Tumor Cancers

Multicancer Detection Tests [e.g., Galleri (GRAIL, Inc.)]

Multicancer detection (MCD) tests are being studied in RCTs to determine the impact of their use as screening tests on occurrence of late-stage cancers and cancer-related mortality. At present, there are no professional medical societies that have issued recommendations on the use of MCD tests for cancer screening (National Cancer Institute, 2026), and published evidence does not support the use of MCD tests for screening for any type of cancer.

Rous et al. (2025) used a state-transition modeling approach to estimate how different screening intervals for a blood-based MCD test might affect stage at diagnosis and cancer-specific mortality in individuals aged 50 to 79 years. The model incorporated test performance by cancer type and stage from a large case-control validation study of the Galleri cfDNA methylation-based MCD assay and applied stage-specific incidence and survival from the SEER18 14-state database (2006-2015 diagnoses; follow-up to December 31, 2018). Two tumor growth scenarios were examined: fast (stage I dwell time 2-4 years) and fast aggressive (stage I dwell time 1-2 years). Screening intervals ranged from 6 months to 3 years, with emphasis on annual and biennial testing added to usual care. Outcomes included diagnostic yield,

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stage shift, and deaths within 5 years of the date that a cancer would have been diagnosed in the absence of the MCD test (lead time accounted for). Under the fast growth scenario, annual screening detected 370 additional cancer signals per year per 100,000 screened, reduced late-stage (III/IV) diagnoses by 49% (210 vs 409), and averted 84 deaths (21%) within 5 years compared with usual care. Biennial screening detected 292 additional signals, reduced late-stage diagnoses by 39% (248 vs 409), and averted 68 deaths (17%). In the fast aggressive scenario, biennial screening averted 54 deaths (14%), and annual screening averted 74 deaths (19%). The PPV was higher with biennial than annual testing (54% vs 43%), and biennial testing prevented more deaths per 100,000 tests (132 vs 84), although there were fewer deaths per calendar year. Across 6- to 36-month intervals, more frequent testing increased early-stage diagnoses, and annual testing consistently projected fewer deaths than biennial or no MCD testing. The authors concluded that adding MCD screening to usual care at annual or biennial intervals could downstage diagnoses and reduce near-term cancer mortality, with annual intervals providing greater absolute benefit and biennial intervals offering higher efficiency per test. These projections rely on idealized assumptions that likely overstate benefits that could not be replicated in the real-world clinical setting, such as 100% uptake, perfect adherence to diagnostic workup and treatment, and perfect accuracy and adherence for confirmatory testing; they also assume that earlier stage at detection translates to lower mortality and that stage-specific survival does not differ between MCD-positive and -negative tumors. Model inputs were limited to SEER18 regions, and performance parameters derived from case-control data may not reflect average-risk, intended-use populations. The study did not evaluate clinical utility directly, and there was no randomized or prospective comparison group; the analysis is observational modeling without masking. Funding was provided by GRAIL, Inc., with multiple authors being employed by or advising the sponsor and having equity interests. Well-designed, comparative studies are needed.

A Hayes Molecular Test Assessment (2025), issued as a follow-up to a Hayes Precision Medicine Research Brief (2024), concluded that the overall very low-quality body of evidence is insufficient to evaluate the effectiveness of the Galleri test (GRAIL, Inc.) for blood-based MCD screening in asymptomatic individuals. The two poor-quality/very poor-quality individual clinical utility studies that Hayes identified did not allow for sufficient evaluation of the impact of testing on individuals' survival outcomes. Hayes identified one article that addressed the test's clinical validity, using the same study population as one of the clinical utility studies. Accordingly, Hayes found insufficient evidence to inform conclusions regarding test performance. Hayes determined that clinical practice guidelines currently offer no/unclear support for the use of Galleri for blood-based screening in asymptomatic individuals.

The Marinac et al. (2025) prospective subanalysis from PATHFINDER evaluated the Galleri MCD blood test in 1,609 participants with a previous cancer history compared with 4,969 participants without prior cancer history, drawn from 6,662 adults aged ≥ 50 years who were enrolled across seven U.S. ambulatory health networks between December 12, 2019, and December 4, 2020; participants with previous cancer history were eligible only if they were ≥ 3 years beyond completion of therapy and without clinical suspicion of cancer at enrollment, with 12 months of end-of-study follow-up. Participants provided blood for cfDNA sequencing; a locked classifier reported cancer signal detected vs not detected and, if positive, a cancer signal origin prediction restricted to the two most likely tumor types. Prior primary cancer sites included breast (47%), melanoma (10%), prostate (9%), colorectal (4%), and lymphoma (4%), with a mean of 11.2 years since the previous diagnosis. A cancer signal was detected in 1.2% (20 of 1,609) of the survivor cohort, yielding 10 cancers in nine participants with a positive test: five second primaries (stage I uterine, stage II sarcoma, stage III ovarian, stage IV lymphoma, and stage IV colorectal) occurring 8 to 15 years after the original diagnosis and five metastatic BC recurrences occurring 4 to 11 years after the original diagnosis. In survivors, the specificity was 99.3%, PPV was 45.0%, NPV was 98.2%, and top-one cancer signal origin accuracy was 88.9%; test performance was similar in those without prior cancer, and the number needed to screen to find a cancer was 179 in survivors, 311 in those without prior cancer, and 263 overall. Among survivors with a negative test, 1.8% (28 of 1,589) were diagnosed with cancer within 1 year (54% second primaries; 46% recurrences), with several undetected cancers representing lower-shedding tumor types and three central nervous system cancers and some diagnoses occurring many months after the negative result. The authors concluded that the Galleri test showed high specificity, a low false-positive rate, and high tumor-type localization accuracy in survivors and detected both second primaries and recurrences up to 15 years after the index cancer, suggesting the potential to address gaps in long-term surveillance when used alongside guideline-recommended screening. The findings are constrained by the observational, descriptive design without prespecified hypothesis testing; lack of randomization or masking; limited racial/ethnic diversity, with predominance of BC survivors; incomplete detail on adherence to standard-of-care screening and survivor-specific surveillance; possible underdetection of low cfDNA shedding and central nervous system tumors; industry funding and author employment/financial ties to the test manufacturer; and 12-month follow-up. Well-designed, comparative studies are needed.

Hurt et al. (2025) performed a prospective cohort study that was conducted over 18 months in a tertiary ambulatory internal medicine clinic to evaluate the feasibility of integrating MCD testing [e.g., Galleri (GRAIL, Inc.)] into routine workflows for asymptomatic participants. Between June 2022 and November 2023, 2,244 participants underwent testing. Of them, 17 (0.76%) had a positive result, and 15 completed a diagnostic evaluation. Cancer was confirmed in 11

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participants (73.3%), including breast, colon, esophageal, lymphoma, ovarian, and pancreatic cancers, while four had no malignancy despite a full workup. While the results are promising, the authors noted the need for further research in larger, more diverse populations and inclusion of data that address long-term outcomes, cost-effectiveness, and clinical utility.

Wade et al. (2025) performed a systematic literature review to evaluate the diagnostic accuracy and clinical effectiveness of blood-based MCD tests for screening asymptomatic individuals who were aged 50 to 79 years. Comprehensive searches across major databases and trial registries identified 36 studies that met the inclusion criteria; these comprised one ongoing RCT, 13 completed cohort studies, 17 completed case-control studies, and five ongoing cohort or case-control studies. Although specificity across tests and studies was consistently high (greater than 96%), sensitivity varied widely; Galleri (GRAIL, Inc.) and CancerSEEK (Exact Sciences) demonstrated sensitivities of 20.8% to 66.3% and 27.1% to 62.3%, respectively, while others such as SPOT-MAS™ (Gene Solutions) and Trucheck™ (Datar Cancer Genetics) reported higher sensitivities (up to 100%). Sensitivity was consistently lower for early-stage cancers than advanced stages. The review was limited by the lack of completed RCTs and failure to report results for critical outcomes (e.g., mortality, morbidity, QOL). The researchers also noted that selecting appropriate studies for inclusion was challenging due to the varied stages of development of MCD tests. Additionally, most of the included studies exhibited a high risk of bias, primarily due to inadequate follow-up of negative cases. The authors determined that while MCD tests appeared to have high specificity, their sensitivity and clinical utility remain uncertain, and current evidence is insufficient to support widespread implementation. Future research should prioritize robust trials, with real-world screening populations and the assessment of patient-relevant outcomes to establish validity and clinical utility. Publications by Schrag et al. (2023) and Klein et al. (2021), previously discussed in this policy, and Nicholson et al. (2023), discussed below, were included in the Wade et al. (2025) systematic literature review.

Kahwati et al. (2025) performed a systematic review to assess the benefits, accuracy, and harms of screening for multiple cancers using a single blood-based MCD test. Overall, 20 studies, involving 109,177 individuals, assessed the test accuracy of 19 different MCD tests; most studies were noted to have a high risk of bias. Seven studies examined prediagnostic performance in asymptomatic individuals over 1 year, while the remainder relied on case-control designs that compared confirmed cancer cases with healthy controls. The reported sensitivity ranged widely from 0.095 to 0.998, specificity ranged from 0.657 to 1.0, and AUC ranged from 0.52 to 1.0. Diagnostic performance studies generally showed higher sensitivity and AUC than prediagnostic studies, but no consistent accuracy patterns emerged. Harms were minimally reported, with only one cohort study providing limited data. A significant limitation of this analysis is the lack of completed, controlled investigations that measure the benefits of screening with MCD tests. In addition, there was substantial variability among the study designs, and the majority of the studies included were at a high risk of bias due to the use of straightforward cases and healthy controls; this likely led to overestimation of discrimination accuracy. The authors determined that the current evidence is inadequate to establish the benefits, risks, and reliability of MCD tests for population-level screening. Publications by Schrag et al. (2023) and Klein et al. (2021), previously discussed in this policy, and Liu et al. (2020), discussed below, were included in the Kahwati et al. (2025) systematic literature review.

The LeeVan and Pinsky (2024) systematic review evaluated the ability of cell-free nucleic acid–based MCD tests to predict cancer status. Overall, 20 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 noted that the majority of the cases of cancer in the studies reviewed were evaluated with MCD tests at diagnosis, which may lead to overestimates of test sensitivity compared with samples from individuals who were asymptomatic. It was also noted that sensitivity varied by cancer type and typically increased with cancer stage. Ultimately, the researchers recognized the lack of published evidence supporting the clinical validity (and clinical utility) of cell-free nucleic acid–based MCD testing and recommended further high-quality studies that investigate MCD assay use in populations of asymptomatic individuals, which is the intended-use population for MCD testing.

The Bittla et al. (2023) systematic review examined the role of ctDNA in early cancer detection by screening 166 records and ultimately including 12 medium- to high-quality studies that involved 25,774 individuals. Eligible studies were observational studies, randomized trials, systematic reviews, and meta-analyses published in English after 2013, enrolling individuals aged 18 years or older. Across the included studies, ctDNA was evaluated as an exposure using targeted assays, NGS, methylation-based tests, and multianalyte approaches, with comparators ranging from tissue biopsy to conventional tumor markers and standard imaging. The primary outcomes were centered on early cancer detection performance (particularly sensitivity, specificity, and concordance with tumor tissue); the secondary outcomes included PFS, OS, recurrence prediction, and tumor localization. The pooled evidence demonstrated limited utility of ctDNA for detecting early-stage tumors smaller than 1 cm, with assay sensitivities ranging from 69% to 98% and specificities near 99% across select platforms such as CancerSEEK, which localized tumor origin in 83% of cases and showed sensitivities from 33% to 100% depending on cancer type. TEC-Seq detected early-stage mutations in 59% to 71% of individuals

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across several cancers, while PanSeer identified cancer up to 4 years before standard diagnosis, with 88% sensitivity and 96% specificity, detecting preclinical cancer in 95% of asymptomatic individuals later diagnosed with malignancy (CI, 89%-98%). As a prognostic biomarker, positive ctDNA was consistently associated with worse OS, including an OR of 4.83 for mortality across solid tumors, and postoperative ctDNA elevation predicted recurrence in 15 of 16 individuals in one study. The authors concluded that while ctDNA shows promise as a prognostic and disease-monitoring tool, its current performance characteristics limit its use as a stand-alone early-detection assay. Limitations of this analysis include the documented clonal hematopoiesis causing false positives, reduced detectability of ctDNA in tumors measuring < 10 mm, heterogeneity in assay methods across studies, and inconsistent concordance with tissue biopsy. The absence of randomized trials, reliance on heterogeneous study designs, and variability in sampling and processing further limit reliability. The clinical implication is that ctDNA may be valuable for surveillance, response assessment, and recurrence prediction, but it should not replace established early-detection methods and requires confirmatory diagnostics when positive.

The Nicholson et al. (2023) multicenter, prospective, observational study (SYMPLIFY) assessed the performance of MCD testing in symptomatic participants who were referred for a specialty evaluation from primary care. Participants were 18 years of age or older and had been referred from primary care with symptoms that were either nonspecific or potentially related to gynecologic, lung, or gastrointestinal (GI) cancers. A sample of blood was obtained from each participant when they presented for further investigation of their symptoms. Overall, 5,461 participants 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, Inc., MCD test, test errors, lack of final diagnosis, participant withdrawal). Participants were tracked until a diagnosis was reached or for a maximum of 9 months. MCD was performed on cfDNA and blinded to clinical outcome. Finally, predictions from the MCD test were compared with the diagnosis obtained via standard processes to determine primary outcomes, including overall PPV and NPV, sensitivity, and specificity. The final outcomes were measured only in participants who had both a valid MCD test and diagnostic resolution. In total, 368 participants (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% (88.5%-98.7%) in stage IV]. When a participant 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. Participants with symptoms that indicate a potential upper GI 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 asserted that this study is the first large-scale, prospective evaluation of an MCD test 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 in individuals who present to primary care clinics with nonspecific symptoms. Further study is recommended to evaluate the impact of MCD testing on the use of resources, clinical decision-making, and clinical outcomes. This study was funded by GRAIL, Inc.

The Kandimalla et al. (2021) retrospective study used 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 cfDNA specimens for early detection and/or population screening of all GI cancers. The study design involved tissue discovery followed by plasma cfDNA validation. Methylation data from 1,781 tumor and adjacent normal tissues and DMRs between individual GI cancers and adjacent normal tissues were studied, including CRC, hepatocellular carcinoma, esophageal SCC, gastric cancer, esophageal adenocarcinoma, and pancreatic ductal adenocarcinoma. By comparing data from tumor vs normal tissues in each GI cancer as well as across all GI cancers, 67,832 regions of interest were identified based on differentially methylated probes, with a p value of < 0.001 and an absolute delta beta of 0.20 across all the comparisons. Three distinct categories of DMR panels were developed to include (1) cancer-specific biomarker panels, with AUC values of 0.98 (CRC), 0.98 (hepatocellular carcinoma), 0.94 (esophageal SCC), 0.90 (gastric cancer), 0.90 (esophageal adenocarcinoma), and 0.85 (pancreatic ductal adenocarcinoma); (2) a pan-GI panel that detected all GI cancers, with an AUC of 0.88; and (3) a multicancer (tissue of origin) prediction panel, EpiPanGI Dx, with a prediction accuracy of 0.85 to 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 of a cfDNA methylation assay that offers strong diagnostic accuracy for multidetection of GI cancers in a noninvasive and cost-effective manner. This study is limited by its retrospective observations, the limited sample size used to represent each stage, and the 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 that includes prospective evaluation is needed to determine the clinical relevance of these findings.

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The Liu et al. (2020) prospective case-control substudy of the Atlas and STRIVE studies (NCT02889978 and NCT03085888) assessed the performance of targeted methylation analysis of cfDNA in detecting and localizing multiple cancer types across all stages, with high specificity. Overall, 6,689 participants [2,482 with cancer (over 50 types), 4,207 without cancer] were grouped into training or validation sets. cfDNA was sequenced, and a panel of over 100,000 informative methylation areas was targeted. From this, a classifier was developed and validated for detection of cancer and localization of tissue of origin. The authors documented consistent performance in both the training and validation sets. In the validation set, the specificity was 99.3%. Stage I to III sensitivity was 67.3% in a preselected 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 to III sensitivity was 43.9% in all cancer types, with an increase in detection as cancer stage increased. The tissue of origin was predicted in 96% of samples with cancer-like signals, and of that group, the tissue of origin localization was accurate in 93%. In conclusion, the researchers indicated that cfDNA sequencing using informative methylation patterns warrants further evaluation in prospective, population-level studies. This study was included in the Kahwati et al. (2025) and LeeVan and Pinsky (2024) systematic reviews discussed above.

Circulating Tumor Tissue–Modified HPV DNA (e.g., NavDx®)

There is currently insufficient evidence to support the use of circulating tumor tissue–modified human papillomavirus (HPV) DNA testing [e.g., NavDx (Naveris)] for the detection and measurement of tumor tissue–modified viral (TTMV) HPV DNA in individuals with HPV-related cancers.

The Jones et al. (2026) systematic review evaluated posttreatment plasma ctDNA monitoring in head and neck squamous cell carcinoma (HNSCC), including HPV-specific ctDNA. Overall, 18 studies met predefined criteria and collectively included 2,447 individuals (median ages ranging from 26-92 years). Most studies were prospective, and sample sizes were modest, with a median of 59 individuals and median follow-up times of 24.5 months. All studies performed posttreatment plasma ctDNA assessment after curative-intent surgery, RT, chemoradiotherapy (CRT), or mixed treatment approaches. In total, 13 studies focused exclusively on HPV-positive disease using assays directed at the HPV-16 genotype in all cases, with subsets also targeting HPV-18, HPV-31, HPV-33, or HPV-35. Across these studies, posttreatment plasma HPV ctDNA demonstrated a consistently high NPV for excluding residual or recurrent disease; the calculated mean sensitivity and specificity in HPV-positive cohorts were 80.2% and 97%, with a corresponding PPV and NPV of 86.7% and 95.8%, respectively. In several cohorts, undetectable posttreatment HPV ctDNA strongly aligned with favorable outcomes, including complete radiological response and confirmed absence of recurrence on biopsy, while reemergence of detectable ctDNA frequently preceded clinical or radiographic recurrence by months. Longitudinal analyses showed that persistent or rising HPV ctDNA after initial clearance was closely associated with subsequent progression, whereas clearance kinetics immediately after surgery or CRT provided early posttreatment prognostic information. Reported lead times varied extensively, ranging from 0 to > 500 days before radiological detection, and consecutive positive tests improved predictive performance in some reports. Although transient, isolated HPV ctDNA elevations were noted in a minority of individuals; these typically resolved on repeat sampling and did not correlate with eventual recurrence. Across the included studies, the authors concluded that plasma HPV ctDNA represents a promising posttreatment surveillance tool, with a high NPV for HPV-associated disease and potential for earlier detection of recurrence compared with imaging or clinical examination. Prominent limitations include modest sample sizes, short follow-up durations, heterogeneous sampling intervals, inconsistent assay methodologies, lack of masking, incomplete reporting, and missing blood draws in key time windows. Additional constraints arose from nonuniform treatment protocols, reliance on surrogate HPV markers in some cohorts, and the absence of validated thresholds for ctDNA positivity. These limitations reduce confidence in the conclusions and indicate that well-designed, comparative studies that include longer surveillance are needed. Publications by Berger et al. (2022), previously discussed in this policy, and Ferrandino et al. (2023), discussed below, were included in this systematic review.

The Hanna et al. (2024) systematic review synthesized evidence on surveillance strategies after curative-intent therapy for HNSCC, including plasma HPV ctDNA for HPV-associated disease. Overall, 80 studies met the inclusion criteria (five meta-analyses/systematic reviews, one randomized trial, one post hoc analysis, 25 prospective studies, and 48 retrospective cohorts, comprising 27,525 individuals). For biomarker surveillance, the panel focused on HPV ctDNA as a liquid biopsy approach specific to HPV-driven oropharyngeal cancer. Approximately 10% of individuals with HPV-positive oropharyngeal cancer lacked detectable pretreatment HPV ctDNA, limiting eligibility for this modality. In the strongest prospective cohort identified (n = 115), 15 recurrences occurred during a median 23-month follow-up in 28 individuals with any posttreatment positive ctDNA. In this cohort, two consecutive positive tests yielded a PPV of 94%, with a median lead time of 3.9 months from test to biopsy-proven disease. A pooled analysis of five ctDNA surveillance studies (n = 600) reported a sensitivity of 0.54, specificity of 0.98, PPV of 93%, and NPV of 95% for detecting recurrence, while a large multicenter cohort reported a PPV of 95% for detecting occult recurrence. The authors concluded that a positive HPV ctDNA result during surveillance warrants confirmatory repeat testing and subsequent targeted clinical and imaging workup, emphasizing that prospective evidence linking ctDNA-triggered detection to improved survival or QOL is lacking.

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The authors highlighted limitations that temper confidence in clinical utility; across the surveillance literature, there are no large, randomized trials demonstrating that imaging or HPV ctDNA surveillance in asymptomatic individuals improves disease-specific outcomes or QOL. Additionally, most ctDNA data are derived from retrospective or observational cohorts with heterogeneous populations, variable recurrence risk, and short follow-up. Accordingly, the authors concluded that HPV ctDNA shows promise for early detection of occult recurrence and may be considered in risk-adapted surveillance when pretreatment ctDNA is detectable but called for prospective studies to determine whether ctDNA-guided surveillance improves outcomes. Well-designed, comparative studies are needed. The authors disclosed financial relationships, including those with a manufacturer associated with HPV ctDNA. The publication by Berger et al. (2022), previously discussed in this policy, was included in this systematic review.

The Campo et al. (2024) systematic review and meta-analysis evaluated the sensitivity, specificity, and accuracy of circulating tumor HPVDNA (ctHPVDNA) and TTMV-HPVDNA by digital drop polymerase chain reaction to determine the efficacy and limitations for its use in posttreatment surveillance of HPV+ oropharyngeal SCC (OPSCC). Twelve studies, comprising 1,311 individuals, were included in the meta-analysis. In 398 individuals, HPVDNA was evaluated by ctHPVDNA. TTMV-HPVDNA was used to evaluate 913. The results showed a pooled sensitivity and specificity of 86% (95% CI, 78%-91%) for ctHPVDNA and 96% (95% CI, 91%-99%) for TTMV-HPVDNA. The pooled DOR was 371.66, and the AUC was 0.81 (95% CI, 0.67-0.91). The authors concluded that the use of cfDNA may be helpful for detecting earlier recurrence in individuals with HPV+ OPSCC; however, these testing methodologies and protocols require standardization before they can be routinely used in a clinical setting. Further research, including prospective studies with larger numbers of individuals and improvement in the test sensitivity, are needed. The publication by Berger et al. (2022), previously discussed in this policy, was included in the Campo et al. systematic review and meta-analysis.

Hayes published a Molecular Test Assessment in 2023 that addressed the use of NavDx for the detection and measurement of circulating TTMV-HPVDNA in HPV-related cancer. The assessment found that the overall body of evidence for the NavDx test is limited. Only one poor-quality study (Berger et al., 2022) that met the inclusion criteria assessed the currently marketed NavDx test. This study evaluated a single use of the test (monitoring for recurrence); it reported limited data for clinical performance, did not compare the NavDx test with the current standard care surveillance schedule or another assessment method, and lacked follow-up in individuals with negative tests. No studies evaluated the clinical utility of the NavDx test to impact treatment decision-making or individuals' clinical outcomes for any use. No studies evaluated the clinical validity of the NavDx test to confirm tumor HPV genotype, assess current treatment response, or identify measurable residual disease (MRD) post treatment. The 2024 annual review of the NavDx Molecular Test Assessment indicated that evidence that is newly published since the prior assessment is unlikely to change the current Hayes rating [Hayes, NavDx (Naveris), 2023; updated 2025].

An ECRI Clinical Evidence Assessment evaluated the effectiveness of NavDx when added to standard surveillance compared with the effectiveness of standard surveillance alone for detecting recurrence of HPV-associated cancer. ECRI concluded that based on evidence from two diagnostic cohort studies, NavDx may add incremental value for identifying HPV-associated OPSCC recurrence when used in addition to standard surveillance. However, available studies primarily focused on HPV-16, which makes it unclear how well NavDx works for other HPV subtypes. Furthermore, there are no published data on the clinical utility of NavDx in the management of individuals or outcomes (ECRI, 2023).

Ferrandino et al. (2023) reported on the accuracy of TTMV-HPVDNA testing via liquid biopsy for the diagnosis and monitoring of patients with HPV-associated OPSCC. In this retrospective observational cohort study that included 399 patients, 163 were in the diagnostic cohort, and 290 were in the surveillance cohort. In the diagnostic cohort, 152 of 163 patients had HPV-associated OPSCC, and 11 of 163 had HPV-negative OPSCC. In this group, the sensitivity of TTMV-HPVDNA in pretreatment diagnosis was 91.5% (95% CI, 85.8%-95.4%; 139 of 152 tests), and the specificity was 100% (95% CI, 71.5%-100%; 11 of 11 tests). In the surveillance cohort, 593 tests were conducted in the 290 patients. Molecularly confirmed pathological recurrences occurred in 23 patients. The TTMV-HPVDNA test exhibited a sensitivity of 88.4% (95% CI, 74.9%-96.1%; 38 of 43 tests) and a 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). The median time from a positive TTMV-HPVDNA test to pathological confirmation was 47 (0-507) days. The authors concluded that this cohort study revealed a 100% specificity of the TTMV-HPVDNA assay when used in a clinical setting for both diagnosis and surveillance. However, the sensitivity was 91.5% for the diagnosis cohort and 88.4% for the surveillance cohort, indicating false negatives in nearly one 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. (This study is included in the systematic review and meta-analysis by Campo et al., 2024.)

The Rettig et al. (2022) cross-sectional study analyzed plasma from 110 participants with initial or recurrent HPV-positive OPSCC (without distant metastases) using a digital droplet polymerase chain reaction–based assay [NavDx (Naveris)].

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This assay assesses tumor tissue–derived HPV DNA in plasma. The objective was to describe the clinical, pathological, and imaging features associated with TTMV-HPVDNA. Circulating TTMV-HPVDNA was found in 98 participants and was most strongly associated with clinical n stage. Few participants (n = 11) had N0-stage disease, and only four of them (36%) had detectable DNA. In participants with clinical stage N1 to N3 disease, 94 of 99 (95%) had detectable DNA. Of participants with undetectable TTMV-HPVDNA, over half (seven of 12) had clinical stage N0 disease. The majority of participants had computed tomography and positron emission tomography scans available; TTMV-HPVDNA prevalence and score increased with progressively higher clinical nodal stage, diameter of largest LN, and higher nodal maximum standardized uptake value on positron emission tomography/computed tomography. In addition, the size of the largest cervical LN was more closely associated with the circulating TTMV-HPVDNA than it was with the size of the primary tumor. The authors concluded that circulating TTMV-HPVDNA detection and score were strongly associated with nodal disease in participants with HPV-positive OPSCC, and the cause of this association as well as the use of this assay for surveillance merit further investigation. This study is limited by a relatively small number of participants, especially in certain subgroups, and potential author biases.

Clinical Practice Guidelines

American Radium Society

Hanna et al. (2024) published the American Radium Society's evidence-based appropriate use criteria for HPV ctDNA surveillance in survivors of nonmetastatic HNSCC:

- It may be appropriate to monitor plasma HPV DNA (if detectable at baseline) for surveillance after completion of therapy for low-risk, HPV-associated oropharynx cancer. The panel agrees that further prospective evidence of the utility of plasma HPV DNA monitoring in detecting subclinical recurrence is needed, including its effects on QOL and/or survival, before considering it to be usually appropriate (strength of evidence: moderate; strength of recommendation: weak).
- The panel agrees that plasma HPV DNA monitoring (if detectable at baseline) may be appropriate in higher-risk HPV-associated oropharynx cancer (strength of evidence: moderate; strength of recommendation: weak).

National Comprehensive Cancer Network (NCCN)

In the NCCN Guidelines on head and neck cancers (v2.2025), the NCCN states the following: "The performance of various plasma cell-free HPV DNA detection assays (preferably validated per CLIA and CAP regulatory guidelines) for a diagnosis of HPV-positive oropharyngeal cancer against a gold standard of E6/E7 mRNA detection is unknown. Sensitivity and specificity against p16-IHC are approximately 90% and 94%, respectively. At this time, persistent cell-free oncogenic HPV DNA detection in plasma (among those positive and quantifiable at diagnosis) may identify patients at increased risk for progression after completion of curative intent therapy. However, without concurrent clinical, radiographic, or pathological correlates represents an outcome without actionable therapeutic implications outside of clinical trials."

Immune Profile Score (Tempus)

The Zander et al. (2025) prospectively designed retrospective study developed and validated the Immune Profile Score (IPS), a multiomic algorithm derived from Tempus targeted DNA sequencing (the 648-gene xT panel; includes TMB) and RNA sequencing (the whole-transcriptome xR panel) to stratify outcomes among individuals with stage IV solid tumors treated with ICIs as a first- or second-line therapy across 16 cancer types [NSCLC (n = 647), gastroesophageal (n = 171), urothelial (n = 137), renal cell carcinoma (n = 131), HNSCC (n = 125), melanoma (n = 102), BC (n = 86), CRC (n = 46), hepatocellular carcinoma (n = 40), and other (n = 115)]. Using a Tempus deidentified real-world database, the authors randomly split an ICI-treated pancancer population into a development cohort (n = 1,707) and a validation cohort (n = 1,600). The IPS scores were scaled from 0 to 100 and dichotomized at prespecified 55th and 60th percentile thresholds, with an indeterminate band (n = 81) excluded from analyses. In the validation cohort (median age, 65 years; 40% female), an IPS-high result (n = 576) was associated with significantly longer OS than an IPS-low result (n = 943) in multivariable Cox models adjusted for regimen and stratified by line of therapy (hazard ratio, 0.45; 90% CI, 0.40-0.52; $p < 0.01$), with similar magnitude when modeled continuously. Prognostic associations were consistent across key biomarker and clinical subgroups, including TMB high and TMB low; PD-L1 positive and negative; microsatellite stable; presence or absence of brain and liver metastases; and major tumor types such as NSCLC, renal cell carcinoma, melanoma, and HNSCC. The effects were smaller in gastroesophageal cancer, hepatocellular carcinoma, BC, and CRC. IPS added prognostic information beyond TMB, PD-L1 IHC, and MSI in likelihood ratio tests; in an exploratory sequence-of-therapy analysis, IPS did not associate with time to next treatment on first-line chemotherapy but did with OS on second-line ICI (hazard ratio, 0.63; 90% CI, 0.49-0.82; significant interaction), while an external non-ICI cohort of individuals with stage IV cancer from The Cancer Genome Atlas showed a weaker but significant association (hazard ratio, 0.75; 90% CI, 0.59-0.95). This suggests the prognostic and possible predictive utility for ICI benefit. The authors concluded that IPS is a generalizable pancancer biomarker that may identify individuals, including those with low TMB or microsatellite-stable or PD-L1–

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negative tumors, who experience improved outcomes on ICI-based regimens. The study is limited by its retrospective design, variability in cohort sizes by cancer type, reliance on OS without durable response analyses to date, and absence of additional clinical or laboratory covariates in the IPS model; furthermore, many authors were employees of or held equity or intellectual property related to the sponsor, which was Tempus AI.

EpiSwitch Checkpoint inhibitor Response Test

Hayes (2024) published a Precision Medicine Research Brief describing the published literature related to the use of the EpiSwitch Checkpoint inhibitor Response Test for the assessment of the likelihood of response to ICI therapy. Hayes did not identify any abstracts addressing the test's clinical utility and only identified one case-control study evaluating test performance. Hayes concluded that there is no/unclear support for the test in clinical practice guidelines and position statements. The paucity of published, peer-reviewed literature precluded further evidence evaluation.

Guardant360 Liquid

Hayes (2024; updated 2025) published a Molecular Test Assessment evaluating the clinical utility of the Guardant360 test to determine eligibility for ICI therapy in the setting of advanced solid cancer. Hayes identified a very small body of very low-quality evidence suggesting that Guardant360 may identify MSI-high tumors that are eligible for ICI therapy, with an implied benefit for selecting treatment considered to be standard care. Hayes concluded that there is weak support for the test over more limited cancer type–specific panels in clinical practice guidelines and position statements when used to determine ICI eligibility. The paucity of published, peer-reviewed literature precluded further evidence evaluation. During their 2025 annual review of this test, Hayes noted that the test now goes by the name Guardant360 Liquid and includes an expanded panel of genomic biomarkers and epigenomic signals. However, Hayes did not identify any newly published studies that met the inclusion criteria for their assessment.

Measurable Residual Disease Assays (e.g., Signatera)

Currently, evidence to support the clinical utility of Signatera use for monitoring response to treatment or for surveillance after treatment is lacking. There is also currently insufficient evidence in the peer-reviewed clinical literature to support the use of other MRD assays in the management or posttreatment prognosis of solid tumor cancers.

Hayes (2026) published a Molecular Test Assessment that evaluated the published literature related to the use of the Guardant Reveal test for detection of MRD; monitoring for recurrence of CRC, BC, or lung cancer recurrence; and monitoring therapy response in advanced-stage solid tumors. Hayes identified five applicable studies, which comprised an overall low-quality body of evidence that met the inclusion criteria for their review. Hayes concluded that there is insufficient published data to assess Guardant Reveal for the noted indications and weak support against the Guardant Reveal test in clinical practice guidelines and position statements. No peer-reviewed studies were found that evaluated the clinical usefulness of Guardant Reveal in guiding the monitoring of individuals, treatment decisions, or clinical outcomes.

A 2024 Hayes Precision Medicine Research Brief addressed Haystack MRD (Quest Diagnostics), a tumor-informed MRD test that detects ctDNA in blood for solid tumor cancers. It is intended to inform the use of adjuvant therapy via residual disease detection, to monitor treatment response to adjuvant therapy, and to surveil disease recurrence. Hayes determined that there currently is not enough published, peer-reviewed literature to evaluate the evidence related to Haystack MRD for any of these indications, finding no abstracts that met their criteria. Hayes identified weak support in clinical practice guidelines and position statements against the use of the Haystack MRD test.

The Zheng et al. (2024) systematic review and meta-analysis evaluated 80 prospective studies and randomized trials published between 2013 and 2023 that enrolled individuals with resectable AJCC stage I to IV cancers and collected blood samples prospectively for postoperative or postadjuvant ctDNA testing. Eligible studies classified individuals by ctDNA status, reported hazard ratios for recurrence-related outcomes or OS, and used either landmark or longitudinal ctDNA assessments. Across the included literature, 2,565 ctDNA-positive and 10,763 ctDNA-negative samples contributed to recurrence analyses, while 766 ctDNA-positive and 2,482 ctDNA-negative samples contributed to OS analyses. The primary exposure was postoperative ctDNA positivity, with recurrence-free survival, DFS, PFS, event-free survival, time to recurrence, DMFS, and OS as outcomes. Pooled analyses demonstrated that postoperative ctDNA positivity was strongly associated with recurrence, with a pancancer hazard ratio of 7.48 (95% CI, 6.39-8.77) and with OS (hazard ratio, 5.58; 95% CI, 4.17-7.48). Sensitivity for recurrence detection varied substantially by testing strategy; landmark sensitivity was 0.50 (95% CI, 0.46-0.55), with a specificity of 0.91 (95% CI, 0.89-0.92), whereas longitudinal assessments achieved a sensitivity of 0.74 (95% CI, 0.68-0.80), with a specificity of 0.89 (95% CI, 0.85-0.93). Longitudinal testing also outperformed landmark testing in diagnostic accuracy, with a higher area under the summary receiver operating characteristic curve (0.88 vs 0.80) and DOR (25.70 vs 9.90). Subgroup analyses showed that adjuvant therapy reduced recurrence risk among ctDNA-positive individuals with CRC, with a pooled hazard ratio of 6.63 (95% CI, 4.24-

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10.35) compared with a hazard ratio of 17.67 (95% CI, 9.47-32.96) without adjuvant treatment. These results suggest that postoperative ctDNA positivity consistently identifies individuals at a substantially increased risk of recurrence and death across multiple cancer types and that longitudinal monitoring provides superior sensitivity for detecting MRD. Limitations of this study include the heterogeneity in ctDNA assays, small number of studies for some cancer types, variable definitions of ctDNA positivity, and influence of differing proportions of ctDNA-negative individuals on sensitivity estimates. Publications by Reinert et al. (2019) and Loupakis et al. (2021), previously discussed in this policy, were included in this systematic review and meta-analysis.

In a Molecular Test Assessment [Hayes, Signatera (Natera Inc.), 2023; updated 2025], 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 that assessed the clinical validity of Signatera but no peer-reviewed articles that reported an impact on clinical decision-making or an improvement in outcomes related to the use of Signatera. Significant questions exist regarding the appropriate selection of individuals for testing and the most effective timing of testing. In addition, the studies identified by Hayes had a wide variation in cancer types and treatments, and the overall quality was poor. At this time, the evidence is insufficient to support the use of Signatera for both monitoring response and detecting recurrence. However, Hayes noted that there are multiple ongoing clinical trials that are evaluating these outcomes. The 2025 annual review of this Hayes assessment indicates that recently published evidence is unlikely to change the current Hayes rating for this test.

Bladder Cancer

Galsky et al. (2026) conducted a post hoc exploratory analysis of ctDNA using the Signatera assay in the CheckMate 274 trial, a phase 3, randomized, double-blinded, multicenter study evaluating adjuvant nivolumab vs placebo in participants with high-risk, muscle-invasive urothelial carcinoma following RC or nephroureterectomy. Eligible participants (n = 709) had undergone R0 surgery within 120 days of randomization, were clinically and radiographically disease free, and met pathological criteria of ypT2-ypT4a or ypN+ disease (if previously treated with neoadjuvant cisplatin) or pT3-pT4a or pN+ disease (if they had not received neoadjuvant cisplatin but were ineligible for or declined adjuvant cisplatin). Baseline ctDNA was analyzed in 133 participants (18.8%), of whom 54 were found to have detectable ctDNA (40.6%). Median DFS was 52.1 months (95% CI, 19.4 months to not estimable) in those with undetectable ctDNA and 5.0 months (95% CI, 2.8 months to not estimable) in those with detectable ctDNA (hazard ratio, 0.30; 95% CI, 0.18-0.48). Among ctDNA-detectable participants, nivolumab improved DFS compared with placebo (7.4 months vs 2.8 months; hazard ratio, 0.35; 95% CI, 0.18-0.66) and improved OS (36.2 months vs 19.3 months; hazard ratio, 0.41; 95% CI, 0.20-0.83). In contrast, among ctDNA-undetectable participants, hazard ratios for DFS and OS were 0.99 (95% CI, 0.51-1.93) and 0.87 (95% CI, 0.41-1.84), respectively, indicating no clear between-group differences. Limitations of this substudy include its post hoc design and its sample size of only 18.8% of the randomized cohort, which may reduce its generalizability. Additionally, OS results have not yet crossed the prespecified threshold for statistical significance. Despite these limitations, the findings suggest that postoperative ctDNA may help identify individuals with urothelial carcinoma at particularly high ROR, offering a possible path toward more personalized adjuvant treatment if validated in additional prospective studies. Numerous authors reported advisory roles, research funding, and employment relationships with pharmaceutical companies, which may pose potential conflicts of interest.

Powles et al. (2025) reported on an international phase 3 trial that prospectively embedded a serial ctDNA surveillance phase after cystectomy for MIBC to identify participants at risk for disease recurrence, with randomization of only those who became ctDNA positive while remaining radiographically disease free. Eligibility for entry into the surveillance phase required an age of ≥ 18 years, ECOG performance status of ≤ 2, surgical staging of (y)pT2-4aN0M0 or (y)pT0-4aN+M0, no radiographic evidence of disease, and enrollment 6 to 24 weeks after cystectomy. Surveillance used tumor-informed assays performed every 6 weeks for 9 months, with a final test at 1 year; either the Signatera or a BGI Genomics assay was used. Between May 2021 and November 2024, 761 participants were enrolled at 157 sites, and 379 tested ctDNA positive at some point during surveillance. Of those who tested positive, 250 met treatment phase criteria and were randomized to atezolizumab or placebo. In total, 377 tested ctDNA negative, of whom 357 met the prespecified criteria for analysis of clinical outcomes. Among the randomized ctDNA-positive cohort, 59% were positive at the initial test, and 41% converted from negative to positive later in surveillance. The primary outcome of the overall study was investigator-assessed DFS; the secondary outcomes included OS and, in exploratory analyses, ctDNA clearance dynamics and outcomes among the persistent ctDNA-negative cohort. The median follow-up was 16.1 months in randomized ctDNA-positive participants and 21.8 months after cystectomy in ctDNA-negative participants. Persistent ctDNA-negative status was associated with low event rates; by the clinical cutoff date, 39 of 357 ctDNA-negative participants (11%) had disease recurrence or had died, with landmark DFS of 95% at the end of the 1-year monitoring period and of 88% at 2 years. In the randomized ctDNA-positive cohort, ctDNA kinetics were informative; clearance by treatment cycle 3 or 5 occurred in 25% overall with atezolizumab vs 14% with placebo, and clearance of high pretreatment ctDNA levels occurred only in those receiving atezolizumab. Participants who cleared with placebo had uniformly low baseline ctDNA and often converted to positivity relatively late after cystectomy. These findings may support integrating serial, tumor-informed

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ctDNA surveillance after cystectomy to risk stratify individuals. In this study, those with sustained ctDNA negativity experienced high 1- and 2-year DFS without adjuvant therapy exposure during monitoring, while conversion to ctDNA positivity identified a population with substantially higher near-term risk in which intensified follow-up and adjuvant treatment were associated with favorable on-treatment ctDNA dynamics and improved clinical outcomes. Study limitations include the use of two different, although methodologically similar, assays and a short median follow-up; evolving postrecurrence therapies may also influence downstream outcomes. The trial was funded by F. Hoffmann-La Roche, which provided study interventions and collaborated in the design, conduct, analysis, and writing; Natera contributed to design discussions and supplied molecular residual disease tests that were purchased at full cost.

Breast Cancer

Ademuyiwa et al. (2025) performed a single-arm, observational biomarker study nested within a clinical trial of neoadjuvant docetaxel and carboplatin in participants with stage II to III, triple-negative BC. The study was conducted at a single academic center, with prospective blood collection at predefined time points. A total of 119 participants contributed 452 evaluable plasma samples. Most participants were under age 65 years and had stage IIA or IIB disease. Blood samples were obtained before neoadjuvant therapy, on treatment, after completion of neoadjuvant therapy, prior to surgery, and throughout the postsurgical surveillance period. Plasma was analyzed using the Guardant Reveal tissue-free epigenomic assay assessing methylation-based ctDNA. Follow-up extended to a median of 50 months. ctDNA was detected at baseline in 56 of 72 evaluable participants (78%), at an early on-treatment time point in 65 of 83 (78%), and after neoadjuvant therapy in two of 55 (3.6%). Postneoadjuvant ctDNA detection was associated with DR ($p = 0.014$); in participants with residual disease at surgery, postneoadjuvant ctDNA positivity was associated with a markedly shorter RFI (hazard ratio, 28.2; $p < 0.0001$). Clearance of ctDNA between baseline and postneoadjuvant time points was associated with lower rates of DR (one of 22) compared with persistent detection (two of two; $p = 0.011$). In the postsurgical setting, surveillance sensitivity for distant metastatic recurrence was 83% (five of six participants with samples collected within 1 year of recurrence), and sample-level specificity among participants without recurrence was 99.5% (197 of 198 samples). Participants with detectable ctDNA at any postsurgical time point had a shorter RFI than those who remained ctDNA negative (median, 8.2 months vs not reached; hazard ratio, 37.7; $p < 0.0001$). The authors concluded that ctDNA detection using a tissue-free epigenomic assay demonstrated high specificity and sensitivity for metastatic recurrence and that ctDNA dynamics across neoadjuvant therapy and surveillance were strongly prognostic. The study is limited by its single-arm design, without a comparison group; lack of randomization or masking; small number of recurrence events; and infrequent postsurgical sampling that constrained assessment of lead intervals. Additional limitations include a substantial number of quality-control failures related to low plasma input volumes, inability to evaluate residual cancer burden, and reliance on biobanked specimens. This publication was included in the Hayes 2026 Guardant Reveal Molecular Test Assessment.

The Elliott et al. (2025) single-center, retrospective cohort study analyzed 290 banked plasma samples from 95 patients with early-stage, ER-positive/HER2-negative or triple-negative BC treated with NCT at a single center to evaluate the Guardant Reveal tumor-agnostic methylation-based MRD (mMRD) ctDNA assay against investigator-defined genomic MRD (gMRD) derived from a 753-gene plasma-only panel. Patients were prospectively enrolled from 2015 to 2021 inclusive, with serial blood draws at baseline, perioperatively, and at follow-up time points; treatment decisions followed local standard of care, and results were not disclosed clinically. The median age was 49 years, 53.7% had ER-positive disease, and 46.3% had triple-negative disease; most received anthracycline- and taxane-based regimens, and the median follow-up from surgery was 2.9 years. Baseline mMRD detection was 72.5% overall and was associated with higher event risk in univariable and multivariable analyses (hazard ratio, 9.4; 95% CI, 1.3-70.3; $p = 0.03$), with a sensitivity and specificity for subsequent recurrence of 95% and 35% overall and subtype-specific sensitivities/specificities of 100%/42% in ER-positive and 91%/27% in triple-negative disease. No mMRD positivity occurred prior to the operation, consistent with clearance during neoadjuvant therapy. Postoperative and follow-up mMRD detection was strongly prognostic for worse event-free survival (hazard ratio, 17.0; 95% CI, 6.0-48.0; $p < 0.0001$) and provided 62.5% sensitivity and 100% specificity for recurrence, with a median molecular lead time of 152 days (range, 15-748 days); all assessable ER-positive patients with postoperative mMRD positivity recurred, whereas sensitivity was lower in triple-negative disease. Exploratory gMRD definitions using gene-list restriction, variant allele fraction thresholds, baseline-informed tracking, and sequence-context filtering for clonal hematopoiesis improved performance but remained inferior to mMRD; unfiltered gMRD yielded implausibly high postoperative positivity, and the combination of gMRD and mMRD did not outperform mMRD alone. The authors concluded that tumor-agnostic mMRD outperformed gMRD across metrics and that mMRD positivity in surveillance identified patients with near-certain recurrence; this supports the further development and prospective evaluation of mMRD assays in this setting. The findings are constrained by the retrospective, observational cohort, single-center design; enrichment for patients who recurred; missed or delayed draws during the COVID-19 period; relatively short follow-up for ER-positive disease; absence of contemporary chemoimmunotherapy or adjuvant cyclin-dependent kinase 4/6 inhibitor use during accrual; use of a locked vendor algorithm for mMRD; and exploratory, unblinded gMRD definitions not produced by the commercial assay. Well-designed, comparative studies are needed. This publication was included in the Hayes 2026 Guardant Reveal Molecular Test Assessment.

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Magbanua et al. (2021, included in the 2023 Hayes Signatera Molecular Test Assessment) evaluated the clinical utility of ctDNA to test for the risk of metastatic recurrence and predictive ability of pCR in participants with early BC. A retrospective ancillary ctDNA study was performed on samples that had been prospectively collected from high-risk participants with early BC who were part of the multicenter neoadjuvant I-SPY2 trial. The eligibility requirements included a 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 participants who had received a MammaPrint high score. On pretreatment testing, 73% of participants were ctDNA positive. The participants who continued to be ctDNA positive 3 weeks after initiation of paclitaxel were significantly more likely to have residual disease after NCT than those who were no longer ctDNA positive. All participants who achieved pCR after NCT were ctDNA negative. In 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, the 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 NCT may assist with clinical assessment and treatment in early BC. The 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.

Cervical Cancer

Mayadev et al. (2025) reported on the CALLA trial, a phase 3, randomized, double-blinded study evaluating adult women with previously untreated, locally advanced cervical cancer (International Federation of Gynecology and Obstetrics 2009 stage IB2-IIB node positive or IIIA-IVA with any nodal status) who had measurable disease, an ECOG performance status of 0 to 1, and adequate organ function. Participants were randomized 1:1 to durvalumab plus concurrent CRT or placebo plus CRT, with plasma collected at baseline, after completion of CRT [cycle 3 day 1 (C3D1)], and 3 months later (C6D1) for tumor-informed ctDNA testing using the Personalis NeXT Personal assay. Among 770 randomized participants, 241 consented to sequencing, and the biomarker-assessable population included 185 at baseline, 186 at C3D1, and 130 at C6D1. Baseline ctDNA detection was high [98.9% (183/185)], enabling analyses based on ctDNA level rather than presence. Participants with baseline ctDNA below the global median of 5,268.2 ppm had more favorable outcomes; hazard ratios for PFS and OS in the low- vs high-ctDNA groups were 0.60 (95% CI, 0.27-1.33) and 0.63 (95% CI, 0.27-1.45) in the durvalumab arm and 0.62 (95% CI, 0.31-1.23) and 0.85 (95% CI, 0.43-1.69) in the CRT arm. The strongest prognostic signals emerged from on-treatment ctDNA. At C3D1, absence of detectable ctDNA was associated with markedly lower risks of progression and death across both treatment arms, with PFS hazard ratios of 0.23 (95% CI, 0.11-0.50) in the durvalumab arm and 0.15 (95% CI, 0.07-0.33) in the CRT arm and corresponding OS hazard ratios of 0.20 (95% CI, 0.09-0.47) and 0.18 (95% CI, 0.08-0.38). By C6D1, ctDNA negativity was even more strongly predictive, with PFS hazard ratios of 0.04 (95% CI, 0.01-0.16) for durvalumab and 0.04 (95% CI, 0.01-0.17) for CRT; OS hazard ratios were similarly 0.04 (95% CI, 0.01-0.20) and 0.04 (95% CI, 0.01-0.19). ctDNA detection preceded radiographic progression by a median of 164 days (95% CI, 85-250 days) at C3D1 and 155 days (95% CI, 135-238 days) at C6D1. In multivariable modeling, disease stage and ctDNA status at C3D1 were independently prognostic for progression ($p = 0.017$ and $p < 0.001$). The consistent prognostic value of ctDNA across time points, particularly its strong, early discrimination of recurrence risk, suggests potential for integrating ctDNA-guided surveillance into clinical practice to enable earlier intervention and more individualized posttreatment monitoring. Limitations of this study include the exclusion of participants who did not reach C3D1 due to progression during CRT and the authors' disclosures of multiple industry relationships, with several investigators employed by or receiving support from AstraZeneca, which was the study sponsor.

Cholangiocarcinoma

Yoo et al. (2025) evaluated participants participating in the multicenter, open-label, randomized, phase 2 STAMP trial who had completely resected, LN-positive extrahepatic cholangiocarcinoma. In the STAMP trial, 101 participants had been assigned 1:1 to eight 3-week cycles of adjuvant capecitabine or gemcitabine-cisplatin, with a prespecified biomarker cohort (n = 89) providing longitudinal plasma for the Signatera personalized, tumor-informed assay (positivity defined as at least two tumor-specific variants). The primary end point was DFS over an extended follow-up period (median, 52.8 months). No between-arm benefit was detected; median DFS was 14.6 months with gemcitabine-cisplatin vs 11.1 months with capecitabine (hazard ratio, 1.02; one-sided 90% CI, 0.76-1.37; $p = 0.47$), and median OS was 35.0 vs 35.7 months (hazard ratio, 1.04; one-sided 90% CI, 0.75-1.44; $p = 0.43$). The biomarker cohort (median age, 67 years; 66% male; tumor site perihilar, 48%; distal, 52%; R0 resection margins, 67%; pathological stages II/III/IV, 43%/44%/13%) contributed 254 postsurgical samples prior to adjuvant chemotherapy, at 12 weeks of adjuvant chemotherapy, and at 24 weeks of adjuvant chemotherapy. Positivity rates for ctDNA were 24.7% prior to adjuvant chemotherapy, 21.6% at 12 weeks, 19.5% at 24 weeks, 70.7% at clinical recurrence, and 52.8% at any time post surgery. Positivity for ctDNA correlated with inferior DFS at every landmark; the hazard ratio for prior to adjuvant chemotherapy was 1.8 (95% CI, 1.06-3.07; $p = 0.029$), for 12 weeks was 7.72 (95% CI, 4.09-14.56; $p < 0.001$), for 24 weeks was 5.24 (95% CI, 2.75-9.97; $p < 0.001$),

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and any time post surgery was 3.81 (95% CI, 2.22-6.54; p < 0.001). Median DFS was 12.1 months in ctDNA-positive vs 42.7 months in ctDNA-negative participants prior to adjuvant chemotherapy; the findings were consistent across perihilar vs distal tumors and across adjuvant regimens. In a multivariable analysis, ctDNA status at 12 weeks outperformed clinicopathologic factors (including cancer antigen 19-9, resection margin, and stage) as a predictor of DFS. Dynamics were informative: compared with persistent negativity (n = 56), persistent positivity (n = 10) and conversion to positive (n = 11) were associated with markedly shorter DFS (hazard ratio, 6.7 and 5.8, respectively; both p < 0.001), whereas conversion from positive to negative (n = 12) yielded outcomes similar to persistent negativity (hazard ratio, 1.4; p = 0.328). Among those who became ctDNA positive during adjuvant therapy, ctDNA often antedated radiological recurrence by months and could rise while cancer antigen 19-9 or carcinoembryonic antigen (CEA) remained normal. The authors concluded that serial ctDNA status and its on-treatment dynamics predicted recurrence during adjuvant therapy in resected extrahepatic cholangiocarcinoma and may help optimize clinical decision-making. Limitations of this study include the modest sample size; open-label design, without masking; absence of a no-therapy control; and biomarker constraints such as suboptimal plasma volumes in some draws. All participants received adjuvant chemotherapy, which may attenuate prognostic separation for OS in the window prior to adjuvant chemotherapy; postrecurrence treatments could also confound survival analyses. Several authors were employees of the ctDNA assay manufacturer, and study drugs and budget support were provided by the industry. Well-designed, comparative studies that include larger cohorts are needed.

Colorectal Cancer

The Camblor et al. (2026) systematic review and diagnostic accuracy meta-analysis compared tumor-informed and tumor-agnostic ctDNA assays for detecting recurrence in resected CRC and evaluated how landmark vs serial sampling influenced performance. The investigators searched PubMed and Embase through October 31, 2025, and included English-language full-text studies from 2015 onward; 33 studies met the criteria, with 27 studies (7,482 individuals; 1,488 recurrences) contributing to landmark analyses and 17 studies (2,865 individuals; 561 recurrences) to serial analyses. Across all studies, serial sampling increased sensitivity vs landmark sampling (0.72 vs 0.51; p = 0.001), without a significant difference in false-positive rates. In stratified analyses, tumor-informed assays achieved higher sensitivity than tumor-agnostic assays in the serial setting (0.88 vs 0.59; p = 0.001), while no significant sensitivity difference was observed at the landmark time point (0.48 vs 0.58; p = 0.070); false-positive rates did not differ significantly between approaches in either setting. Subgroup analyses found no significant performance differences in tumor-informed methods (panel based vs genome wide) or in tumor-agnostic methods (single-omic vs multiomic). A Deek test suggested no publication bias in landmark studies but indicated small-study overperformance in serial studies. The authors concluded that sampling strategy is pivotal and that when longitudinal monitoring is feasible, tumor-informed assays provide the most reliable recurrence detection, whereas a single postoperative landmark test yields broadly comparable performance across approaches, making logistical considerations more relevant at that time point. The authors concluded that aligning assay selection with clinical intent (particularly favoring tumor-informed assays for serial surveillance) may optimize detection of recurrence after curative-intent surgery. Limitations of this study include the substantial heterogeneity among tumor-agnostic serial studies; evidence of publication bias in the serial dataset; imbalances in sample size across settings and assay subtypes; a reliance on indirect comparisons due to few within-study, head-to-head evaluations; and restricted generalizability, given the exclusion of stage IV and rectal-only populations. The analysis focused on cross-sectional diagnostic accuracy and could not assess lead time or clinical benefit because time-to-recurrence outcomes were inconsistently reported; the authors emphasized the need for standardized end points and prospective studies to clarify real-world impact. The publication by Tie et al. (2025), discussed below, was included in this systematic review and meta-analysis.

The Negro et al. (2025) systematic review and meta-analysis evaluated ctDNA as a marker of MRD and recurrence risk in adults with stage II CRC who underwent curative-intent surgery. Seven studies met the inclusion criteria, comprising prospective and retrospective cohort designs, one matched case-control study, and one RCT. Two studies focused exclusively on colon cancer, while five included broader CRC populations. Across the included studies, ctDNA was measured in plasma using tumor-informed or tumor-agnostic assays, most commonly at 4 to 10 weeks after surgery, with some studies also assessing postadjuvant chemotherapy and including longitudinal follow-up. Sample sizes ranged from 27 to 852 individuals, with postoperative ctDNA positivity rates across stage II subgroups varying from 8% to 17%. The pooled meta-analysis included 126 ctDNA-positive and 964 ctDNA-negative individuals and demonstrated that postoperative ctDNA positivity was associated with a higher recurrence risk, with a pooled risk ratio of 3.66 (95% CI, 1.25-10.72; p = 0.002). Subgroup analyses indicated that tumor-informed methods (pooled risk ratio, 4.87; 95% CI, 2.22-10.66; p < 0.0001), analyses restricted to colon cancer (risk ratio, 4.93; 95% CI, 3.34-7.28; p < 0.00001), and chemotherapy-naive individuals (risk ratio, 5.58; 95% CI, 3.43-9.10; p < 0.00001) all showed strong associations between ctDNA positivity and recurrence risk. Five studies evaluated ctDNA after completion of adjuvant chemotherapy, consistently demonstrating markedly worse outcomes in ctDNA-positive individuals, with hazard ratios of 11.0 (95% CI, 1.8-68; p = 0.001), 11.0 (95% CI, 5.2-23; p < 0.0001), and 11.58 (95% CI, 1.33-101; p = 0.001). Four studies included serial surveillance testing, showing that ctDNA detected recurrence earlier than conventional markers, including a median

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61-day lead time over CEA (61 days vs 167 days; p = 0.04) and an approximately 8-month lead over radiographic imaging (8.2 months vs 16.9 months; p = 0.001). Variability in ctDNA assays, detection thresholds, sampling time points, and study designs introduced heterogeneity into this study and may influence pooled effect estimates. Additional limitations include small stage II subgroups in several studies and low postoperative ctDNA positivity rates, which may contribute to false-negative results. Despite these constraints, the evidence suggests that ctDNA may be a sensitive biomarker for identifying individuals at high ROR after stage II CRC resection and may help refine decisions regarding adjuvant therapy and surveillance intensity. Standardization of ctDNA methodologies and validation in large prospective trials remain essential before broad implementation.

Tie et al. (2025) published 5-year results from the prospective multicenter DYNAMIC trial, an investigation of ctDNA-guided adjuvant chemotherapy decisions in participants with stage II colon cancer. The DYNAMIC trial randomized 455 participants in a 2:1 ratio to either ctDNA-guided management (Haystack MRD) or standard clinicopathologic management. Those participants whose tests were ctDNA positive (detected at either 4 or 7 weeks after surgery) received oxaliplatin-based or fluoropyrimidine chemotherapy. Participants with ctDNA-negative results did not receive adjuvant therapy. The primary outcome was 2-year recurrence-free survival. The secondary outcomes included adjuvant chemotherapy use, OS, ctDNA clearance, and long-term outcomes. The researchers found that chemotherapy use was reduced in the ctDNA-guided treatment group compared with the standard treatment group (15% vs 28%), and the 2-year recurrence-free survival rate was similar (93.5% vs 95.4%). The recurrence-free survival rate at 5 years was 88% in the ctDNA-guided group vs 87% in the standard group, and the OS rate in the ctDNA-guided group was 93.8% compared with 93.3% in the standard group. Of the ctDNA-positive participants treated with chemotherapeutic agents, 87.5% achieved ctDNA clearance by the end of treatment, which is predictive of more favorable long-term outcomes. Notably, higher postoperative ctDNA burden correlated with a worse prognosis, and persistent ctDNA after chemotherapy predicted recurrence in nearly 100% of cases. The authors suggested that the ctDNA-guided approach to treatment management reduced chemotherapy use, without compromising survival, and may serve as a useful and important biomarker for individualized adjuvant treatment decision-making in individuals with stage II colon cancer. Limitations of this study include the lack of generalizability to larger and more diverse populations; this study was conducted primarily in Australian medical facilities. In addition, the small number of ctDNA-positive participants prevented detailed subgroup analyses. Additional well-designed studies are needed to confirm these findings.

The Emiloju et al. (2024) retrospective analysis focused on the use of Signatera to detect MRD in ctDNA in patients with stage II to IV CRC. The researchers examined the clinical records of 120 patients with CRC who had undergone at least one tumor-informed ctDNA assay. In total, 476 ctDNA assays were performed in the 120 patients. Overall, 70% of the assays were administered to patients who had recurrent disease, most often to monitor the effectiveness of the treatments provided. Only 16% of the total assays that were performed led to a change in clinical decision-making. Overall, 62 patients experienced a total of 110 recurrences (some patients had more than one recurrence). The authors determined that the results of this study show that although ctDNA for MRD detection may be helpful for certain individuals with CRC, 84% of the total assays included in this analysis resulted in no change to clinical decision-making, which highlights the need for additional study in high-quality clinical trials.

The Nakamura et al. (2024) prospective, multicenter observational analysis evaluated whether tumor-informed, ctDNA-based MRD status predicts DFS and OS after curative-intent resection for CRC and whether ctDNA dynamics relate to adjuvant treatment effects. The cohort included 2,240 participants with stage II to III colon cancer or stage IV CRC who underwent R0 resection, with a median follow-up of 23 months. ctDNA was assayed using the Signatera personalized, multiplex polymerase chain reaction–NGS, tumor-informed platform during a prespecified MRD window (2 to 10 weeks post surgery, before any adjuvant chemotherapy) and serially in a surveillance window. Survival analyses used landmarking to address immortal time bias and multivariable Cox models to compare ctDNA status with clinicopathologic factors. MRD positivity was strongly associated with inferior outcomes; the hazard ratio for DFS was 11.99 (95% CI, 10.02-14.35) and for OS was 9.68 (95% CI, 6.33-14.82), with 24-month DFS of 20.57% vs 85.10% and 24-month OS of 83.65% vs 98.50% in MRD-positive vs MRD-negative participants. Across surveillance, any ctDNA positivity conferred markedly worse prognosis (DFS hazard ratio, 33.56; OS hazard ratio, 19.51). In multivariable models, ctDNA positivity was the strongest prognostic factor for both DFS and OS, outperforming nodal status, T stage, MSI, and *BRAF* V600E as factors. Among those who recurred radiologically (n = 500), ctDNA positivity in the MRD window or during surveillance was associated with shorter overall and postrecurrence survival (for MRD window: OS hazard ratio, 2.71), independent of recurrence site. Exploratory biomarker analyses showed that MRD positivity predicted inferior DFS consistently across actionable subgroups; notably, participants with *BRAF* V600E and MRD negativity had a low recurrence rate (7.89%) vs universal recurrence among those who were MRD positive (11 of 11). In high-risk, stage II/III colon cancer, MRD-positive participants appeared to benefit from adjuvant chemotherapy (adjusted hazard ratio, 0.23), whereas MRD-negative participants had no statistically significant benefit (adjusted hazard ratio, 0.70; p = 0.091). Among MRD-positive participants treated with adjuvant chemotherapy, ctDNA clearance at 3 and 6 months correlated with superior DFS and OS, and sustained clearance across serial time points identified a markedly favorable group compared with transient

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clearance or no clearance (24-month DFS: 89.0% with sustained clearance vs 3.33% with transient clearance); true spontaneous clearance without clinical recurrence was rare at 1.9% (two of 105). The authors concluded that ctDNA-based MRD monitoring after resection provides powerful risk stratification for recurrence and mortality and that ctDNA clearance may serve as an early indicator of adjuvant treatment efficacy, potentially informing escalation or de-escalation strategies as tested in ongoing randomized trials within the platform. Limitations of this study include the observational, nonrandomized design for adjuvant therapy decisions and the median 23-month follow-up that may not capture longer-term survival effects. Financial conflicts were disclosed by several authors. Well-designed, comparative studies are needed. This publication was included in the Hayes Guardant Reveal 2026 Molecular Test Assessment.

The Kotani et al. (2023) interim analysis from GALAXY, the observational arm of a prospective, multicenter registry, evaluated presurgical and postsurgical ctDNA using a personalized, tumor-informed assay (Signatera) among 1,039 participants with resectable, stage II to IV or recurrent CRC who were enrolled across 92 institutions. Blood was collected before and 4, 12, 24, 36, 48, and 72 weeks after surgery, with a median follow-up of 16.74 months. Four weeks after surgery, 18.0% (187 of 1,039) were ctDNA positive, and 82.0% (852 of 1,039) were ctDNA negative. Postsurgical ctDNA positivity was strongly associated with recurrence across stages, with 61.4% of ctDNA-positive participants experiencing relapse vs 9.5% of ctDNA-negative participants (hazard ratio, 10.0; 95% CI, 7.7-14.0; $p < 0.0001$; 18-month DFS, 38.4% vs 90.5%); in multivariable analysis among pathological stage II to III disease, it was the most significant prognostic factor for recurrence (hazard ratio, 10.82; 95% CI, 7.07-16.6; $p < 0.001$). Compared with CEA measured at 12 weeks, ctDNA provided more informative relapse stratification in discordant cases (70% relapse when ctDNA positive/CEA negative vs 12% when ctDNA negative/CEA positive). In adjusted analyses in participants with high-risk, stage II or stage III disease, adjuvant chemotherapy conferred substantial benefit in those who were ctDNA positive at 4 weeks (adjusted hazard ratio, 6.59; 95% CI, 3.53-12.3; $p < 0.0001$), whereas no significant benefit was observed in those who were ctDNA negative (adjusted hazard ratio, 1.71; 95% CI, 0.80-3.7; $p = 0.167$; 18-month DFS: 94.9% with adjuvant chemotherapy vs 91.5% with observation). ctDNA dynamics from 4 to 12 weeks were also prognostic; compared with a persistently negative status, conversion to positive (hazard ratio, 14.0; 95% CI, 8.5-24.0) or persistent positivity (hazard ratio, 21.0; 95% CI, 14.0-31.0) was associated with markedly worse DFS. Among the 182 ctDNA-positive participants with clearance data, adjuvant chemotherapy increased the cumulative incidence of ctDNA clearance by 24 weeks (adjusted hazard ratio, 8.50; 95% CI, 4.2-17.3), and failure to achieve clearance on adjuvant chemotherapy was associated with inferior DFS (adjusted hazard ratio, 11; 95% CI, 5.2-23.0). The authors concluded that postsurgical ctDNA may be predictive of adjuvant chemotherapy benefit, potentially informing escalation for ctDNA-positive and de-escalation for ctDNA-negative postoperative management strategies. Key limitations of this analysis include the observational, nonrandomized design for treatment comparisons, with potential selection confounding despite multivariable adjustment and landmark analyses; relatively short follow-up for definitive assessment of longer-term outcomes; and limited feasibility of randomizing ctDNA-positive participants to observation in this setting. The study reported multiple author relationships, including employment by and equity in the ctDNA assay manufacturer and other industry relationships.

The Fakih et al. (2022) retrospective, single-center cohort study evaluated the comparative surveillance strategies of a ctDNA assay (Signatera) with those of standard radiographic imaging and CEA levels per NCCN Guidelines in patients with resected CRC. Of 48 patients with curatively resected CRC, 15 had disease recurrence during surveillance. Confirmation via imaging was made in nine patients, and positive ctDNA confirmed disease recurrence in eight patients. Confirmation by CEA levels occurred in three patients, and confirmation by combined imaging with CEA levels occurred in 11 patients. 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 ($p > 0.99$). The combination of imaging plus the measurement of CEA levels (sensitivity, 73.3%; 95% CI, 44.8%-91.1%) had a numerical advantage compared with ctDNA in identifying recurrence ($p = 0.55$). In addition, the 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, small number of recurrences, and short follow-up. The authors concluded that the findings show that the ctDNA assay (Signatera) may not supply definitive advantages as a surveillance strategy compared with the standard imaging combined measurement of CEA levels when performed per NCCN Guidelines. Additional prospective studies that focus on the correlation between low-burden lung recurrence and negative ctDNA findings should be investigated further. This publication was included in the 2023 Hayes Signatera Molecular Test Assessment.

Cutaneous Melanoma

The Liu et al. (2025) systematic review and meta-analysis examined 12 observational cohort studies involving 1,063 individuals with unresectable, previously untreated, stage III or IV melanoma who received ICIs. Eligible studies required pathological confirmation of melanoma, assessment of ctDNA, and reportable OS or PFS outcomes. Across studies conducted between 2018 and 2023, ctDNA was measured before treatment, after treatment initiation, or both. Detectable ctDNA at baseline was associated with significantly poorer OS, with a pooled hazard ratio of 3.19 (95% CI, 2.22-4.58; $p < 0.001$), and shorter PFS, with a hazard ratio of 2.08 (95% CI, 1.61-2.69; $p < 0.001$). Detectable ctDNA during treatment

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demonstrated even stronger associations, with OS (hazard ratio, 4.57; 95% CI, 3.03-6.91; p < 0.001) and PFS (hazard ratio, 3.79; 95% CI, 2.13-6.75; p < 0.001) both significantly worse in individuals with measurable ctDNA. The authors noted limitations, including few available studies, variable detection techniques, inconsistent timing of ctDNA measurement, and relatively short follow-up in some cohorts, all of which may limit generalizability and introduce methodological variation. The study's results suggest that ctDNA may help stratify prognosis before initiation of ICI therapy and may provide a dynamic indicator of treatment response and disease progression during therapy.

Hepatocellular Carcinoma

The Galli et al. (2025) systematic review evaluated circulating blood biomarkers for MRD after curative-intent treatment of hepatocellular carcinoma. Inclusion required at least one postintervention blood test, with association with recurrence or time-to-event outcomes. Overall, 91 studies met the criteria (74 with results; 17 ongoing). Evidence was grouped as circulating DNA (comprising ctDNA, cfDNA, methylated DNA, and virus-host chimera DNA; 24 studies); circulating tumor cells (20); circulating RNA, including exosomal RNA (eight); and miscellaneous immune and protein markers (22). Across circulating DNA studies, postoperative biomarker positivity or higher biomarker levels consistently predicted recurrence irrespective of treatment modality, with reported sensitivities of 59% to 80% and several prospective cohorts showing that ctDNA outperformed α-fetoprotein for MRD detection. Circulating RNA signatures (e.g., K19 and CD44 mRNA ratios, selected miRNA/long noncoding RNA) were associated with recurrence, but most lacked head-to-head comparison with α-fetoprotein. The authors concluded that ctDNA is one of the most mature novel approaches for blood-based MRD in hepatocellular carcinoma and warrants prospective, interventional trials to establish clinical utility, with the potential to enrich adjuvant therapy for MRD-positive individuals while sparing toxicity in MRD-negative individuals. Limitations of this systematic review include methodological heterogeneity in assays, cutoffs, sampling schedules, and end points; limited or absent intention-to-treat frameworks; sparse direct comparisons with α-fetoprotein for several modalities; lack of standardized follow-up windows; and the absence of biomarker-driven trials. Additional concerns include variable preinterventional detection rates and population and etiologic differences that may limit generalizability. Well-designed, comparative, interventional studies are needed.

Lung Cancer

Black et al. (2025) conducted a prospective, longitudinal cohort analysis in TRACERx that included 431 participants with stage I to III NSCLC undergoing definitive resection, using a tumor-informed ctDNA assay (NeXT Personal) to evaluate pre- and postoperative landmark (10 to 120 days) MRD and longitudinal surveillance against clinical outcomes over a median follow-up of 5 years. Prior to the operation, ctDNA was detected in 82.7% (329 of 398; median, 829 ppm; 25% < 80 ppm); positivity predicted worse RFS and OS in lung adenocarcinoma (RFS: hazard ratio, 4.74, 95% CI, 2.59-8.71; OS: hazard ratio, 5.77, 95% CI, 2.49-13.40), with multivariable associations persisting after adjustment. At the landmark time point, ctDNA was detected in 28.6% (72 of 252), including 43.1% < 80 ppm, yielding a specificity of 92.5%, sensitivity of 61.5%, PPV of 86.2%, and NPV of 76.0% for relapse. Landmark positivity strongly predicted inferior outcomes (RFS: hazard ratio, 7.17, 95% CI, 4.80-10.73; OS: hazard ratio, 5.79, 95% CI, 3.74-8.97). Stratification by quantitative landmark level revealed a stepwise gradient: ctDNA not detected (best), < 80 ppm (intermediate), and ≥ 80 ppm (worst), independent of age, sex, histology, stage, smoking, and adjuvant therapy. In lung adenocarcinoma, combining pre- and postoperative status refined risk further. Preoperative negative/landmark negative had the most favorable course; preoperative positive/landmark negative formed a distinct intermediate-risk group vs preoperative positive/landmark positive (RFS: hazard ratio, 0.18 for preoperative positive/landmark negative vs preoperative positive/landmark positive). Among 35 participants who were ctDNA positive post surgery/prior to adjuvant therapy with paired postadjuvant samples, ctDNA clearance after adjuvant therapy was associated with improved RFS (hazard ratio, 5.56) and OS (hazard ratio, 7.49). All who cleared had completed four cycles of platinum chemotherapy, whereas none receiving less than four cycles cleared (Fisher p = 0.0028). Longitudinally, any postoperative ctDNA positivity predicted relapse with 86% sensitivity, 91% specificity, a PPV of 90%, and an NPV of 88%, providing a median molecular lead time of 158 days (range, 0-1,732 days); persistently positive ctDNA and conversion from negative to positive conferred markedly elevated relapse risks, with poor 3-year RFS (5.97% and 20.7%, respectively). The authors concluded that ultrasensitive, tumor-informed ctDNA, especially detections below 80 ppm and their kinetics through adjuvant therapy, enables high-resolution risk stratification, informs adjuvant treatment benefit, and anticipates the timing of relapse; they proposed the use of a ctDNA-guided surveillance and treatment schema that integrates pre- and postoperative results. The study used a single-cohort design, without randomized treatment assignment; was limited to participants with available tumor tissue for panel design; and was conducted before widespread neoadjuvant immunotherapy or the era of adjuvant tyrosine kinase inhibitors. Reliance on retrospective ctDNA analysis of prospectively collected samples further tempers generalizability. The authors disclosed multiple industry relationships, including platform developer employment and financial ties. Overall, the findings are suggestive of clinical validity but remain limited by the observational design; well-designed, comparative studies are needed.

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The Chen et al. (2025) PRISMA-guided systematic review and meta-analysis synthesized 52 studies published from 2016 to 2024 to evaluate ctDNA as a prognostic tool in NSCLC across four predefined time points: baseline, perioperative, post full-course therapy, and surveillance. The included studies were required to be original observational studies or randomized trials in individuals with NSCLC that reported ctDNA status as a binary variable and at least one prognostic end point (relapse-/disease-/event-free survival, OS, recurrence, or lead time). The cohorts spanned AJCC stages I to IV and had sample sizes of 12 to 330 individuals. ctDNA positivity vs negativity served as the exposure; comparators were ctDNA-negative individuals in the same time point. The primary outcomes were RFS and OS; the secondary outcomes were recurrence risk and lead time from ctDNA detection to radiographic/clinical recurrence. Across all studies, baseline ctDNA positivity was associated with inferior RFS (hazard ratio, 2.23; 95% CI, 1.82-2.75). In resectable disease, postoperative ctDNA positivity was strongly associated with worse RFS (hazard ratio, 5.64; 95% CI, 3.88-8.19) and worse OS (hazard ratio, 4.17; 95% CI, 2.22-7.84). After completion of full-course therapy, pooled RFS was worse in both the resectable (hazard ratio, 5.82; 95% CI, 3.12-10.87) and unresectable (hazard ratio, 2.72; 95% CI, 1.99-3.72) cohorts. For OS, baseline ctDNA positivity predicted poorer outcomes in both resectable (hazard ratio, 4.15; 95% CI, 2.45-7.02) and unresectable disease (hazard ratio, 1.74; 95% CI, 1.49-2.03), with additional OS detriment after full-course therapy in unresectable disease (hazard ratio, 3.38; 95% CI, 1.97-5.80). Recurrence risk rose with later time points; the baseline recurrence risk was 1.67 (95% CI, 1.27-2.20), post–full-course recurrence risk was 3.13 (95% CI, 2.09-4.67), and surveillance recurrence risk was 5.42 (95% CI, 3.20-9.18). Eighteen studies reported a lead time from ctDNA to radiological/clinical recurrence, with a median of study-specific medians of 2.93 months (range, 1.70-12.60 months). The results suggest that ctDNA positivity identifies higher-risk individuals at each treatment landmark and often anticipates radiographic relapse by several months, supporting its potential use for risk stratification and closer surveillance while underscoring the need for standardized assays and prospective trials that test ctDNA-triggered interventions. The Egger test detected publication bias in this study for OS at baseline ($p = 0.001$) and after full-course therapy ($p = 0.012$) and for RFS after full-course therapy ($p = 0.006$). Additional weaknesses of this study include the heterogeneity of the underlying studies, including variable ctDNA definitions and end points, and the studies' predominantly retrospective, small cohorts.

The Leite da Silva et al. (2025) systematic review and meta-analysis evaluated whether longitudinal plasma ctDNA kinetics predict treatment outcomes in advanced NSCLC. The authors included observational studies and clinical trials that enrolled individuals with confirmed NSCLC who were undergoing systemic therapy and performed serial plasma ctDNA assessments. Overall, 32 studies met the criteria, encompassing 3,047 individuals treated with targeted therapies (n = 1,940), immune checkpoint blockade (n = 826), or chemotherapy (n = 281), with ctDNA measured at baseline and during early treatment. The primary outcomes were PFS and OS. Across 31 studies, ctDNA decrease or clearance was associated with improved PFS (hazard ratio, 0.32; 95% CI, 0.26-0.40; $p < 0.01$; $I^2 = 63\%$), with ctDNA clearance showing greater benefit (hazard ratio, 0.27; 95% CI, 0.20-0.36). Subgroup analyses were consistent across targeted therapy (hazard ratio, 0.34; 95% CI, 0.24-0.46) and immune checkpoint blockade (hazard ratio, 0.33; 95% CI, 0.24-0.46). Mutation-specific analyses showed improved PFS in individuals with *EGFR*-mutant NSCLC achieving *EGFR* ctDNA clearance (hazard ratio, 0.30; 95% CI, 0.22-0.41). For OS, pooled results from 25 studies demonstrated benefit with ctDNA decrease or clearance (hazard ratio, 0.31; 95% CI, 0.23-0.42; $p < 0.01$; $I^2 = 47\%$), including consistent effects across targeted therapy (hazard ratio, 0.41; 95% CI, 0.28-0.58) and immune checkpoint blockade (hazard ratio, 0.32; 95% CI, 0.25-0.41). *EGFR* ctDNA clearance similarly correlated with improved OS (hazard ratio, 0.31; 95% CI, 0.19-0.50). Sensitivity analyses supported the robustness of associations across study designs, assay types, and ctDNA assessment time points, and meta-regression found no significant differences by NSCLC subtype, smoking status, or sex. Study limitations include the heterogeneity in study designs, treatments, definitions of ctDNA decrease, assay types, and sampling intervals, which may affect comparability. The possibility of publication bias could not be completely excluded. Overall, the findings suggest that ctDNA clearance or reduction may be associated with improved survival across systemic therapies, suggesting its potential value as an early prognostic biomarker. Prospective trials are still needed to determine whether modifying therapy based on ctDNA kinetics improves outcomes.

The Lu et al. (2025) systematic meta-analysis synthesized 30 observational studies, which included 3,287 individuals with resectable NSCLC, to evaluate postoperative, ctDNA-based MRD testing for predicting recurrence and survival. Eligibility required prospective or retrospective studies of pathologically resectable NSCLC in which individuals underwent ctDNA testing vs conventional recurrence detection (imaging or laboratory tests) and/or studies that reported RFS and/or OS. The meta-analysis distinguished landmark testing (a single postoperative assessment within 3 months) from longitudinal monitoring (serial postoperative assessments) and compared tumor-informed assays (personalized panels) with tumor-agnostic assays (fixed panels), with one tumor-naive study included. Pooled landmark performance yielded a sensitivity of 0.44 (95% CI, 0.38-0.50), specificity of 0.95 (0.93-0.97), PPV of 0.70 (0.61-0.79), NPV of 0.79 (0.71-0.87), AUC of 0.73 (0.69-0.77), and DOR of 16.06 (10.57-24.41). Longitudinal monitoring improved performance, with a sensitivity of 0.78 (0.70-0.85), specificity of 0.92 (0.85-0.96), PPV of 0.76 (0.69-0.84), NPV of 0.84 (0.77-0.92), AUC of 0.91 (0.88-0.93), and DOR of 41.31 (17.07-99.98). Subgroup analyses showed that in landmark testing, tumor-informed assays had a higher specificity and AUC than tumor-agnostic assays (specificity, 0.97 vs 0.93; AUC, 0.81 vs 0.70), whereas tumor-agnostic assays had slightly higher sensitivity (0.44 vs 0.42). With longitudinal monitoring, tumor-informed assays maintained

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higher specificity (0.96 vs 0.88), while tumor-agnostic assays showed modestly higher sensitivity (0.79 vs 0.76) and AUC (0.91 vs 0.86). Additional stratification found a follow-up effect whereby landmark studies with a median follow-up of > 36 months showed a specificity of 0.99 (0.96-1.00) but a lower sensitivity of 0.31 (0.20-0.45); longitudinal studies with < 36 months of follow-up had a sensitivity of 0.85 (0.75-0.92) and AUC of 0.94 (0.91-0.96). Limitations of this meta-analysis include incomplete reporting of key clinical covariates across studies. The findings suggest that serial ctDNA monitoring after curative-intent surgery may provide substantially higher sensitivity while maintaining high specificity, and assay choice entails trade-offs; tumor-informed testing optimizes specificity for ruling in recurrence, whereas tumor-agnostic testing offers slightly higher sensitivity in longitudinal use and broader mutation detection. A conflict-of-interest statement disclosed employment relationships for three coauthors with a diagnostics company.

Martin et al. (2024) performed a retrospective analysis of the effect of ctDNA monitoring on the identification and subsequent management of recurrence in 108 patients with resected early-stage NSCLC. Signatera was used to detect and measure ctDNA at 3-month intervals after cancer resection with curative intent had been performed. The study took place between October 2021 and March 2023 and at least one ctDNA measurement post operation and one computed tomography scan report post operation were required for inclusion in the analysis. ctDNA-positive test results were the primary outcome measured. A secondary outcome that was assessed was the change in treatment plan after a ctDNA-positive result. A total of 11.1% (n = 12) of the study patients were found to have ctDNA-positive results at one or more measurement intervals after surgery. Of them, eight patients (66.7%) had a clinically identifiable recurrence. The remaining four patients had limited clinical follow-up assessments. Overall, 10 of the study patients developed recurrent disease, and eight of these patients had ctDNA positivity. Brain-only metastases were found in the remaining two patients who had recurrence. Of the 12 patients with ctDNA positivity, 100% had a change in postoperative clinical care; all 12 patients with ctDNA-positive results underwent an early positron emission tomography scan, with 66.6% found to be positive for characteristics of malignancy. The authors concluded that routine monitoring of ctDNA after curative intent therapy may improve risk stratification and prognostic ability in this population. However, the small number of patients in this study, coupled with its retrospective design, limits its quality. In addition, some patients were not adherent to the regularly scheduled testing intervals, and the providers involved in the care of the patients were not blinded to ctDNA results when making decisions regarding the patients' surveillance and adjuvant treatments. Lastly, several of the authors were employees of the company that manufactures the assay that was used in this study. Further high-quality, prospective studies that include larger cohorts and longer follow-up times are needed to validate these early results and help develop best-practice strategies.

The Zaman et al. (2023) systematic review and meta-analysis assessed the prognostic value of MRD analysis via ctDNA or cfDNA in NSCLC. Overall, 27 studies, including 3,419 individuals, were included in the analysis. Eleven studies, including 1,359 individuals, reported on the association of baseline ctDNA with PFS, and 16 studies, including 1,659 individuals, reported on dynamic changes in ctDNA associated with PFS. The analysis revealed that individuals with negative baseline ctDNA trended toward improved PFS (pooled hazard ratio, 1.35; 95% CI, 0.83-1.87; $p < 0.001$; $I^2 = 96\%$) compared with individuals who were ctDNA positive. In addition, when early reduction/clearance of ctDNA levels occurred after treatment, individuals had improved PFS (pooled hazard ratio, 0.71; 95% CI, 1.85-3.65; $I^2 = 89.4\%$) compared with individuals with no reduction/persistence in ctDNA levels. Only good- and fair-quality studies (based on assessment via the Newcastle-Ottawa Scale) exhibited improvement in PFS (pooled hazard ratio, 1.95, 95% CI, 1.52-2.38 and pooled hazard ratio, 1.99, 95% CI, 1.09-2.89, respectively); this did not occur in poor-quality studies that were included in the analysis. The authors noted 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 of PFS and OS in individuals who undergo targeted therapies for advanced NSCLC. The authors recommended additional studies that include serial ctDNA testing to further support clinical utility in the management of advanced NSCLC.

Merkel Cell Carcinoma

The Akaike prospective observational study evaluated the clinical utility of ctDNA as a biomarker for surveillance and prognosis in participants diagnosed with Merkel cell carcinoma (MCC). The study included 319 total participants, who were divided into a discovery cohort (n = 167) and a validation cohort (n = 152). Plasma specimens, imaging results, and clinical data were available for each participant. Signatera, a tumor-informed ctDNA assay, was used to monitor disease status. The researchers found the assay to have high diagnostic accuracy; the assay identified MCC at enrollment, with 95% sensitivity in the discovery cohort and 94% in the validation cohort and specificities of 90% and 86%, respectively. Further, a positive ctDNA result during the surveillance period was strongly related to the ROR, with hazard ratios of 6.8 in the discovery cohort and 20 in the validation cohort. The PPV for recurrence within 1 year after a positive ctDNA result was 69% in the discovery cohort and 94% in the validation cohort; the NPV at 135 days after negative test results was 94% in the discovery cohort and 93% in the validation cohort. Compared with those who tested negative, participants who tested positive for ctDNA within 4 months after treatment were significantly more likely to have disease recurrence at 1 year (74% vs 21%). Based on these results, the authors suggested that the use of ctDNA for surveillance in individuals

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with MCC could (1) help identify those at a higher risk who may benefit from intensified monitoring or adjuvant treatment and (2) potentially reduce reliance on frequent imaging, especially for those at a lower risk. The results are promising, but several limitations were noted, including the lack of long-term follow-up (median follow-up, 295 days). Although ctDNA testing showed a strong 1-year predictive value for recurrence, further investigation for longer-term outcomes and the impact on disease-specific survival is needed. In addition, the study was performed in the U.S. across several tertiary care facilities, which limits generalizability to broader, global populations. Because the sensitivity and specificity of ctDNA were determined based on the status of clinical disease that was assessed at the time of the first blood draw for ctDNA, any newly identified disease during the study follow-up period was not taken into account, which could impact accuracy. Lastly, several authors were affiliated with and/or had potential conflicts of interest related to the manufacturer of the ctDNA test that was used in the study (Signatera). Further high-quality research that investigates the utility of ctDNA assays for the identification of candidates who are most likely to benefit from adjuvant treatments as well as for the monitoring of tumor response to immunotherapy, with long-term outcomes, is recommended.

Squamous Cell Carcinoma

The Helou et al. (2025) systematic review and meta-analysis evaluated nonviral HNSCC across 47 studies (publication years 2001-2024). Eligible studies enrolled a total of 3,574 individuals with HNSCC and used a nonviral plasma ctDNA assay for diagnosis, prognosis, and/or treatment response. The study population most commonly had oral cavity (35%) or oropharyngeal (22%) tumors, with AJCC stage IVA/IVB disease comprising 29% and follow-up ranging from 3 to 60 months (median, 18.5 months); assays were primarily NGS- (60%) or polymerase chain reaction–based (45%), with a reported median sensitivity of 80% (range, 38%-100%) and specificity of 99% (60%-100%) across 16 studies. The exposure was ctDNA detection (baseline and posttreatment MRD) compared with ctDNA-negative status, and the primary outcomes were OS, PFS, and recurrence-free survival or DFS. Pretreatment ctDNA detection ranged from 50% to 100% (median, 83%) and posttreatment detection from 28% to 100% (median, 48%); ctDNA signals anticipated clinical or radiological recurrence in a median of 80% of individuals, with a median lead time of 4.6 months (range, 1-27 months). Pooled analyses showed that ctDNA positivity was associated with worse OS (hazard ratio, 10.26; 95% CI, 3.58-29.40; $p < 0.0001$; $I^2 = 0\%$), with additional pooled signals for *SEPT9*-methylated ctDNA (hazard ratio, 1.15; 95% CI, 1.08-1.23; $p < 0.0001$; $I^2 = 72\%$) and mutated ctDNA (hazard ratio, 1.96; 95% CI, 1.28-3.00; $p = 0.002$; $I^2 = 0\%$). Residual ctDNA after treatment was strongly associated with inferior PFS (five-study pooled hazard ratio, 7.32; 95% CI, 4.17-12.86; $p < 0.00001$; $I^2 = 0\%$) and recurrence-free survival (three-study pooled hazard ratio, 7.33; 95% CI, 2.75-19.58; $p < 0.0001$; $I^2 = 0\%$); individual studies reported shorter median recurrence-free survival with detectable ctDNA (8 months vs > 33 months; $p < 0.001$) and worse DFS linked to *SEPT9* positivity (hazard ratio, 2.72; 95% CI, 1.30-5.68; $p = 0.008$) or higher ctDNA levels (hazard ratio, 4.43; 95% CI, 1.21-16.18; $p = 0.024$). Limitations of this analysis include the between-study variability in assay platforms, targets, timing, and definitions of outcomes, which introduces clinical and analytical heterogeneity. The authors noted that four studies demonstrated no significant survival associations and highlighted that ctDNA analysis is not yet a clinical standard, with barriers including assay diversity and lack of standardization. However, the observed 4.6-month median lead time for recurrence detection suggests that ctDNA may inform earlier surveillance and risk stratification in nonviral HNSCC, pending prospective standardization and head-to-head evaluations against imaging.

The Yang et al. (2024a) systematic review and meta-analysis evaluated the relationship between ctDNA and prognosis in individuals with HNSCC. Studies were eligible if they analyzed ctDNA or methylated ctDNA in relation to survival outcomes. Across 22 eligible studies published between 2002 and 2024, the systematic review encompassed 5,062 individuals, with 16 studies using polymerase chain reaction–based assays and six using NGS. Eleven studies collected baseline blood samples, and 13 collected posttreatment samples. Across 10 studies evaluating ctDNA/methylation status and OS, positivity was associated with worse outcomes, with a pooled hazard ratio of 2.00 (95% CI, 1.35-2.96; $p = 0.0006$). Subgroup analyses showed poorer OS in individuals who were ctDNA positive (hazard ratio, 3.39; 95% CI, 1.51-7.62; $p = 0.003$) or methylation positive (hazard ratio, 1.47; 95% CI, 1.03-2.09; $p = 0.03$). For PFS or recurrence-free survival, five studies contributed data, demonstrating worse outcomes with ctDNA/methylation positivity (hazard ratio, 3.54; 95% CI, 1.05-11.85; $p = 0.04$). A further subgroup analysis indicated an association between ctDNA positivity alone and inferior PFS/recurrence-free survival (hazard ratio, 4.56; 95% CI, 1.11-18.72; $p = 0.04$). Limitations identified by the authors include heterogeneity arising from different ctDNA detection methods, varied definitions of ctDNA positivity, differing sequencing targets, and potential publication bias. The findings suggest that both ctDNA positivity and methylation positivity may be associated with poorer survival; however, variability across studies and assay methods underscores the need for standardized approaches to improve reliability and clinical applicability.

Uveal Melanoma

The Noronha et al. (2025) systematic review and meta-analysis included seven studies (four prospective cohorts and three clinical trials) that included 518 individuals with histologically confirmed, metastatic UM who underwent at least one plasma-based ctDNA assessment; studies were eligible if they were observational or interventional, evaluated ctDNA in adults (≥ 18 years), and reported survival outcomes. The primary outcomes were OS and PFS. Pooled analyses showed

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that baseline ctDNA positivity was associated with worse PFS (hazard ratio, 2.34; 95% CI, 1.56-3.51; $p < 0.01$; $I^2 = 0\%$) and worse OS (hazard ratio, 3.32; 95% CI, 2.09-5.29; $p < 0.01$; $I^2 = 48\%$). In individuals treated with tebentafusp, longitudinal ctDNA kinetics were informative; ctDNA clearance correlated with superior OS (hazard ratio, 0.19; 95% CI, 0.07-0.49; $p < 0.01$; $I^2 = 46\%$), and any ctDNA decrease was also associated with longer OS (hazard ratio, 0.42; 95% CI, 0.22-0.80; $p < 0.01$; $I^2 = 0\%$). The overall risk of bias was judged to be moderate due to residual confounding (e.g., tumor burden, prior therapies) and variability in systemic regimens, and a slightly asymmetric funnel plot suggests possible publication bias. Heterogeneity in ctDNA platforms (droplet digital polymerase chain reaction vs NGS), assay thresholds for positivity and reduction, timing of blood draws, and end point definitions, together with the limited number of studies and imbalance in study designs, represents a threat to this study's reliability and may inflate or attenuate pooled effects. Clinically, baseline ctDNA detection may stratify prognosis at metastatic diagnosis, and on-treatment ctDNA reduction or clearance during tebentafusp therapy may identify individuals with improved survival. However, routine implementation awaits assay harmonization and validation in prospective, randomized settings.

Clinical Practice Guidelines

American Society of Clinical Oncology (ASCO)

ASCO's 2022 update (Henry et al.) to their guidelines on the use of biomarkers for systemic therapy in metastatic breast cancer indicates that there were insufficient data to recommend routine use of ctDNA to monitor response to therapy among patients with metastatic breast cancer (type: informal consensus; evidence quality: low; strength of recommendation: moderate).

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

Merker et al. (2018) published a joint literature review surveying the clinical use of ctDNA testing on behalf of ASCO and the CAP. They concluded that although certain ctDNA tests have demonstrated clinical validity and utility in select advanced-stage cancers, there is insufficient evidence supporting the clinical validity and utility for most assays and stages of cancer at this time. The review concluded that there is no evidence of the clinical utility and little evidence of the clinical validity of ctDNA tests in early-stage cancer, treatment monitoring, residual disease detection, or cancer screening.

European Society for Medical Oncology (ESMO)

A 2022 ESMO publication (Pascual et al.) made recommendations on the use of ctDNA for the detection of MRD. ESMO advised that for patients treated for early-stage cancers, there is strong evidence supporting the clinical validity of ctDNA analysis to assess the risk of disease relapse for several cancer types. However, ESMO did not recommend the use of ctDNA MRD testing in routine clinical practice, as there is insufficient evidence for its clinical utility in directing treatment decisions. ESMO advised that there is insufficient evidence supporting the use of ctDNA in advanced cancer monitoring during therapy and insufficient evidence that its use improves clinical outcomes, adding that its use in cancer screening has not been validated.

Comprehensive Genomic Profiling and Solid Tumor Profiling, Including Whole-Exome, Whole-Genome, and/or Whole-Transcriptome Sequencing

Molecular assays that use whole-exome, whole-genome, and/or whole-transcriptome sequencing to detect clinically meaningful somatic variations in tumor tissue have been developed. There is currently insufficient evidence in the peer-reviewed clinical literature to support the use of these tests to provide molecular information that is intended to inform clinical decision-making for individuals with cancer.

Hayes (2025) published a Precision Medicine Research Brief describing the published literature related to the use of the OncoDEEP suite of comprehensive molecular profiling tests, comprising OncoDEEP NGS, OncoDEEP I+, and OncoDEEP Package+, for guiding treatment decisions for solid tumors. Hayes did not identify any abstracts addressing the tests' clinical validity and only two studies evaluating clinical utility, both of which were without a control group or comparison with another technology, placebo, or sham. Hayes concluded that there is weak support for OncoDEEP in clinical practice guidelines and position statements. The paucity of published, peer-reviewed literature precluded further evidence evaluation.

The Katz et al. (2025) systematic review and meta-analysis evaluated genomic sequencing in childhood and adolescent/young adult solid tumors, with a prespecified focus on clinical yield and downstream decisions, drawing on 24 original studies that included 5,278 individuals and 5,359 tumor samples; of these samples, 5,207 were analyzable. The median of reported median ages of the individuals was 11.5 years. The included evidence base comprised observational cohorts, prospective programs, and clinical trials that variably used whole-exome sequencing, whole-genome sequencing, and whole-transcriptome sequencing, often alongside targeted panels or methylation arrays, with notable between-study

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heterogeneity in tumor sampling at diagnosis vs relapse and in definitions of actionability. Pooled outcomes showed a 57.9% proportion of actionable alterations (95% CI, 49.0%-66.5%) and a 22.8% proportion with changes to decision-making based on test results (95% CI, 16.4%-29.9%); publication bias assessments were mixed but generally not definitive. Although several programs explicitly incorporated whole-exome sequencing, whole-genome sequencing, and whole-transcriptome sequencing, this review did not provide modality-stratified pooled estimates, and the authors emphasized that methodological variability (including the sequencing approach itself, inclusion of fresh-frozen as well as FFPE tumor specimens, and decentralized vs tumor-board–mediated interpretation) limits modality-specific inferences about clinical utility. The authors concluded that genomic sequencing, as implemented across these studies, identifies a substantial proportion of potentially actionable alterations and can inform treatment decisions. The evidence presented in this study does not isolate any incremental clinical impact of whole-exome sequencing, whole-genome sequencing, or whole-transcriptome sequencing. The findings are further limited by the lack of randomization and masking and nonstandardized definitions of actionability. The publication by Summers et al. (2022), previously discussed in this policy, is included in this systematic review and meta-analysis.

Hayes (2024) published a Precision Medicine Research Brief describing the published literature related to the use of the Tempus xR test for whole-transcriptome RNA sequencing for solid tumors to help inform clinical management decisions. Hayes did not identify any abstracts addressing the tests' clinical validity or clinical utility. Hayes concluded that there is weak support for the Tempus xR test in clinical practice guidelines and position statements. The paucity of published, peer-reviewed literature precluded further evidence evaluation.

The Owen et al. (2024) multisite, retrospective cohort study explored the use of RNA-NGS for the detection of fusions and splicing variations in patients with NSCLC using the Tempus xR assay (tissue-based whole-transcriptome RNA sequencing) and the Tempus xT assay, version 4 (tissue-based DNA sequencing). The primary outcome that was assessed was detection rates of NCCN Guideline–based structural variants that were uniquely identified by RNA-NGS. Included patients (n = 5,570) had sufficient tissue quantity for both RNA-NGS and DNA-NGS testing. Overall, the frequency of actionable structural variant (aSV) detection was 8.8% (n = 491), with 86.7% of those variants detected via DNA-NGS. Performing concurrent DNA-NGS and RNA-NGS led to the detection of 15.3% more patients with aSVs compared with the use of only DNA-NGS (491 vs 426 patients, respectively). No significant association was found between the assay that was used for aSV identification and aSV-targeted therapeutic adoption or clinical outcome. These results suggest that the identification of aSV by concurrent RNA-NGS and DNA-NGS is higher across NCCN Guideline–recommended biomarkers than by DNA-NGS alone, leading the authors to conclude that RNA-NGS should be considered for routine use in the clinical care of individuals with advanced NSCLC. The study was limited by the use of just one commercial NGS platform, and since the study was retrospective, there were incomplete longitudinal data regarding therapy selection and time-to-next-treatment analyses, which led to small cohort sizes and potential difficulty detecting statistical differences between DNA-NGS and RNA-NGS. In addition, differences in assay failure rates between DNA-NGS and RNA-NGS were not accounted for in this study. Further high-quality, prospective studies are recommended to validate these findings and further investigate the use of RNA-NGS as a companion diagnostic test.

Pleasance et al. (2022) performed whole-genome and -transcriptome sequencing and analysis (WGTA) on samples from 570 participants, who had various types of advanced or metastatic cancer, to identify and prioritize clinically actionable genomic variations and potentially inform treatment decisions. Participants were enrollees in the Personalized OncoGenomics program (NCT02155621), a single-arm prospective trial with the primary objectives of (1) determining the feasibility of integrating WGTA in advanced cancer populations; (2) understanding the frequency of clinically actionable findings using WGTA and the potential rate of WGTA-informed therapy; and (3) obtaining a better understanding of the genomics involved in advanced and pretreated cancers. In the Pleasance et al. study, DNA-based information was combined with RNA-based information to produce comprehensive WGTA profiles, which were then reviewed by a multidisciplinary team. In total, 83% of participants had clinically actionable targets, and of them, 37% went on to receive WGTA-informed treatment. The researchers found RNA expression data to be the most informative, with contributions to 67% of WGTA-informed treatments. Overall, 25% of treatments were guided by RNA expression only, and 46% of WGTA-informed treatments resulted in a clinical benefit. Based on these results, the authors contended that their study exhibits the potential benefit of using whole-genome and -transcriptome sequencing in the individualized care of individuals with cancer. A noted barrier in the study is the limited access to drugs that were related to the WGTA results obtained; this underscores the need for access to approved off-label treatments and clinical trials to enhance the potential for clinical action. An additional limitation is the lack of the evaluation of the impact of WGTA on OS.

Hayes (2022; updated 2025) published a Molecular Test Assessment evaluating the clinical utility of the MI Profile test as a broad molecular profiling tool to identify biomarkers present in a solid tumor and to then assign matched therapy specific to those biomarkers. The use of the MI Profile for the primary purpose of testing predetermined biomarkers having molecularly related FDA-approved therapies for a particular cancer was out of scope for this evaluation. During the initial

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2022 review as well as during annual reviews thereafter, Hayes did not identify any published abstracts that met their inclusion criteria for this report focused on the use of the MI Profile test as a broad molecular profiling tool.

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 Act of 1988. More information is available at: https://www.fda.gov/medicaldevices/deviceregulationandguidance/ivdregulatoryassistance/ucm124105.htm. (Accessed April 7, 2026)

A list of nucleic acid–based tests that have been cleared or approved by the FDA Center for Devices and Radiological Health is available at: https://www.fda.gov/medical-devices/in-vitro-diagnostics/nucleic-acid-based-tests. (Accessed April 7, 2026)

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Policy History/Revision Information

Date	Summary of Changes

10/01/2026	**Applicable Codes**
	• Updated list of applicable CPT/HCPCS codes to reflect quarterly edits:
	  ○ Added 0668U, 0682U, 0683U, 0686U, 0688U, 0689U, 0693U, 0695U, and 0696U
	  ○ Removed 0585U
	Supporting Information
	• Archived previous policy version 2026T0588QQ

Instructions for Use

This Medical Policy provides assistance in interpreting UnitedHealthcare standard benefit plans. When deciding coverage, the member specific benefit plan document must be referenced as the terms of the member specific benefit plan may differ from the standard plan. In the event of a conflict, the member specific benefit plan document governs. Before using this policy, check the member specific benefit plan document and any applicable federal or state mandates. 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.

This Medical Policy may also be applied to Medicare Advantage plans in certain instances. In the absence of a Medicare National Coverage Determination (NCD), Local Coverage Determination (LCD), or other Medicare coverage guidance, CMS allows a Medicare Advantage Organization (MAO) to create its own coverage determinations, using objective evidence-based rationale relying on authoritative evidence (Medicare IOM Pub. No. 100-16, Ch. 4, §90.5).

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

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