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

Policy Number: 2023T0588BB
Effective Date: October 1, 2023

Instructions for Use

Table of Contents **Page**

Community Plan Policy

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

Medicare Advantage Coverage Summaries

- Genetic Testing
- Laboratory Tests and Services

Application

UnitedHealthcare Commercial

This Medical Policy applies to all UnitedHealthcare Commercial benefit plans.

UnitedHealthcare Individual Exchange

This Medical Policy applies to Individual Exchange benefit plans in all states except for Colorado.

Coverage Rationale

Solid Tumor Testing

Breast Cancer Gene Expression Profiling (GEP)

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

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

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

The use of more than one predictive GEP for the same tumor in an individual with breast cancer is unproven and not medically necessary due to insufficient evidence of efficacy.

Note: This does not apply to BCI testing, which can be used once in the evaluation of the role of extended endocrine therapy in a breast cancer that may have already had GEP to determine the role of adjuvant chemotherapy.

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

Lung Cancer

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

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

Prostate Cancer Gene Expression Profiling (GEP)

The use of the 17 gene mRNA score (e.g., Oncotype DX® Genomic Prostate Score [GPS]) is proven and medically necessary for individuals with biopsy-proven, untreated, localized adenocarcinoma of the prostate (no clinical evidence of metastasis or lymph node involvement) when:
- Test is ordered by a urologist or medical oncologist; and
- Results will be used to assist with treatment decision-making when the individual has not yet received treatment for prostate cancer and is a candidate for either active surveillance or definitive therapy and all of the following:
  - Life expectancy greater than 10 years; and
  - Risk group (as per NCCN) is one of the following:
    - Very-Low-Risk Prostate Cancer; or
    - Low-Risk Prostate Cancer: or
    - Favorable Intermediate-Risk Prostate Cancer

The use of the 22 gene mRNA score (e.g., Decipher® Prostate RP genomic classifier) is proven and medically necessary to inform adjuvant treatment if adverse features (e.g., high-grade disease, Gleason score 8 or higher, extracapsular extension, positive surgical margins, seminal vesicle invasion) are found after radical prostatectomy or with PSA persistence or recurrence.

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

Thyroid Cancer or Indeterminate Thyroid Nodule Testing

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

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

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

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

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

CGP of confirmed anaplastic thyroid cancer is proven and medically necessary. For all other primary thyroid cancers see criteria for FoundationOne® CDx below.

Uveal Melanoma Gene Expression Profiling (GEP)

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

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

Companion Diagnostics via Tissue Sample for Solid Tumor Cancers

Specific biomarker identification for solid tumors is considered medically necessary when biomarker confirmation is required per the "Indications and Usage" of the U.S. FDA-approved prescribing label prior to initiation of therapy.

FoundationOne® CDx (0037U only) testing using tumor tissue is considered proven and medically necessary when all the following criteria are met:

- Individual has an unresectable or metastatic primary solid tumor (excluding primary CNS tumors in individuals less than 18 years of age); and
- Immune checkpoint inhibitor therapy (e.g., pembrolizumab, nivolumab, cemiplimab, atezolizumab, avelumab, durvalumab, ipilimumab, relatimab) is being considered for treatment; and
- There has been progression of disease and there are no satisfactory alternative treatment options; and
- No Comprehensive Genomic Profiling (CGP) has been performed previously for this primary tumor type

Repeat testing with FoundationOne CDx on tumor tissue after initial use of FoundationOne CDx is considered unproven and not medically necessary due to insufficient evidence of efficacy.

Any other CGP test for solid tumors not addressed above (e.g., oncomap™ ExTra, NeoTYPE® Discovery Profile for Solid Tumors, MSK-IMPACT®, TheraMap™ Solid Tumor, CANCERPLEX®, Solid Tumor Profile Plus, Tempus xT) is considered unproven and not medically necessary for use as a companion diagnostic due to insufficient evidence of efficacy.

Companion Diagnostics via Plasma Sample/Liquid Biopsy (Cell-Free DNA [cfDNA] or Circulating Tumor DNA [ctDNA]) for Solid Tumor Cancers

Specific biomarker identification for solid tumors via Liquid Biopsy is considered medically necessary when biomarker confirmation is required per the "Indications and Usage" of the U.S. FDA-approved prescribing label prior to initiation of therapy.

FoundationOne® Liquid CDx (0239U only) is proven and medically necessary for advanced or metastatic breast cancer, metastatic non-small cell lung cancer, metastatic castration-resistant prostate cancer (mCRPC) or recurrent ovarian, fallopian tube, or primary peritoneal cancer when all of the following criteria are met:

- No CGP has been performed previously for this primary tumor type; and
- The individual is not medically fit for invasive biopsy or tumor tissue testing is not feasible; and
- Treatment with an FDA-approved drug for use in the individual's cancer is being considered

Guardant360® CDx (0242U only) comprehensive Liquid Biopsy is proven and medically necessary when the individual has a recurrent, relapsed, refractory, metastatic, or advanced NCSLC that did not originate from the central nervous system and all of the following criteria are met:

- NSCLC has been pathologically confirmed; and
- No CGP has been performed previously for this primary tumor type; and
- The individual is not medically fit for invasive biopsy or tumor tissue testing is not feasible; and
- Treatment with an FDA-approved drug for use in the individual's cancer is being considered

Circulating tumor cell (CTC) testing (e.g., CellSearch®) is unproven and not medically necessary for all indications due to insufficient evidence of efficacy.

Liquid Biopsy (using cfDNA/ctDNA) for any other tumor genetic analysis or tumor screening (e.g., ColonSentry®, Epi proColon®, FoundationOne® Heme, Tempus xF) is considered unproven and not medically necessary for use as a companion diagnostic due to insufficient evidence of efficacy.

Hematological Cancer Testing

Testing at Initial Diagnosis

Clonality assessment at initial diagnosis (e.g., ClonoSeq® Clonality ID) on one specimen only is proven and medically necessary when ordered by a hematologist or oncologist for individuals with:

- Acute lymphoblastic leukemia
- Multiple myeloma

The use of multigene panels (50 genes or fewer) at initial diagnosis is medically necessary when ordered by a hematologist or oncologist for individuals with:

- Acute lymphoblastic leukemia
- Acute myeloid leukemia
- Multiple myeloma
- Myelodysplastic syndrome suspected
- Myeloproliferative neoplasm

Measurable Residual Disease (MRD) Testing After Treatment

MRD testing (e.g., ClonoSeq® MRD) is proven and medically necessary when ordered by a hematologist or oncologist for individuals with all of the following:

- Acute lymphoblastic leukemia or multiple myeloma; and
- Testing occurs after completing a course of therapy

Companion Diagnostics for Hematological Cancers

Specific biomarker identification for hematologic cancers is considered medically necessary when biomarker confirmation is required per the "Indications and Usage" of the US FDA-approved prescribing label prior to initiation of therapy.

CGP (e.g., FoundationOne Heme) for hematological malignancies is unproven and not medically necessary due to insufficient evidence of efficacy.

Due to insufficient evidence of efficacy, molecular testing such as GEP, multigene NGS panels and CGP is unproven and not medically necessary for all indications other than those previously described as proven, including but not limited to:

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

Documentation Requirements

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 documentation requirements outlined below are used to assess whether the member meets the clinical criteria for coverage but do not guarantee coverage of the service requested.

CPT Codes*	Required Clinical Information
Molecular Oncology Testing for Cancer Diagnosis, Prognosis, and Treatment Decisions	
0018U, 0022U, 0026U, 0171U, 0179U, 0245U, 0288U, 0306U, 0307U, 0332U, 0388U, 81228, 81229, 81277, 81445, 81479, 81449, 81518, 81519, 81520, 81521, 81522, 81523, 81546, 81599	Medical notes documenting the following, when applicable: • Cancer type and stage including, if applicable, tumor size and nodal status • Results of other biomarker testing (e.g., estrogen receptor, HER-2 neu), if applicable • Proposed treatment based on results of genetic testing (if available)

*For code descriptions, refer to the Applicable Codes section.

Definitions

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

Chromosome Microarray Analysis (CMA): A laboratory analysis that identifies genome-wide copy number variations at the chromosome level, such as aneuploidies, microdeletions and duplications, rearrangements, and amplification. CGH is one technology that can be used for a Chromosome Microarray test, and another example is a single nucleotide polymorphism (SNP) array (Peterson et al., 2018).

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

Favorable Intermediate-Risk Prostate Cancer: Clinical/pathological features must include all of the following: PSA less than 20, Gleason score of 3+3 or 3+4, and no less than 50% positive biopsy cores (NCCN Prostate Cancer, v1.2023).

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

Liquid Biopsy: Testing performed on a blood sample to identify cancer cells from a tumor in the blood or for DNA from tumor cells that are circulating in an individual's blood. Liquid Biopsy may be used for early detection of cancer or to help identify effective treatments or to monitor for return of cancer (National Cancer Institute [NCI]).

Low-Risk Prostate Cancer: Clinical/pathological features must include all of the following: PSA less than 10, Gleason score ≤ 3+3, no more than 3 biopsy cores positive with no more than 50% cancer in each core and palpable disease (T1-T2a) (NCCN Prostate Cancer, v1.2023).

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

Predictive Molecular Markers: Biomarkers which can be used to evaluate the likelihood of benefit from a specific clinical intervention, or the differential outcomes of more than one intervention (Mehta et al., 2010).

Prognostic Molecular Markers: Biomarkers which can be used to evaluate overall outcome, such as the likelihood of recurrence of cancer after standard treatment (Mehta et al., 2010).

Very-Low-Risk Prostate Cancer: Clinical/pathological features must include all of the following: PSA less than 10, Gleason score ≤ 3+3, no more than 3 biopsy cores positive with no more than 50% cancer in each core and non-palpable disease (T1c) (NCCN Prostate Cancer, v1.2023).

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

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

Applicable Codes

The following list(s) of procedure and/or diagnosis codes is provided for reference purposes only and may not be all inclusive. Listing of a code in this policy does not imply that the service described by the code is a covered or non-covered health service. Benefit coverage for health services is determined by 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
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

CPT Code	Description
0013M	Oncology (urothelial), mRNA, gene expression profiling by real-time quantitative PCR of five genes (MDK, HOXA13, CDC2 [CDK1], IGFBP5, and CXCR2), utilizing urine, algorithm reported as a risk score for having recurrent urothelial carcinoma
0016M	Oncology (bladder), mRNA, microarray gene expression profiling of 219 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as molecular subtype (luminal, luminal infiltrated, basal, basal claudin-low, neuroendocrine-like)
0017M	Oncology (diffuse large B-cell lymphoma [DLBCL]), mRNA, gene expression profiling by fluorescent probe hybridization of 20 genes, formalin-fixed paraffin-embedded tissue, algorithm reported as cell of origin
0005U	Oncology (prostate) gene expression profile by real-time RT-PCR of 3 genes (ERG, PCA3, and SPDEF), urine, algorithm reported as risk score
0018U	Oncology (thyroid), microRNA profiling by RT-PCR of 10 microRNA sequences, utilizing fine needle aspirate, algorithm reported as a positive or negative result for moderate to high risk of malignancy
0019U	Oncology, RNA, gene expression by whole transcriptome sequencing, formalin-fixed paraffin embedded tissue or fresh frozen tissue, predictive algorithm reported as potential targets for therapeutic agents
0021U	Oncology (prostate), detection of 8 autoantibodies (ARF 6, NKX3-1, 5'-UTR-BMI1, CEP 164, 3'-UTR-Ropporin, Desmocollin, AURKAIP-1, CSNK2A2), multiplexed immunoassay and flow cytometry serum, algorithm reported as risk score
0022U	Targeted genomic sequence analysis panel, non-small cell lung neoplasia, DNA and RNA analysis, 23 genes, interrogation for sequence variants and rearrangements, reported as presence or absence of variants and associated therapy(ies) to consider
0026U	Oncology (thyroid), DNA and mRNA of 112 genes, next-generation sequencing, fine needle aspirate of thyroid nodule, algorithmic analysis reported as a categorical result ("Positive, high probability of malignancy" or "Negative, low probability of malignancy")
0036U	Exome (i.e., somatic mutations), paired formalin-fixed paraffin-embedded tumor tissue and normal specimen, sequence analyses
0037U	Targeted genomic sequence analysis, solid organ neoplasm, DNA analysis of 324 genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability and tumor mutational burden
0045U	Oncology (breast ductal carcinoma in situ), mRNA, gene expression profiling by real-time RT-PCR of 12 genes (7 content and 5 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as recurrence score
0047U	Oncology (prostate), mRNA, gene expression profiling by real-time RT-PCR of 17 genes (12 content and 5 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a risk score
0048U	Oncology (solid organ neoplasia), DNA, targeted sequencing of protein-coding exons of 468 cancer-associated genes, including interrogation for somatic mutations and microsatellite instability, matched with normal specimens, utilizing formalin-fixed paraffin-embedded tumor tissue, report of clinically significant mutation(s)
0050U	Targeted genomic sequence analysis panel, acute myelogenous leukemia, DNA analysis, 194 genes, interrogation for sequence variants, copy number variants or rearrangements
0069U	Oncology (colorectal), microRNA, RT-PCR expression profiling of miR-31-3p, formalin-fixed paraffin-embedded tissue, algorithm reported as an expression score
0089U	Oncology (melanoma), gene expression profiling by RTqPCR PRAME and LINC00518, superficial collection using adhesive patch(es)
0090U	Oncology (cutaneous melanoma), mRNA gene expression profiling by RT-PCR of 23 genes (14 content and 9 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a categorical result (i.e., benign, intermediate, malignant)
0091U	Oncology (colorectal) screening, cell enumeration of circulating tumor cells, utilizing whole blood, algorithm, for the presence of adenoma or cancer, reported as a positive or negative result

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
0118U	Transplantation medicine, quantification of donor-derived cell-free DNA using whole genome next-generation sequencing, plasma, reported as percentage of donor-derived cell-free DNA in the total cell-free DNA
0120U	Oncology (B-cell lymphoma classification), mRNA, gene expression profiling by fluorescent probe hybridization of 58 genes (45 content and 13 housekeeping genes), formalin-fixed paraffin-embedded tissue, algorithm reported as likelihood for primary mediastinal B-cell lymphoma (PMBCL) and diffuse large B-cell lymphoma (DLBCL) with cell of origin subtyping in the latter
0153U	Oncology (breast), mRNA, gene expression profiling by next-generation sequencing of 101 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a triple negative breast cancer clinical subtype(s) with information on immune cell involvement
0171U	Targeted genomic sequence analysis panel, acute myeloid leukemia, myelodysplastic syndrome, and myeloproliferative neoplasms, DNA analysis, 23 genes, interrogation for sequence variants, rearrangements and minimal residual disease, reported as presence/absence
0179U	Oncology (non-small cell lung cancer), cell-free DNA, targeted sequence analysis of 23 genes (single nucleotide variations, insertions and deletions, fusions without prior knowledge of partner/breakpoint, copy number variations), with report of significant mutation(s)
0204U	Oncology (thyroid), mRNA, gene expression analysis of 593 genes (including BRAF, RAS, RET, PAX8, and NTRK) for sequence variants and rearrangements, utilizing fine needle aspirate, reported as detected or not detected
0211U	Oncology (pan-tumor), DNA and RNA by next-generation sequencing, utilizing formalin-fixed paraffin-embedded tissue, interpretative report for single nucleotide variants, copy number alterations, tumor mutational burden, and microsatellite instability, with therapy association
0239U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free DNA, analysis of 311 or more genes, interrogation for sequence variants, including substitutions, insertions, deletions, select rearrangements, and copy number variations
0242U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free circulating DNA analysis of 55-74 genes, interrogation for sequence variants, gene copy number amplifications, and gene rearrangements
0244U	Oncology (solid organ), DNA, comprehensive genomic profiling, 257 genes, interrogation for single-nucleotide variants, insertions/deletions, copy number alterations, gene rearrangements, tumor-mutational burden and microsatellite instability, utilizing formalin-fixed paraffin-embedded tumor tissue
0245U	Oncology (thyroid), mutation analysis of 10 genes and 37 RNA fusions and expression of 4 mRNA markers using next-generation sequencing, fine needle aspirate, report includes associated risk of malignancy expressed as a percentage
0250U	Oncology (solid organ neoplasm), targeted genomic sequence DNA analysis of 505 genes, interrogation for somatic alterations (SNVs [single nucleotide variant], small insertions and deletions, one amplification, and four translocations), microsatellite instability and tumor-mutation burden
0262U	Oncology (solid tumor), gene expression profiling by real-time RT-PCR of 7 gene pathways (ER, AR, PI3K, MAPK, HH, TGFB, Notch), formalin-fixed paraffin-embedded (FFPE), algorithm reported as gene pathway activity score
0285U	Oncology, response to radiation, cell-free DNA, quantitative branched chain DNA amplification, plasma, reported as a radiation toxicity score
0287U	Oncology (thyroid), DNA and mRNA, next-generation sequencing analysis of 112 genes, fine needle aspirate or formalin-fixed paraffin-embedded (FFPE) tissue, algorithmic prediction of cancer recurrence, reported as a categorical risk result (low, intermediate, high)

CPT Code	Description
0288U	Oncology (lung), mRNA, quantitative PCR analysis of 11 genes (BAG1, BRCA1, CDC6, CDK2AP1, ERBB3, FUT3, IL11, LCK, RND3, SH3BGR, WNT3A) and 3 reference genes (ESD, TBP, YAP1), formalin-fixed paraffin-embedded (FFPE) tumor tissue, algorithmic interpretation reported as a recurrence risk score
0296U	Oncology (oral and/or oropharyngeal cancer), gene expression profiling by RNA sequencing at least 20 molecular features (e.g., human and/or microbial mRNA), saliva, algorithm reported as positive or negative for signature associated with malignancy
0297U	Oncology (pan tumor), whole genome sequencing of paired malignant and normal DNA specimens, fresh or formalin-fixed paraffin-embedded (FFPE) tissue, blood or bone marrow, comparative sequence analyses and variant identification
0298U	Oncology (pan tumor), whole transcriptome sequencing of paired malignant and normal RNA specimens, fresh or formalin-fixed paraffin-embedded (FFPE) tissue, blood or bone marrow, comparative sequence analyses and expression level and chimeric transcript identification.
0299U	Oncology (pan tumor), whole genome optical genome mapping of paired malignant and normal DNA specimens, fresh frozen tissue, blood, or bone marrow, comparative structural variant identification
0300U	Oncology (pan tumor), whole genome sequencing and optical genome mapping of paired malignant and normal DNA specimens, fresh tissue, blood, or bone marrow, comparative sequence analyses and variant identification
0306U	Oncology (minimal residual disease [MRD]), next-generation targeted sequencing analysis, cell-free DNA, initial (baseline) assessment to determine a patient specific panel for future comparisons to evaluate for MRD
0307U	Oncology (minimal residual disease [MRD]), next-generation targeted sequencing analysis of a patient-specific panel, cell-free DNA, subsequent assessment with comparison to previously analyzed patient specimens to evaluate for MRD
0313U	Oncology (pancreas), DNA and mRNA next-generation sequencing analysis of 74 genes and analysis of CEA (CEACAM5) gene expression, pancreatic cyst fluid, algorithm reported as a categorical result (i.e., negative, low probability of neoplasia or positive, high probability of neoplasia)
0314U	Oncology (cutaneous melanoma), mRNA gene expression profiling by RT-PCR of 35 genes (32 content and 3 housekeeping), utilizing formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as a categorical result (ie, 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 (ie, Class 1, Class 2A, Class 2B)
0326U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free circulating DNA analysis of 83 or more genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability and tumor mutational burden
0329U	Oncology (neoplasia), exome and transcriptome sequence analysis for sequence variants, gene copy number amplifications and deletions, gene rearrangements, microsatellite instability and tumor mutational burden utilizing DNA and RNA from tumor with DNA from normal blood or saliva for subtraction, report of clinically significant mutation(s) with therapy associations
0331U	Oncology (hematolymphoid neoplasia), optical genome mapping for copy number alterations and gene rearrangements utilizing DNA from blood or bone marrow, report of clinically significant alterations
0332U	Oncology (pan-tumor), genetic profiling of 8 DNA-regulatory (epigenetic) markers by quantitative polymerase chain reaction (qPCR), whole blood, reported as a high or low probability of responding to immune checkpoint–inhibitor therapy
0333U	Oncology (liver), surveillance for hepatocellular carcinoma (HCC) in high-risk patients, analysis of methylation patterns on circulating cell-free DNA (cfDNA) plus measurement of serum of AFP/AFP-L3 and oncoprotein des-gamma-carboxy-prothrombin (DCP), algorithm reported as normal or abnormal result

CPT Code	Description
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), evaluation of 17 DNA biomarkers using droplet digital PCR (ddPCR), cell-free DNA, algorithm reported as a prognostic risk score for cancer recurrence
0362U	Oncology (papillary thyroid cancer), gene-expression profiling via targeted hybrid capture–enrichment RNA sequencing of 82 content genes and 10 housekeeping genes, fine needle aspirate or formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as one of three molecular subtypes
0363U	Oncology (urothelial), mRNA, gene expression profiling by real-time quantitative PCR of 5 genes (MDK, HOXA13, CDC2 [CDK1], IGFBP5, and CXCR2), utilizing urine, algorithm incorporates age, sex, smoking history, and macrohematuria frequency, reported as a risk score for having urothelial carcinoma
0364U	Oncology (hematolymphoid neoplasm), genomic sequence analysis using multiplex (PCR) and next-generation sequencing with algorithm, quantification of dominant clonal sequence(s), reported as presence or absence of minimal residual disease (MRD) with quantitation of disease burden, when appropriate
0368U	Oncology (colorectal cancer), evaluation for mutations of APC, BRAF, CTNNB1, KRAS, NRAS, PIK3CA, SMAD4, and TP53, and methylation markers (MYO1G, KCNQ5, C9ORF50, FLI1, CLIP4, ZNF132 and TWIST1), multiplex quantitative polymerase chain reaction (qPCR), circulating cell-free DNA (cfDNA), plasma, report of risk score for advanced adenoma or colorectal cancer
0379U	Targeted genomic sequence analysis panel, solid organ neoplasm, DNA (523 genes) and RNA (55 genes) by next-generation sequencing, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability, and tumor mutational burden
0388U	Oncology (non-small cell lung cancer), next-generation sequencing with identification of single nucleotide variants, copy number variants, insertions and deletions, and structural variants in 37 cancer-related genes, plasma, with report for alteration detection
0391U	Oncology (solid tumor), DNA and RNA by next-generation sequencing, utilizing formalin-fixed paraffin-embedded (FFPE) tissue, 437 genes, interpretive report for single nucleotide variants, splicesite variants, insertions/deletions, copy number alterations, gene fusions, tumor mutational burden, and microsatellite instability, with algorithm quantifying immunotherapy response score
0409U	Oncology (solid tumor), DNA (80 genes) and RNA (36 genes), by next-generation sequencing from plasma, including single nucleotide variants, insertions/deletions, copy number alterations, microsatellite instability, and fusions, report showing identified mutations with clinical actionability
81228	Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of genomic regions for copy number variants, comparative genomic hybridization [CGH] microarray analysis
81229	Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants, comparative genomic hybridization (CGH) microarray analysis
81277	Cytogenomic neoplasia (genome-wide) microarray analysis, interrogation of genomic regions for copy number and loss-of-heterozygosity variants for chromosomal abnormalities

CPT Code	Description
81425	Genome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis
81426	Genome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator genome (e.g., parents, siblings) (List separately in addition to code for primary procedure)
81427	Genome (e.g., unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained genome sequence (e.g., updated knowledge or unrelated condition/syndrome)
81445	Targeted genomic sequence analysis panel, solid organ neoplasm, 5-50 genes (eg, ALK, BRAF, CDKN2A, EGFR, ERBB2, KIT, KRAS, MET, NRAS, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed; DNA analysis or combined DNA and RNA analysis
81449	Targeted genomic sequence analysis panel, solid organ neoplasm, 5-50 genes (eg, ALK, BRAF, CDKN2A, EGFR, ERBB2, KIT, KRAS, MET, NRAS, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed; RNA analysis
81450	Targeted genomic sequence analysis panel, hematolymphoid neoplasm or disorder, 5-50 genes (eg, BRAF, CEBPA, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MLL, NOTCH1, NPM1, NRAS), interrogation for sequence variants, and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis
81451	Targeted genomic sequence analysis panel, hematolymphoid neoplasm or disorder, 5-50 genes (eg, BRAF, CEBPA, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MLL, NOTCH1, NPM1, NRAS), interrogation for sequence variants, and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; RNA analysis
81455	Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes (eg, ALK, BRAF, CDKN2A, CEBPA, DNMT3A, EGFR, ERBB2, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MET, MLL, NOTCH1, NPM1, NRAS, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; DNA analysis or combined DNA and RNA analysis
81456	Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm or disorder, 51 or greater genes (eg, ALK, BRAF, CDKN2A, CEBPA, DNMT3A, EGFR, ERBB2, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MET, MLL, NOTCH1, NPM1, NRAS, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed; RNA analysis
81479	Unlisted molecular pathology procedure
81504	Oncology (tissue of origin), microarray gene expression profiling of > 2000 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as tissue similarity scores
81518	Oncology (breast), mRNA, gene expression profiling by real-time RT-PCR of 11 genes (7 content and 4 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithms reported as percentage risk for metastatic recurrence and likelihood of benefit from extended endocrine therapy
81519	Oncology (breast), mRNA, gene expression profiling by real-time RT-PCR of 21 genes, utilizing formalin-fixed paraffin embedded tissue, algorithm reported as recurrence score
81520	Oncology (breast), mRNA gene expression profiling by hybrid capture of 58 genes (50 content and 8 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as a recurrence risk score
81521	Oncology (breast), mRNA, microarray gene expression profiling of 70 content genes and 465 housekeeping genes, utilizing fresh frozen or formalin-fixed paraffin-embedded tissue, algorithm reported as index related to risk of distant metastasis
81522	Oncology (breast), mRNA, gene expression profiling by RT-PCR of 12 genes (8 content and 4 housekeeping), utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as recurrence risk score

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

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

Diagnosis Code	Description
C90.10	Plasma cell leukemia not having achieved remission
C90.11	Plasma cell leukemia in remission
C90.12	Plasma cell leukemia in relapse
C91.00	Acute lymphoblastic leukemia not having achieved remission
C91.01	Acute lymphoblastic leukemia, in remission
C92.02	Acute myeloblastic leukemia, in relapse
C92.40	Acute promyelocytic leukemia, not having achieved remission
C92.41	Acute promyelocytic leukemia, in remission
C92.42	Acute promyelocytic leukemia, in relapse
C92.50	Acute myelomonocytic leukemia, not having achieved remission

Diagnosis Code	Description
C92.51	Acute myelomonocytic leukemia, in remission
C92.52	Acute myelomonocytic leukemia, in relapse
C92.60	Acute myeloid leukemia with 11q23-abnormality not having achieved remission
C92.61	Acute myeloid leukemia with 11q23-abnormality in remission
C92.62	Acute myeloid leukemia with 11q23-abnormality in relapse
C92.A0	Acute myeloid leukemia with multilineage dysplasia, not having achieved remission
C92.A1	Acute myeloid leukemia with multilineage dysplasia, in remission
C92.A2	Acute myeloid leukemia with multilineage dysplasia, in relapse
C95.90	Leukemia, unspecified not having achieved remission
C95.91	Leukemia, unspecified, in remission
C95.92	Leukemia, unspecified, in relapse

Description of Services

Technologies used for molecular profiling of 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 number of genes evaluated can range from a single gene to the whole exome or genome of a tumor. For the purposes of this policy, multi-gene analysis generally refers to a gene panel containing five or more genes, though some exceptions may apply as noted specifically in the policy. In some tests, expression patterns of defined genes are combined in a defined manner to provide an expression signature, a score, or a classifier for potential diagnosis and or prognosis of disease or to predict impact of intervention. Results of molecular profiling may assist individuals and healthcare providers with determining prognosis and selection of more effective and targeted cancer therapies (Chantrill et al., 2015).

Clinical Evidence

Breast Cancer

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

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

Harnan and colleagues (2019) conducted a systematic review and economic analysis to determine the efficacy and cost-effectiveness of the tumor profiling tests Oncotype DX, MammaPrint, Prosigna, EndoPredict, and immunohistochemistry 4 (IHC4). Studies included individuals with estrogen receptor-positive (ER+), HER2-, stage I, or II cancer with zero to three positive lymph nodes (LN+). The review included 153 articles on all five tests. In all five tests, the proportions of individuals who were lymph node-negative (LN0) getting endocrine monotherapy, 9% to 33%, were categorized as high-risk, according to the literature. For individuals who were LN+, three tests: Prosigna, EPClin, and IHC4 plus clinical factors (IHC4+C), categorized more (38% to 76%) individuals who were LN+ than those who were LN0 as high-risk according to the studies of endocrine

monotherapy. Oncotype DX categorized high-risk in the LN0 and LN+ subsets as equal. Oncotype DX classified more individuals as low-risk in LN+ when compared to other tests (57% in Oncotype DX vs. 4% to 28% in other tests), but worse 10-year distant recurrence/relapse-free survival/distant recurrence/ relapse-free interval outcomes (82% in Oncotype DX vs. 95% to 100% in other tests). An increase of 1% to a decrease of 23% was seen in UK studies and a reduction of 0% to 64% across European studies on the net change of individuals who were recommended chemotherapy or decision pre/posttest. Limitations included gaps in the literature, the risk of bias, and limited data relating to the ability of Oncotype DX and MammaPrint to predict benefits from chemotherapy. Additional long-term studies can show the impacts and changes in chemotherapy decisions for Oncotype DX and MammaPrint. The authors concluded that the evidence indicates that all the tests deliver prognostic data regarding the risk of relapse, although greater variation was seen in individuals with LN+ status than those with LN 0 status.

Oncotype Dx Breast

Oncotype Dx Breast (Genomic Health, Redwood City, CA) is a test that analyzes the expression of a panel of 21 genes within a tumor to determine a Recurrence Score (RS) which may correspond to a likelihood of BC recurrence within 10 years. The test was initially developed for women with early-stage invasive breast cancer with early-stage cancers that are LN0, and subsequently evidence was gathered on individuals with up to 3 ipsilateral nodes positive. These individuals are typically treated with anti-hormonal therapy, such as tamoxifen or aromatase inhibitors, and Oncotype Dx® can help determine if chemotherapy should be added to the treatment regimen (Evaluation of Genomic Applications in Practice and Prevention [EGAPP] Working Group, 2016).

In a 2022 systematic review and network meta-analysis, Davey et al. evaluated the Oncotype DX 21-gene RS for its ability to estimate locoregional recurrence (LRR) in ER+/HER2- BC. The review uncovered 16 articles together with 21,037 individuals. The average RS was 17.1, and the average follow-up was 66.4 months. Employing standard RS cut-offs, 49.7% of individuals had RS < 18 (3944/7935), 33.8% had RS 18–30 (2680/7935), and 16.5% had RS > 30 (1311/7935). Those with RS 18–30 and RS > 30 were significantly more likely to experience LRR than those with RS < 18. Using the TAILORx cut-off, 16.2% of individuals had RS < 11 (1974/12,208), 65.8% had RS 11–25 (8036/12,208), and 18.0% with RS > 30 (2198/12,208). LRR rates were comparable for individuals with RS 11–25; however, those with RS > 25 had a considerable risk of LRR versus those with RS < 11. The authors concluded that RS testing correctly estimates the risk of LRR for individuals being treated with the intent to cure early-stage ER+/HER2− BC. RS testing is a valid method to measure the risk of distant disease recurrence; however, awareness of its ability to predict LRR is significant to create effective locoregional control of the breast and axilla. Future prospective, randomized studies can confirm the predictive value of RS for estimating LRR and the application of RS to create suitable locoregional control in high-risk cases.

In 2021, Kalinsky et al. published the results of a prospective randomized clinical trial (RCT) to find the effect of chemotherapy on invasive disease-free survival in individuals with positive lymph-node disease and determine whether the RS based on the 21 gene assay(Oncotype Dx) influenced the outcome. A total of 5018 women with hormone-receptor-positive, HER2- BC, 1 to 3 positive axillary lymph nodes, and an RS of 25 or lower were randomly grouped into an endocrine therapy alone subset or a chemotherapy with endocrine (chemoendocrine) therapy subset. The intention-to-treat analysis included the participants who declined the assigned treatment, with 402 (16.2%) participants allocated to chemoendocrine therapy and 144 (5.8%) given to endocrine treatment. The trial did not show a clinically applicable or statistically significant rise in invasive disease-free survival with the addition of adjuvant chemotherapy to endocrine therapy in the global population with the same characteristics. For this trial, 67% of post-menopausal participants had no chemotherapy advantage. Dissimilarity, adjuvant chemotherapy led to a relative growth of 40% in invasive disease-free survival and a relative rise of 42% in distant relapse-free survival (RFS) among premenopausal women. Invasive disease-free survival at five years was 91.9% among post-menopausal women in the endocrine-only group and 91.3% in the chemoendocrine group, with no chemotherapy advantage. In the group of premenopausal women, invasive disease-free survival at five years was 89.0% with endocrine-only therapy and 93.9% with chemoendocrine treatment, with a comparable rise in distant relapse-free survival. The trial showed that between premenopausal women with 1 to 3 positive lymph nodes (N1) and an RS of 25 or less, individuals who received chemoendocrine therapy had a lengthier invasive disease-free survival and distant RFS than those who received endocrine-only treatment. In contrast, post-menopausal women with the same characteristics did not profit from adjuvant chemotherapy.

Hayes conducted a Molecular Test Assessment on Oncotype DX Breast RS for individuals with LN+ breast cancer to determine the capability of the test to estimate the risk of distant recurrence and the probable advantage of chemotherapy to guide effective treatment choices. Limited evidence suggests that the Oncotype DX test may evaluate the risk of distant recurrence and the benefit of chemotherapy for individuals with N1, ER+, and HER2- breast cancer. Consistent, supportive evidence of

prognostic ability was uncovered; however, the evidence supporting predictive ability of the test was not consistent. The Oncotype Dx test results may change an individual's treatment plan for those N1, ER+, and HER2- individuals, but evidence on health outcomes was not reported. Oncotype DX may improve outcomes for that population by lessening the total population of individuals treated with chemotherapy, thereby avoiding detrimental side effects. Insufficient evidence was found to support that Oncotype DX tests can estimate the risk of distant recurrent and the potential benefit of chemotherapy to guide effective treatment decisions for individuals with 4 to 9 positive lymph nodes (N2), ER+, HER2-, invasive BC. (Hayes, Oncotype DX Breast Recurrence Score [Genomic Health Inc.] for Lymph Node–Positive Patients, 2020, updated 2022).

In a 2020 Hayes Molecular Test Assessment, the Oncotype DX breast RS test was assessed as a prognostic indicator for 9-year distant BC recurrence and chemotherapy benefit for individuals diagnosed with ER+, HER2, and node-negative (N0) invasive BC. The evidence uncovered in the assessment suggests that the Oncotype DX test can estimate the risk of distant recurrence and the likely benefit of chemotherapy for guiding proper treatment decisions for individuals. However, added studies regarding the RS range are necessary for subgroup populations to predict the likelihood of chemotherapy benefits. Clinical utility studies are essential for reporting the health outcomes after RS-based treatment (Hayes, Oncotype DX Breast Recurrence Score for Lymph Node–Negative Patients [Genomic Health Inc.] 2020, updated 2022).

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

Wang et al. (2019) examined the value of Oncotype Dx when determining the prognosis in female individuals with BC and tumor stage 1-2 (tumor is 20-55mm), LN+ and no evidence of metastasis (T1-2 N1M0). The study reviewed 4059 cases to categorize them to prognostic stages IA and IIB and used data derived from the National Cancer Institute's limited use Surveillance, Epidemiology, and End Results (SEER) 18 registry databases, released in November 2017. Cases in the SEER database was linked to RS results from assays performed by Genomic Health. All cases with RS had negative HER2, and the authors selected female ER+ invasive ductal carcinoma (IDC) cases in T1-2N1M0 stage with Oncotype RS results diagnosed between 2004 and 2012. Individuals were categorized into low-risk (RS < 11), intermediate-risk (RS 11–25), and high-risk (RS > 25) groups. The median age of the individuals was 59 years. Of these participants, 2898 (71.4%) had stage T1 cancer, 1854 (45.7%) had stage N_{1mic} cancer, 743 (18.3%) had grade 3 cancer, and 3746 (92.3%) had positive PR status. They were stratified into the RS low-risk group (794, 19.6%), the RS intermediate-risk group (2667, 65.7%), and 598 (14.7%) were in the RS high-risk group. The high-risk group tended to have younger individuals, larger tumors, a higher percentage of grade 3 disease, negative PR, and more advanced cancer staging. They also had more frequent use of chemotherapy. Otherwise, the RS groups did not differ much in race, N stage, surgery, or radiation. In terms of pathological prognostic stages, there were 2781 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 breast cancer specific survival (BCSS) and overall survival (OS), were compared between RS and pathological staging groups using a variety of statistical analysis. The median follow-up period was 57 months. The results showed a statistically significant correlation ($p < .001$) between the RS groups and pathological stage results. In the low and high-risk RS groups, the BCSS and OS were similar between RS and pathological staging groups. In the intermediate RS group, however, survival rates differed significantly between RS staging and pathological staging. The survival rates were inversely correlated with the escalation of prognostic stages. Similar trends were seen in the high-risk group but were not statistically significant. In this retrospective study, RS was an independent prognosticator for BCSS, and with pathological stage for OS. The authors concluded that Oncotype Dx could complement the prognostic staging system in N+ individuals.

Altman et al. (2018) examined the utility of Oncotype Dx in women with ER+ and HER2+ BC. Individuals were identified from the Surveillance and Epidemiology End Results program database that met criteria and were stratified using the TAILORx RS cutoffs. Of individualist met the criteria in the database, only 5% had Oncotype Dx testing. In that cohort, 17% were high-risk, 49% were intermediate, and 34% were low-risk. Chemotherapy use in those not tested was 66%. In those that were tested, the use of chemotherapy trended according to recurrence risk score, suggesting that the score was used in treatment decisions. In high-risk individuals, 67% had chemotherapy, 30% of intermediate risk, and 19% of low-risk individuals. However, this study does not provide information on the clinical utility of Oncotype Dx in women with HER2 positive BC since clinical outcomes were not captured.

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

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

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

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

MammaPrint (also referred to as the "Amsterdam Signature" or "70-Gene Signature")

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

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

In NBREaST II, a prospective, neoadjuvant study, Göker et al. (2022) measured the treatment response and 5-year survival outcome in the molecular subgroups by combining the MammaPrint and BluePrint. MammaPrint and BluePrint were carried out on 256 individual core needle biopsies (CNB) to quantify chemosensitivity or endocrine sensitivity in the molecular subgroups.

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

Crozier et al. (2022) prospectively collected 139 matched CNB and surgical resection (SR) specimens from women with established early-stage breast cancer (EBC) registered in the ongoing FLEX study (NCT03053193). The goal was to decide the concordance of MammaPrint and BluePrint results among CNB and SR to safeguard reliable prognostic information that can be apprehended from a CNB. FLEX is an ongoing, multi-institutional prospective study of individuals with Stage I–III EBC. Overall, 121 individuals from the FLEX study database with diagnostic MammaPrint and BluePrint results with matched CNB and SR specimens were involved in the study. In total, 50 individuals had High-Risk CNB and SR specimens, and 60 had Low-Risk CNB and SR specimens, resulting in 90.9% total agreement (κ = 0.817), 95.2% negative predictive value (NPV), and 86.2% PPV. The authors concluded the concordance of BluePrint between CNB and SR to be 98.3%. For more than 97% of individuals in this study, treatment decisions and probable outcomes are precise and consistent based on MammaPrint testing of the CNB. According to the authors, this analysis is the most extensive powered study using prospective real-world numbers to assess the concordance of a genomic assay on matched CNB and SR samples. The limitation of the study is the lack of data maturity, as individual follow-up data is not available to correlate outcomes with MammaPrint and BluePrint results from the CNB and SR samples. The authors concluded that the high concordance rates of MammaPrint and BluePrint result among paired samples strongly support the value of these assays to acquire reliable prognostic data on core biopsy tissue, which can guide prompt and proper treatment decisions.

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

Soliman et al. (2020) conducted a prospective case-only trial (IMPACt) enrolling 452 individuals with state I-II, HR+, HER2- BC to evaluate the variation in treatment decision and physician assurance based on the 70-gene ROR signature and the 80-gene molecular subtype signature (80-GS, BluePrint) in early-stage BC. According to clinical risk assessment via the MINDACT criteria, 63.4% (n = 227/358) of individuals were categorized as low-risk, and 36.6% (n = 131/358) of individuals were classed as high-risk of distant recurrence. For individuals with clinically minimal risk, 77.5% (176/227) were suggested not to have chemotherapy by their doctors, while 62.6% (82/131) of individuals with clinically high-risk were recommended treatment plans that involved chemotherapy. The 70-GS categorized 62.5% (n = 224/358) of individuals as low-risk and 37.5% (n = 134/358) as high-risk. Following the 70-GS results, doctors elected to change the chemotherapy treatment (CT) recommendation in 24.0% (n = 86/358) of all cases. After-70-GS treatment plans were, 88.5% (n = 317/358) agreeing with 70-GS results, 83.6% (n =

112/134) for CT in 70-GS high-risk individuals, 91.5% (n = 205/224) for no CT in 70-GS low-risk individuals. The IMPACt trial displayed that the majority (88.5%) of treatment plans were accordant with 70-GS results, showing that doctors make treatment decisions based on the 70-GS result in clinical practice. Physicians also described a rise in confidence in 72.2% of their suggested treatment plans after receiving the 70-GS results. A limitation of the study is that individuals were enrolled both before and after the MINDACT trial results were published, which may have impacted physicians' decisions for treatment. The authors concluded that these results propose that doctors feel the proper individuals (high-risk) are being presented chemotherapy, and they feel confident sparing 70-GS individuals with low-risk from the high clinical and financial burden of chemotherapy. The trial shows that doctors can avoid overtreatment and the adverse effects of chemotherapy treatments for individuals who are not likely to obtain meaningful clinical benefits.

In 2019, Wuerstlein and colleagues reported on the prospective, observational multicenter WSG-PRIMe study designed to gauge the effect of MammaPrint and BluePrint on adjuvant chemotherapy treatment decisions in individuals with early stage breast cancer specifically to show an overall switch percentage of at least 15% regarding chemotherapy. These individuals had MammaPrint considered as part of their standard clinical procedure. Included in the study were 452 individuals who were HR+ and Her2-. Physicians supplied preliminary treatment recommendations created on clinicopathological factors. Post-test therapy recommendations and actual therapy were documented after prospective risk classification by MammaPrint/BluePrint was revealed. MammaPrint allocated 63.5% of participants to the low-risk group and 36.5% to the high-risk group. In (125/430, 29.1%) of individuals (95% CI 24.8–33.6%), the recommendation transformed from chemotherapy to no chemotherapy or vice versa. Chemotherapy had been recommended to 164 individuals (38.1%) pre-test. In 60/164 (36.1%) of the individuals who were recommended chemotherapy, the therapy recommendation converted to the omission of chemotherapy post-test; most (59/60, 98%) of these changes happened in low-risk individuals, according to MammaPrint. On the contrary, deletion of chemotherapy been suggested to 266 individuals (61.9%) pre-test; in 65/266 (24.4%) cases, recommendations converted to chemotherapy post-test; most (64/65, 98.4%) of these modifications happened in MammaPrint high-risk individuals. The physician observance of MammaPrint risk calculation was 92.3% for low-risk and 94.3% for high-risk scores. Three-fourths (n = 59/79, 74.7%) of physicians initially recommending chemotherapy converted to no chemotherapy subsequent low-risk MammaPrint results (72.7% in pN0, 77.1% in pN1); on the contrary, nearly nine tenths (n = 64/72, 88.9%) of physicians originally recommending chemotherapy omission from treatment converted to chemotherapy recommendations following high-risk MammaPrint outcomes (88.1% in pN0, 92.3% in pN1). The authors concluded that the WSG-PRIMe study proves that the use of the gene expression profiles, MammaPrint and BluePrint, has a powerful influence on adjuvant therapy recommendations. The results showed that physicians changed their ultimate recommendation for systemic therapy in 29.1% of cases subsequent MammaPrint testing. The study verified that there is improved, genomically determined individualization of treatment regimens that can lead to a reduced risk of over- or 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 breast cancer.

The randomized, phase 3 clinical MINDACT trial included 6693 women with early-stage BC with the primary goal to assess whether, among individuals with high-risk clinical features and a low-risk gene-expression profile who did not receive chemotherapy, the lower boundary of the 95% confidence interval for the rate of 5-year survival without distant metastasis would be 92% (i.e., the non-inferiority boundary) or higher. Women at low clinical and genomic risk did not receive chemotherapy, while those at high clinical and genomic risk did receive such therapy. For individuals with discordant risk results, either the genomic risk or the clinical risk was used to determine the use of chemotherapy. The researchers found that among women with early-stage BC who were at high clinical risk and low genomic risk for recurrence, the receipt of no chemotherapy on the basis of the 70-gene signature led to a 5-year rate of survival without distant metastasis that was 1.5 percentage points lower than the rate with chemotherapy. Given these findings, approximately 46% of women with BC who are at high clinical risk might not require chemotherapy (Cardoso et al., 2016).

EndoPredict

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

In a Hayes Molecular Test Assessment, the clinical validity, clinical utility, and analytical validity of EndoPredict were evaluated. The assessment uncovered limited but positive evidence suggesting EPclin may estimate the 10-year risk of distant recurrence (DR) for individuals with ER+, HER2-, N0, and early-stage BC; however, it remains unclear if the test can prospectively

distinguish low-risk individuals from others or if the test is equally applicable for premenopausal women. There is limited evidence suggesting EndoPredict may estimate the 10-year risk of DR for individuals with ER+, HER2-, N1, and early-stage BC, and conflicting results to determine if the EPclin low-risk group was genuinely associated with a low-risk of DR in this population. For the EndoPredict test to estimate the likelihood of DR 5 to 15 years from diagnosis and the absolute benefit of chemotherapy at ten years for individuals with ER+, HER2-, N0/N1, early-stage cancer, there are limited studies and data to support the test results. No prospectively designed studies were found regarding the clinical validity of EndoPredict; additional studies are necessary to examine diverse demographics and possibly improve health outcomes resulting from the EndoPredict test (Hayes, EndoPredict [Myriad Genetics Laboratories Inc.], 2020, updated 2022).

In the prospective, translational, randomized phase II ABCSG-34 trial directed by Dubsky et al. (2020), the ability of EndoPredict to predict response to NCT or NET was assessed. HR+, HER2- samples were gathered from participants, and EndoPredict testing was completed to produce a 12-gene MS. Participants were randomized to have either NCT or NET based on menopausal status, HR expression, grade, and Ki67. The outcome measured was calculated via the residual cancer burden (RCB). Overall, 134 individuals who were HR+ received NCT, and 83 received NET as their preoperative SoC treatment. Out of 134 participants who received NCT, nine had low-risk disease according to the 12- gene MS, and 125 had high-risk disease. The 12-gene MS exhibited strong sensitivity for NCT (100%, 95% CI 89.4%e100%), even though specificity was small (8.9%, 95% CI 4.2%e16.2%). Of the participants treated with NET, 44 out of 83 had low-risk disease, and 39 had high-risk disease. According to the authors, this is the first study that prospectively proves the predictive probability of the 12-gene MS for its response to NET. The RCB 0-I outcome for individuals with NET in the low-risk 12-gene MS subset was 27% in contrast to 7.7% in individuals with high-risk MS. The data presented in this trial shows that the 12-gene MS offers supplementary predictive data beyond the traditional clinicopathologic factors used to evaluate risk and is a valuable instrument preoperatively.

Sestak et al. (2020) retrospectively investigated a cohort of individuals with invasive lobular carcinoma (ILC) from previously conducted clinical trials (ABCSG-6, ABCSG-8, TransATAC). The main objective of the study was to determine the prognostic value of EPclin, either alone or in combination with clinical parameters, for DR in women with ILC. All participants had received 5 years of endocrine treatment as the only adjuvant therapy. Information compiled from the 3 clinical trials included data from 2630 postmenopausal women with ER+, HER2- BC. As part of that group, 470 (19.5%) had ILC, 1944 (80.5%) had IDC and 216 (8.2%) had another histological subtype. The researchers found that in this study, EPclin was highly prognostic in women with ILC [HR = 3.32 (2.54–4.34), $p < 0.0001$] and provided better prognostic value than the Clinical Treatment Score [CTS; HR = 2.17 (1.73–2.72)]. Further, they found that EPclin was prognostic in women with IDC (n = 1,944) overall [HR = 2.36 (2.11–2.65), $p < 0.0001$], though not to the level of ILC. They concluded that EPclin provided substantial prognostic information and risk stratification for women with ILC (included in Hayes, EndoPredict [Myriad Genetics Laboratories Inc.]).

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

Breast Cancer Index (BCI)

BCI (BioTheranostics, San Diego, CA) is a prognostic biomarker assay that analyzes the combination of two indices: HOXB13:IL17BR and five cell cycle-associate gene index (BUB1B, CENPA, NEK2, RACGAP1, RRM2). The test is performed on a FFPE tissue block.

In a 2021 publication, Noordhoek et al. documented the results of their prospective-retrospective translational study of individuals that had been part of the IDEAL trial. The IDEAL trial was a prospective phase III study randomizing 1824 individuals with HR+ BC to receive either 2.5 or 5 additional years of letrozole after having completed an initial 5 years of adjuvant endocrine therapy. In this study, the predictive component of the BCI assay, the HOXB13/IL17BR ratio (H/I) was specifically

examined. The main goal of the study was to determine whether BCI (H/I) can predict extended endocrine benefit. All IDEAL participants that had available tumor specimens were eligible and BCI testing was done with blinding to clinical outcome. The BCI test was performed on primary tumor material from 908 IDEAL participants, with primary endpoint being recurrence free interval. The authors found that BCI (H/I) was predictive for benefit from EET in the overall cohort for participants with both high and low scores. In addition, the test was able to predict benefit from 2.5 versus 5 years of EET. The researchers concluded that these results expand the clinical utility of BCI testing to a larger group of individuals and for use as a predictive endocrine response biomarker in individuals with early-stage HR+ BC.

In a 2020 Molecular Test Assessment, Hayes appraised the BCI test's analytical validity, clinical validity, and clinical utility for individuals diagnosed with HR+, N0, early-stage, invasive BC. The assessment demonstrated that there is insufficient evidence to support the BCI test in the prediction of likelihood of benefit from extended endocrine therapy (EET) (> 5 years), or to estimate the risk of late (post-five years from diagnosis) and cumulative distant recurrence risk over ten years for individuals with HR+, N0, invasive BC who were treated with five years of primary adjuvant endocrine therapy. BCI may have predictive ability for both late and cumulative distant recurrence risk, but more studies including large, prospective, randomized trials examining diverse populations and health outcomes related to use of the BCI test are required (Hayes, Breast Cancer Index [BioTheranostics Inc.] for Lymph Node–Negative Patients, 2020, updated 2022).

Hayes also conducted a Molecular Test Assessment to evaluate the BCI test's analytical validity, clinical validity, and clinical utility for individuals diagnosed with HR+, N1 invasive BC who had been treated with five years of primary adjuvant chemoendocrine therapy. The assessment exposed insufficient evidence to support that the BCI test could predict the likelihood of benefit from EET (> 5 years) and estimate the risk of late (post-five years from diagnosis) and cumulative distant recurrence risk over ten years for individuals with HR+, with N1 invasive BC. They were treated with five years of primary adjuvant chemoendocrine therapy. More extensive studies that support all claims made by the laboratory are necessary to demonstrate if BCI tests can lead to positive health outcomes (Hayes, Breast Cancer Index [BioTheranostics Inc.] for Lymph Node–Positive (1-3) Patients, 2020, updated 2022).

A study by Bartlett et al. (2019) examined the use of BCI to predict benefit from EET for individuals that were randomized in a previous trial called the Adjuvant Tamoxifen-To Offer More? (aTTom) trial. In the original aTTom trial, there were 6956 individuals with, 583 HR+ N+ individuals meeting the inclusion criteria for this analysis. The primary study objective was to determine whether the BCI (H/I) status (High versus Low) was predictive of the benefit of 10 versus 5 years for tamoxifen. Among the 292 individuals in 5-year arm, 92 had a recurrence-free interval (RFI) event and there were 77 RFI events in the 291 in the 10-year arm. Of the total 583 individuals, 49% were classified as BCI (H/I)-High and had a significant benefit from 10 versus 5 years of EET as the ROR was 27% and 37% for individuals with 10- and 5-year EET, respectively. BCI (H/I)-Low individuals did not show a benefit from an additional 5 years of EET (HR: 1.07; 95% CI 0.69-1.65; -0.2% absolute risk reduction; p = 0.768). The researchers concluded that this data supports the level 1B evidence for BCI for EET (Included in Hayes, Breast Cancer Index [BioTheranostics Inc.] for Lymph Node–Positive [1-3]).

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

Zhang et al. (2017) examined the predictive ability of BCI results, when integrated with tumor size and grade Breast Cancer Index Model (BCIN), to accurately identify outcomes in a well annotated retrospective series of N+ individuals. A total of 402 participants with N1 who were treated with adjuvant endocrine therapy with or without chemotherapy using a prespecified model. The primary endpoint was time to DR. BCIN classified 20% of participants as low-risk with a 15-year DR rate of 1.3% and 321 individuals as high-risk with a DR risk of 29%. When the results were unblinded and compared to participant outcome, BCI

alone was significantly prognostic (p < .0001), and when tumor size was added the prognostic ability was even further improved (p < .0003) but only incrementally with adding tumor grade (p = .01). Overall, BCIN identified 20% of individuals who were N+ with a limited ROR over 15 years that could avoid extended endocrine treatment. Further studies on combined genomic and clinical algorithmic predictions are needed on N+ individuals (included in Hayes, Breast Cancer Index (BioTheranostics Inc.) for Lymph Node–Positive (1-3) Patients).

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

Zhang et al. (2013) examined the role of BCI within the Stockholm TAM cohort and a multi-institutional cohort. The Stockholm TAM (n = 317) was a randomized prospective trial comparing adjuvant tamoxifen versus control, conducted from 1976 through 1990, and stored formalin FFPE blocks from hormone positive, N0 individuals were used in this study. The multi-institutional cohort (n = 358) was made up of ER+, N0 BC individuals identified from University of Pittsburgh Medical Center and Massachusetts General Hospital who were diagnosed between 1990 and 2000 with FFPE tumor blocks available. Pathologists scored the historical samples using current standard criteria. The stratification scores determined by BCI were compared against current pathological grading and reviewed against available outcome data. For both cohorts, the BCI score was the most significant prognostic factor for distant recurrence rate for 0-5 years and for 5-10 years. The authors concluded that BCI could help inform therapeutic decision making for not only distant recurrence but for extended therapy decisions beyond 5 years (included in Hayes Breast Cancer Index (BioTheranostics Inc.) for Lymph Node–Negative Patients).

Other Breast Cancer Profiling Assays

Gene expression profiling assays for BC treatment other than those previously described, including but not limited to BluePrint, Breast Cancer Gene Expression Ratio (also known as Theros H/I), DCISionRT, Oncotype DX DCIS, the 41-gene signature assay, and the 76-gene "Rotterdam signature" assay are unproven and not medically necessary due to insufficient evidence of efficacy.

DCISionRT

DCISionRT (Prelude Corporation, Laguna Hills, CA) is a risk assessment test for patients with ductal carcinoma in situ which is designed to quantify the individual's10-year risk of DCIS recurrence and determine whether radiation therapy would be of benefit. DCISionRT assesses 7 genes along with other clinical risk factors to provide a DCISionRT score ranging from 0 to 10. Scores 0-3 are considered low risk and scores 3-10 are considered elevated risk.

Hayes published a Molecular Test Assessment evaluating the use of the DCISionRT test to aid in decision-making for breast-conserving surgery (BSC) alone or BCS plus RT by supplying an individual with the DS in addition to their 10-year risk of invasive recurrence and total recurrence subsequent BCS alone or BCS plus RT. The assessment concluded that there is inadequate evidence to support the usage of the DCISionRT test due to the absence of studies. Additionally, no peer-reviewed articles were uncovered that described the test's analytical validity and improvement for individual results because of DCISionRT testing (Hayes, DCISionRT [Prelude Corp.], 2022).

Shah et al. (2021) documented the results of the PREDICT study; a prospective, multi-institutional observational registry designed to evaluate the clinical utility of testing with DCISionRT on clinical recommendations regarding RT for individuals who had undergone BCS for a diagnosis of DCIS. The study included 539 women over the age of 25 who had been treated with BCS for unilateral DCIS. All women were eligible to receive RT and received DCISionRT testing as part of the study. Prior to testing, 69% of all participants had received a recommendation of treatment with RT. After testing with DCISionRT, 46% of those that had previously received recommendation for RT had a change in recommendation to not receive RT. Conversely, for women who were not initially recommended to undergo RT, 35% had a change in recommendation for treatment to include RT. In summary, a change in RT treatment plan was made for 42% of women in the study, with a net reduction in overall RT recommendation of 20%. The elevated DS had the strongest association with an RT recommendation (odds ratio 43.4) compared to other factors such as age, grade, size, and margin status. The authors concluded that DCISionRT testing made a significant difference, including an absolute net decrease in RT recommendations overall in women with DCIS who had

undergone BCS, and was the factor most strongly associated with RT recommendations compared with traditional measures used to drive treatment decisions. The authors also noted limitations to the study. One such limitation was the lack of patient or physician-reported outcomes regarding satisfaction or quality (pending at time of publication). In addition, data on recommendations for RT were only based on two points in time; pre-testing and post-testing. Finally, there is a lack of long-term clinical outcomes and data on subsequent resource utilization related to treatment decisions. These items are planned for further evaluation and assessment when longer follow-up data become available. This study was included in the 2022 Hayes DCISionRT (Prelude Corp.) Molecular Test Assessment.

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

Bremer et al. (2018) reported on the development and cross-validation of DCISionRT (PreludeDX). DCISionRT is a risk assessment test that uses a combination of molecular and clinicopathologic factors to generate a biological signature which calculates an individualized DS. The relationship between DS and 10-year risk of invasive breast cancer (IBC) or any ipsilateral breast event (IBE) was assessed in this study. Benefit of radiotherapy was evaluated as a function of DS, by risk group. Study population included 526 individuals diagnosed with a primary DCIS and treated with BCS, with or without radiotherapy, from two study sites. The study used archived tissue samples. Treatments for the study participants were neither randomized nor strictly rules based. The researchers found a significant association with IBC and IBE risk. In study participants who had been treated without RT, the DS identified a low group with 10-year IBC risk of 4% (7% IBE) and an elevated risk group with IBC risk of 15% (23% IBE). The elevated risk group received significant RT benefit in analysis of DS and RT by group. In a clinicopathologically low-risk-subset, 42% of participants were reclassified into the elevated risk group by using DS. When an interaction analysis of DS and RT was performed, participants whose DS was elevated had significant RT benefit over baseline. The authors concluded that DS appeared to be prognostic for risk and for predicting benefit of RT for individuals with DCIS status-post BCS and was able to identify a clinically meaningful low-risk group and an elevated 10-year risk group, whose members may receive significant benefit from RT over baseline. However, further clinical validations are required to provide more evidence on the capabilities, both prognostic and predictive, of the biological signature and DS.

Oncotype Dx DCIS

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

Hayes conducted a Molecular Test Assessment evaluating the Oncotype DX Breast DCIS Score test to predict the risk of 10-year local or invasive cancer recurrence and create a baseline consideration of benefit from radiation therapy in women diagnosed with DCIS. The assessment revealed favorable but inadequate evidence supporting the usage of the Oncotype DX Breast DCIS Score. Studies with an added diverse population are needed to support the premise that the DCIS Score offers prediction for 10-year local recurrence and creates a baseline consideration of absolute benefit from radiation. Additionally,

appraisal of outcomes when test results are used for risk assessment and medical management selection are needed (Hayes, Oncotype DX Breast DCIS Score [Genomic Health Inc.] 2018, updated 2022).

In a review of the literature regarding prognosis and treatment of DCIS, Gorringe et al. (2017) discussed the available studies and value of the 12 gene expression assay. The two primary studies to date both demonstrated that the test had some prognostic value, but the low-risk group still had a chance of recurrence over 10 years of 10-13%, and there was no difference in outcome between intermediate and high-risk groups. The authors noted that on 50% of individuals in each study the clinicopathological data was incomplete, which could have been important to understanding outcome. In addition, the cases were taken from a prolonged timeframe, nearly a decade, in which advances in surgical and other treatments vastly improved and could have confounded the results.

BluePrint

BluePrint (Agendia, Amsterdam, The Netherlands), a complementary test to MammaPrint, measures the expression of 80 genes to classify the tumor as one of three subtypes. The tumor subtype is used to predict future behavior of the cancer, long term prognosis and response to systemic therapy.

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

Clinical Practice Guidelines

American Society of Clinical Oncology (ASCO)

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

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

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

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

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

In their 2016 evidence-based guideline on the use of biomarkers to guide decisions on adjuvant systemic therapy for women with early-stage invasive BC, ASCO (Harris et al., 2016a; Harris et al., 2016b) found sufficient evidence of clinical utility for the biomarker assays Onco*type* DX, EndoPredict, PAM50, BCI, and urokinase plasminogen activator and plasminogen activator inhibitor type 1 in specific subgroups of BC. No biomarker except for estrogen receptor, progesterone receptor, and HER2 was found to guide choices of specific treatment regimens. Treatment decisions should also consider disease stage, comorbidities, and personal preferences.

For this guideline, the ASCO panel considered only prognosis and prediction for individuals with newly diagnosed, nonmetastatic, primary BC. Prognosis was defined as an indication of future risk of an event (recurrence, distant metastases, or death) independent of the effect of prior or anticipated therapy. Prediction was defined as the ability of a specific biomarker to indicate the likelihood of benefit from a particular therapy or a class of agent (e.g., endocrine, biologic, or chemotherapy).

ASCO considers the conclusions on prognostic and predictive biomarkers in early-stage invasive BC to be limited by the lack of prospective confirmatory studies; findings of insufficient clinical utility; and, in many cases, a lack of data on clinical validity and reproducibility of assays. The expert panel awaits the completion and publication of several randomized trials to establish the clinical utility of some of these assays. Extensive research is needed to validate some of the biomarker candidates described and to identify promising new biomarkers. ASCO believes that cancer clinical trials are vital to inform medical decisions and improve cancer care and that all individuals should have the opportunity to participate.

American College of Radiology (ACR)

Kaufman et al. (2014) reports on the ACR expert panel appropriateness criteria review of Oncotype Dx DCIS and reports that their review of the literature found that the 12 gene assay was of minimal benefit in predicting who may benefit, or not, from radiotherapy. They conclude that further validation is necessary before routine use of this genetic profile can be used for clinical decisions.

European Society for Medical Oncology (ESMO)

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

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

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

National Comprehensive Cancer Network (NCCN) Clinical Guidelines

NCCN breast cancer guidelines indicate that “gene expression assays provide prognostic and therapy-predictive information that complements tumor (T), node (N), distant metastasis (M) and biomarker information. Use of these assays is not required for

staging." The 21-gene assay (Oncotype Dx) is preferred by the NCCN Breast Cancer Panel since it has been clinically validated for the prognosis and prediction of chemotherapy benefit. While other gene expression assays can provide prognostic information, they do not necessarily predict chemotherapy benefit. NCCN notes that the Breast Cancer Index (BCI) test is predictive of benefit of extended adjuvant endocrine therapy (NCCN Breast Cancer, v4.2022).

National Institute for Health and Care Excellence (NICE)

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

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

Thyroid Cancer/Indeterminate Thyroid Nodules

In 2022, Lee et al. conducted a systematic review and meta-analysis to appraise the diagnostic performance of the second-generation molecular tests in diagnosing thyroid nodules with indeterminate fine-needle aspiration biopsy results. Contained within the examination were 15 studies: 7 Afirma Genomic Sequencing Classifier (GSC), 6 ThyroSeq v3, and 2 ThyGeNext. Studies on ThyGeNext were excluded from the meta-analysis due to their small sample sizes. Pooled data for GSC studies on 472 thyroid nodules displayed a sensitivity of 96.6 (95% confidence interval: 89.7–98.9%), specificity of 52.9% (23.4–80.5%), PPV of 63% (51–74%), and NPV of 96% (94–98%). Pooled data for ThyroSeq studies on 530 thyroid nodules presented a sensitivity of 95.1% (91.1–97.4%), specificity of 49.6% (29.3–70.1%), PPV of 70% (55–83%), and NPV of 92% (86–97%). There was not a statistically significant variance in the diagnostic performances of GSC and Thyroseq (p-values for sensitivity = 0.89, specificity = 0.82, PPV = 0.43, NPV = 0.17). Limitations to the study include the small number of studies contained within the meta-analysis, no long-term analysis of the utility of the tests, and only two studies evaluated on ThyGeNext. The authors concluded from the review that high sensitivity and NPV in GSC and ThyroSeq V3 may help rule out malignancy amid thyroid nodules with indeterminate cytology results. There was no difference in diagnostic performances between the two molecular tests displaying that either test is suitable for the malignancy of thyroid nodules. Studies by Livhits et al. (2021) and Endo et al. (2019), previously discussed in this policy, were included in this systematic review by Lee et al.

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

In 2022, Babazadeh et al. reported on the clinical utility of Afirma XA testing during two years of clinical use. Afirma XA became available in 2018 and assesses 593 genes, including 905 potential variants and 235 fusions. Afirma XA was performed on 136

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

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

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

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

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

Angell et al. (2019) reported on their clinical and analytical validation of the Afirma® XA, which uses whole transcriptome RNA-sequencing to detect gene variations and fusions from a panel of over 500 genes in thyroid fine needle aspiration (FNA) samples. From the same sample, DNA and RNA were purified using 943 blinded FNAs and multiple methodologies were used for comparison, including whole-transcriptome RNA-seq, targeted RNA-seq, and targeted DNA-seq. To define performance for fusions between whole transcriptome RNA-seq and targeted RNA-seq, 695 additional blinded FNAs were used. Of variants detected in DNA at 5 or 20% variant allele frequency, 74 and 88% were also detected by XA, respectively, and XA variant detection was 89% compared to another RNA-based detection method. Analytical validation studies showed high intra-plate

reproducibility (89%-94%), inter-plate reproducibility (86–91%), and inter-lab accuracy (90%). Multiple variants and fusions formerly described across the spectrum of thyroid cancers were identified by XA, some of which have approved or investigational targeted therapies. The sensitivity of XA as a standalone test was 49% in 190 Bethesda III/IV nodules. Limitations of measuring variants in expressed RNA were identified, including the fact that some variants and fusions that were identified by an alternative method were not identified by XA; the researchers were not able to determine the reason for the difference, nor which tests was "correct". The authors concluded that the data from this study supports the clinical and analytical validity of XA for GSC suspicious or for Bethesda V/VI nodules. The asserted that XA may also enhance genomic insight when the Afirma GSC is used first for Bethesda III/IV nodules as a rule-out test and results are GSC suspicious and may ultimately help to inform personalized clinical decision-making in individuals with thyroid nodules and thyroid cancer. Further studies addressing the clinical utility of this test are needed.

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

In a meta-analysis of the gene expression classifier (GEC) for the diagnosis of indeterminate thyroid nodules, Santhanam et al. (2016) evaluated 7 out of 58 potential studies. The reference standard for determination of benign or malignant nodules was the histopathology of the thyroidectomy specimen. A QUADAS-2 report for all studies included in the final analysis was tabulated for risk of bias and applicability. The pooled sensitivity of the GEC for malignant histology was 95.7% (95% CI 92.2-97.9, I (2) value 45.4%, $p = 0.09$), and the pooled specificity was 30.5% (95% CI 26.0-35.3, I (2) value 92.1%, $p < 0.01$). Overall, the diagnostic odds ratio was 7.9 (95% CI 4.1-15.1). Although the meta-analysis revealed a high pooled sensitivity and low specificity for the Afirma GEC, individuals with a benign GEC were not followed long enough to ascertain the actual false-negative rates of the index test.

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

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

Pagan et al. (2016) investigated the prevalence of genetic alterations in diverse subtypes of thyroid nodules beyond papillary thyroid carcinomas (PTC) in 851 variants and 133 fusions in 524 genes. After adding a cohort of tissue samples, the authors found 38/76 (50%) of histopathology malignant samples and 15/75 (20%) of benign samples to harbor a genetic alteration. In a

direct comparison of the same FNA also tested by an RNA-based gene expression classifier (GEC), the sensitivity of genetic alterations alone was 42%, compared to the 91% sensitivity achieved by the GEC. The specificity based only on genetic alterations was 84%, compared to 77% specificity with the GEC. Due to the finding that variants are also found in benign nodules, the authors conclude that testing only GEC suspicious nodules may be helpful in avoiding false positives and altering the extent of treatment when selected mutations are found. Sipos et al. (2016) retrospectively evaluated the long-term follow-up of patients with a 'benign' Afirma GEC to determine impact on management compared to published data. During 36 months of follow-up, 17 of 98 patients (17.3%) had thyroid surgery; the majority (88%) being performed within 2 years. According to the authors, this represents a reduction in thyroid surgeries compared to patients that did not have a GEC performed on suspicious lesions. Limitations of this study are small patient population and non-randomization of patients.

MicroRNAs (miRNA) are small noncoding RNAs that regulate gene expression. Research has demonstrated that a number of miRNAs are differentially expressed between benign and malignant thyroid nodules which have led to the development of miRNA based diagnostic lab tests, and in some cases, labs may offer miRNA testing in conjunction with gene variant and expression analysis. Wylie et al. (2016) conducted a study examining genetic variant and miRNA analysis on archived pathology samples from the University of Michigan. The samples consisted of an initial set of 235 aspirates representing 118 nodules with benign cytology, including 13 with surgical outcome (12 benign, 1 malignant), 73 with malignant cytology, including 51 with surgical outcome (1 benign, 50 malignant), and 44 with indeterminate cytology, all with available surgical outcome. The second set of aspirates consisted of 42 distinct nodules with indeterminate cytology and surgical outcome. Thirty-one miRNAs were analyzed as well as 17 genetic alterations in the BRAF, RAS, RET and PAX8 genes, considered standard mutation testing. Furthermore, 54 samples that were negative by the 17-mutation panel were interrogated using a miRNA classification algorithm, commercially available as the ThyraMIR Thyroid miRNA Classifier, which analyzes in parallel 20 genes through next generation sequencing and 46 mRNA transcripts. The authors found that standard mutation testing alone had a sensitivity of 61%, consistent with the literature. Machine learning was utilized to group miRNA analysis into two groups of miRNAs, classifier A and classifier B. When miRNA classifier A was included in the analysis, the sensitivity rose to 78%, and 94% with classifier B. The authors calculated that this leads to a low residual risk of cancer (8%) among specimens negative by mutation and miRNA testing and corresponds to a calculated improvement from 78–90% NPV to 94–98% NPV at 20–40% cancer prevalence. These results contributed to the development of ThyraMIR. In the small cohort that underwent evaluation by ThyraMIR, the authors report a diagnostic sensitivity of 85% and specificity of 95%.

Clinical Practice Guidelines

American Thyroid Association (ATA)

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

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

- Malignant cytology-while studies have been presented in the literature that suggest that *BRAF* and other multi-gene panels may be useful in prognosis and treatment decisions, more studies are needed to establish the impact of molecular profiling involving multiple mutations or other genetic alterations on clinical management of individuals with primary thyroid medullary cancer.
- Post-operative radioiodine (RAI) therapy. Molecular testing to guide postoperative RAI use is not recommended at this time.

American Association of Endocrine Surgeons (AAES)

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

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

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

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

National Comprehensive Cancer Network (NCCN)

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

Hematological Malignancies

In a 2018 multicenter study including 2,035 individuals, Grinfeld et al. sequenced coding exons from 69 identified myeloid cancer genes in individuals diagnosed with myeloproliferative neoplasms. Using this information, a genomic classification was developed to predict outcomes for individuals. In all, 33 of the genes had driver mutations in at least 5 individuals, with JAK2, CALR or MPL as the only abnormality in 45% of participants. Volumes of driver mutations increased in parallel with age and advancement of disease. Demographic variables, germline polymorphisms and driver mutations independently predicted disease and eight genomic subgroups with distinct clinical phenotypes were defined. Ultimately, prognostic models which could generate tailored prediction of clinical outcomes in individuals with chronic-phase myeloproliferative neoplasms and myelofibrosis were created and predicted/observed outcomes correlated in internal cross-validation of a training group and an

independent external group. The authors concluded that their characterization may enable personalized prediction of outcomes and support individuals diagnosed with myeloproliferative neoplasms.

Song et al. (2017) conducted a review of the literature comparing the clinical utility of a variety of genomic profiling techniques in the treatment of myelodysplasias (MDS). They noted that the common defects in MDS that should be identified are del5q, trisomy 8, del20q, del7q, monosomy 7 and complex karyotypes. Each aberration has different prognostic and management challenges, so accurate identification of genomic abnormalities is important for a clear diagnosis and to optimize treatment strategies. The authors compared findings from the literature for routine cytogenetics, FISH, spectral karyotyping (SKY), SNP array, CGH, and SNP+ CGH for the ability to detect the common defects in MDS. The authors concluded that no single technology provides all the information necessary for the clinician to create informed treatment plans, and that a combination of techniques is required. The authors favored routine cytogenetics, FISH and SNP+ CGH, but noted that additional efforts are needed to standardize testing and bioinformatics, and further technological advances are needed to overcome the limitations of diverse techniques.

Evans et al. (2016) studied the diagnostic utility of SNP+ CGH array to identify unexplained cytopenia in 83 MDS patients and compared results with 18 normal bone marrow controls. Array analysis was done in parallel with standard cytogenetics, FISH, flow cytometry, and morphology. Forty-five percent of patients were diagnosed with MDS, 33% were normal, and 8% had other pathological disorders. 57% of the MDS patients had normal cytogenetics, but the SNP+ CGH array found significant cryptic chromosome aberrations. In MDS patients with abnormal cytogenetics, the array essentially matched the chromosome results and didn't add any new information. Overall, the SNP+ CGH array analysis contributed significantly to the diagnostic yield in indeterminate morphology cytopenic patients.

Weinhold et al. (2016) reported clinical outcomes of GEP testing in relation to treatment type for subgroups of patients (n = 1217) with multiple myeloma (MM) who participated in the University of Arkansas for Medical Sciences Total Therapy (TT) trials. Using log-rank tests for GEP data, the researchers identified 70 genes linked to early disease-related death. The UAMS GEP70 risk score is based on the ratio of the mean expression level of up-regulated to down-regulated genes among the 70 genes. Most up-regulated genes are located on chromosome 1q, and many down-regulated genes map to chromosome 1p. The predictor enabled the reliable identification of patients with shorter durations of complete remission, event-free survival, and overall survival that constitute 10-15% of newly diagnosed MM patients. The authors' reported that impact of treatment differs between molecular subtypes of MM and that GEP gives important information that can help in clinical decision-making and treatment selection. Future studies should address whether strategies maximizing exposure to proteasome-inhibitors can further improve outcome in the MS subgroup. The authors' note that comparison of GEP data of multiple paired samples showed differences in risk signatures, indicating the co-existence of HiR and LoR subclones (manuscript in preparation). Possibly, cells of a LoR subclone were collected at relapse in these patients. the addition of thalidomide significantly improved outcome of LoR cases from maintenance and that outcome of LoR was improved further by the addition of bortezomib. The authors comment that they could not detect a significant improvement for HiR cases, but this may be due to a lack of statistical power.

Peterson et al. (2015) conducted a study to determine the clinical utility and diagnostic yield, plus examine the rationale, of including microarray analysis in the diagnosis of hematological neoplasias. Twenty-seven patients with hematological malignancies were evaluated by chromosome analysis, FISH and CGH or CGH+ SNP arrays. Nearly 90% of chromosome abnormalities found in the patients were also identified by microarray. Of 183 CNVs found, 52% were additional anomalies that were not found by routine cytogenetics or FISH. 65% were < 10 Mb in size. Balanced rearrangements were not found by microarray, but of 19 rearrangements that appeared "balanced" by routine cytogenetics, 7 had alterations found by microarray at the breakpoints. The authors concluded that CGH provided clinicians with advantages in identification of cryptic imbalances and clonal abnormalities in non-dividing cells with poor chromosome morphology and therefore had potential to be integrated as a patient management tool.

Laurie et al. (2014) compared the SNP array results of 278 symptomatic CLL patients with > 50,000 subjects from the GENEVA consortium of genome wide association studies, which analyzed people with a range of medical conditions and healthy controls. The CLL patients were also analyzed by FISH to determine performance and concordance between the SNP array and FISH. When a parameter of 20% abnormal cells was used as a cutoff, the concordance rate between the SNP array and FISH was 98.9%. The array found 8.4% of cases with UPD which cannot be detected by FISH. In 214 CLL patients with SNP results, 1112 genetic anomalies were found, of which 628 were considered acquired. This was a higher percentage and anomalies were unique in the CLL group when compared to the GENEVA cohort and suggests that late stage CLL has recurrent acquired

anomalies that do not occur in precursor conditions or in the general population. The clinical significance of this finding is not clear, however, SNP based array was demonstrated to be a valid analysis tool.

Kolquist et al. (2011) examined the clinical utility of CGH in myelodysplasias. They noted that only half of myelodysplasias (MDS) patients show genomic abnormalities using routine cytogenetics, yet this group of patients is characterized by ineffective hematopoiesis, cytopenia, and a 30% risk of developing acute myeloid leukemia (AML). They hypothesized that using CGH to test patients who were cytogenetically normal would reveal cryptic genomic alternations that would improve prognosis, managing disease progression, and determining the suitability and efficacy of molecularly targeted therapy. They analyzed 35 samples by CGH derived from patients with a diagnosis and suspicion of MDS who also had known abnormal karyotypes. 80% of samples had new chromosomal aberrations that had not been revealed by cytogenetics or FISH. An additional 132 cryptic abnormalities were found including deletions of known oncogenes, such as NF1, RUNX1, RASSF1, CCND1, TET2, DNMT3A, HRAS, PDGFRA and FIP1L1. Overall, the authors concluded that CGH in combination with routine cytogenetics provided additional clinically relevant information that could better direct the care of the patients analyzed.

Detection of Measurable Residual Disease (MRD) in Hematologic Malignancies

Hayes performed a Molecular Test Assessment on the FDA-approved clonoSEQ test for measurement of MRD when used to monitor changes in disease burden during and after treatment of B-cell acute lymphoblastic leukemia and multiple myeloma (MM) using bone marrow (BM) samples and in patients with chronic lymphocytic leukemia (CLL) using BM or peripheral blood (PB) samples. Although the overall body of evidence is low in quality, data from 2 studies addressing clinical validity suggest that clonoSEQ has a lower sensitivity threshold for MRD detection than other types of MRD detection tests (allele-specific oligonucleotide-PCR and flow cytometry) in individuals with CLL or MM. At this time, no peer-reviewed evidence was identified that reported improved clinical outcomes resulting from clonoSEQ testing (Hayes, clonoSEQ [Adaptive Biotechnologies], 2022).

In a systematic review and meta-analysis Short et al. (2022) assessed MRD impact on clinical outcomes in AML. Studies reporting association between MRD and overall survival (OS) or disease-free survival (DFS) in AML were included in the review (n = 48). In studies including only individuals in complete remission, estimated 5 year OS for MRD-negative group was 67% (95% Bayesian credible interval [CrI], 53-77%) and for MRD-positive group was 31% (95% CrI, 18-44%). Greater DFS and OS was associated with MRD-negative results regardless of analytic sensitivity or MRD threshold used. Of those in complete remission, studies using MRD cutoff of less than 0.1% showed the greatest benefit related to MRD negativity. Beneficial impact associated with MRD negativity was seen regardless of timing of assessment or type of assay performed. Noted is the lack of survival reporting for individuals with lesser responses or according to specific MRD level in most of the studies analyzed, so no estimate of impact can be made in those situations. In addition, current MRD assay for AML can only achieve a sensitivity of 1×10^{-4} to 1×10^{-5}. As such, absence of detectable MRD doesn't rule out residual disease that may eventually lead to relapse. In this systematic review, using a threshold of 0.1%, 5 year DFT of 63% indicates that a significant portion of MRD-negative individuals will still relapse. In opposition, a small percentage (16%) of individuals who were MRD-positive were still disease free at 5 years. Overall, the authors concluded that for individuals with AML in remission, MRD-negativity correlates with higher DFS and OS, which provides further support for the use of MRD in individuals with AML.

A 2021 NICE innovation briefing states that the clonoSEQ test for MRD shows improved standardization, sensitivity and specificity when compared with other techniques for MRD assessment. However, there is a lack of randomized studies in the evidence at this time.

Wierda et al. (2021) published an expert review and consensus recommendations addressing the use of measurable residual disease (MRD), also referred to as *minimal* residual disease, to evaluate disease burden during and after treatment of chronic lymphocytic leukemia (CLL). They note that undetectable MRD status at the end of treatment has been associated with prognostic significance in CLL, corresponding with favorable, progression-free and overall survival rates with use of chemoimmunotherapy. Because of this, assessment of MRD is being studied in CLL clinical trials, and the need for further standards for terminology and clinical outcomes reporting is recognized. This consensus represents the outcome of a 174-member panel of international and interdisciplinary experts who collaborated to pinpoint key questions on the issues surrounding MRD in CLL and provide recommendations for further study. The authors provide recommendations for standardized nomenclature, methodology, assay requirements, tissue to be used, timing/frequency of MRD assessment (at least 2 months after completion of last treatment and in alignment with response assessment), and the significance of undetectable MRD (U-MRD). The authors state that current guidelines do not recommend routine MRD testing in practice for CLL at this time; this is the subject of study in clinical trials.

In a 2019 expert consensus, Short et al. provided recommendations for assessment of MRD in adults with ALL, affirming that MRD which has persisted after initial therapy is a compelling predictor of survival and relapse in individuals with ALL, but nothing the controversial nature surrounding the best use of this information to inform clinical decision-making. The document addresses MRD assessment methods as well as the prognostic/predictive impact of MRD in ALL, directing that in adults undergoing frontline treatment, bone marrow should be used to assess MRD as per the following timeframe: after the end of induction, in early consolidation (approximately 3 months after start of therapy) and then approximately every 3 months for at least 3 years. In individuals with relapsed or refractory ALL undergoing salvage therapy, MRD should be evaluated, at a minimum, at the time of morphological remission and at the end of treatment. The document further outlines recommended therapeutic approaches based on MRD results. The authors not that NGS holds substantial promise in refining risk assessment and improving clinical decision-making in ALL, but large prospective studies to further evaluate this technology and the utility of peripheral blood MRD assessment are needed.

The efficacy of targeted NGS to identify MRD in patients with acute myeloid leukemia (AML) was studied by Jongen-Lavrencic et al. (2018). Between 2001 and 2013, a total of 482 patients ranging in age from 18-65 with newly diagnosed AML were included. NGS of 54 genes that are often present in AML patients was performed at diagnosis and after induction therapy during complete remission. The end points analyzed were 4-year relapse, relapse free survival and overall survival. Results were compared with flow cytometry (FC). The authors discovered an average of 2.9 mutations per patient, of which at least one single mutation could serve as an indicator of residual disease, in 430 patients. These patients then had NGS testing repeated on bone marrow after induction therapy, and they were in complete remission. Persistent mutations were found in 52% and were highly variable across the genes analyzed. DTA mutations were most common, persisting at rates of 79%, whereas *RAS* pathway mutations cleared, persisting at an average rate of about 9%. The authors noted that DTA mutations are common gene mutations in individuals with age related clonal hematopoiesis, and likely represent non-leukemic clones rather than persistent malignant disease. After DTA mutations were excluded, the detection of MRD was associated with a significantly higher relapse rate than no detection (55% vs. 32%), lower relapse-free survival (37% vs. 58%) and overall survival (42% vs. 66%). The results of NGS were compared to FC in a subset of 340 patients. Concordant results for detection or non-detection of MRD were found in 69% of patients. The four-year relapse rate was 73% among patients in whom both assays were positive, 52% among those who had residual disease on sequencing but not on flow cytometry, 49% among those who had residual disease on flow cytometry but not on sequencing, and 27% among those in whom both assays were negative. Multivariate analysis found that combining the two assays gave a high prognostic value to the rate of relapse ($p < .001$), relapse free survival ($p < .001$) and overall survival ($p = .003$).The authors concluded that persistent mutations associated with clonal hematopoiesis did not have prognostic value, whereas the detection of MRD during complete remission using NGS with FC had significant additive prognostic value.

The Food and Drug Administration (FDA) reviewed data submitted by Adaptive Technologies on their ClonoSeq assay, which included data from currently ongoing studies (FDA, 2018). They noted that clinical validity was demonstrated in a retrospective analysis of 273 patients with ALL, on ongoing study of 323 patients with multiple myeloma, and separate study of 706 patients with multiple myeloma. Patients who had a negative MRD results had a longer event free survival.

An important prognostic factor in B-lymphoblastic leukemia (B-ALL) is early response to combination induction chemotherapy. End of induction response is typically measured by multiparametric flow cytometry (FC) or allele-specific oligonucleotide polymerase chain reaction (ASO-PCR). The analytical sensitivity for FC is 0.01%, and ASO-PCR is .001%, but requires the development of patient specific probes. Wood et al. (2018) reviewed the clinical validity of a new technical approach of using high throughput sequencing (HTS) of IGH and TRG genes to FC for determining minimal residual disease (MRD). The study used 619 paired pretreatment and end-of-induction bone marrow samples from Children's Oncology Group studies AALL0331 and AALL0232 (clinicaltrials.gov). The samples were evaluated by HTS and FC for event free survival and overall survival. Using an MRD threshold of 0.01%, HTS and FC show similar 5-year event free survival and overall survival rates. There was high discordance between HTS and FC in number of patients identified; HTS identified 55 more patients (39%), and these patients had worse outcomes than FC MRD negative patients. HTS also identified 19% of standard risk patients without MRD at any detectable level, which was correlated with excellent outcomes. Overall HTS had a high sensitivity and lower false-negative rate than FC in this analysis.

Avet-Loiseau et al. (2015) reported on the use of FC and NGS in the Intergroup Francophone du Myélome/ Dana-Farber Cancer Institute (IFM/DFCI) 2009 trial to measure MRD in the IFM arm of the study. This trial enrolled 700 patients under 66 years of age and randomized them to either receive either 8 cycles of VRD (Velcade-Revlimid-Dexamethasone) (arm A), or 3 VRD cycles, high-dose melphalan, followed by two consolidation VRD cycles (arm B). All patients received a lenalidomide maintenance for 12 months. A total of 246 patients were evaluated by NGS using the LymphoSight platform, and before maintenance, 87

patients were negative, 80 were low-positive, and 79 were positive. After maintenance, 178 were tested, and 86 patients were negative, 52 were low-positive, and 40 were positive. Using a cutoff of 10^{-6}, patients below this threshold had a pre-maintenance progression free survival (PFS) of 86%, vs 53% for patient > 10^{-6}. In the post-maintenance group, these numbers were 90% and 59% respectively. When compared with results from 7 color FC, of 72 patients who were positive with FC, 67 were also positive with NGS. In the FC negative group, of the 163 patients, 51 were positive by NGS. In this subgroup, the 3-year PFS was 86% for the NGS negative patients compared to 66% for the NGS negative patients in the pre-maintenance group. In the post-maintenance group, the numbers were 91% and 65% respectively. The authors concluded that NGS was able to predict PFS in this study.

Clinical Practice Guidelines

American Society of Clinical Oncology (ASCO)/Cancer Care Ontario (CCR)

In a joint clinical practice guideline, ASCO and CCR (Mikhael et al., 2019) provided recommendations on treatment of multiple myeloma. Recommendations regarding use of MRD in management of multiple myeloma included:

- There is currently insufficient evidence to make modifications to maintenance therapy based on depth of response, including MRD status (Type: informal consensus/evidence based; Evidence quality: low/intermediate, benefit outweighs harm; Strength of recommendation: moderate).
- The goal of initial therapy for transplant-eligible patients should be achievement of the best depth of remission. MRD-negative status has been associated with improved outcomes, but it should not be used to guide treatment goals outside the context of a clinical trial (Type: evidence based; Evidence quality: high, benefit outweighs harm; Strength of recommendation: moderate).
- It is recommended that depth of response be assessed with each cycle. Frequency of assessment once best response is attained or on maintenance therapy may be assessed less frequently but at minimum every 3 months (Type: evidence based; Evidence quality: low, benefit outweighs harm; Strength of recommendation: weak).
- Depth of response for all patients should be assessed by International Myeloma Working Group (IMWG) criteria regardless of transplant eligibility (Type: evidence based; Evidence quality: high, benefit outweighs harm; Strength of recommendation: moderate).
- There is insufficient evidence to support change in type and length of therapy based on depth of response as measured by conventional IMWG approaches or MRD (Type: informal consensus; Evidence quality: low, harm outweighs benefit; Strength of recommendation: moderate).

College of American Pathologists (CAP) and American Society of Hematology (ASH)

CAP and ASH convened a panel of experts to review the literature and establish a guideline for appropriate lab testing for the initial diagnosis of acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) and ambiguous acute leukemias (ALs). The experts reviewed the literature and using an evidence-based methodology intended to meet recommendations from the Institute of Medicine, a set of guidelines was developed. The guidelines were reviewed by an independent panel and were made available for public comment. The outcome was 27 guidelines addressing clinical information required by the pathologist and recommended laboratory testing. Chromosome microarray is broadly addressed as one potential test in several statements that refer to "molecular genetic testing," which may also include FISH, RT-PCR, or DNA methylation studies. These include:

- "In addition to morphologic assessment (blood and BM), the pathologist or treating clinician should obtain sufficient samples and perform conventional cytogenetic analysis (i.e., karyotype), appropriate molecular-genetic and/or FISH testing, and FCI. The flow cytometry panel should be sufficient to distinguish acute myeloid leukemia (including acute promyelocytic leukemia), T-ALL (including early T-cell precursor leukemias), B-cell precursor ALL (B-ALL), and AL of ambiguous lineage for all patients diagnosed with AL. Molecular genetic and/or FISH testing does not, however, replace conventional cytogenetic analysis." [Statement 5. Strong Recommendation].
- "For patients who present with extramedullary disease without BM or blood involvement, the pathologist should evaluate a tissue biopsy and process it for morphologic, immunophenotypic, cytogenetic, and molecular genetic studies, as recommended for the BM." [Statement 11. Strong Recommendation].
- "For patients with suspected or confirmed AL, the pathologist or treating clinician should ensure that flow cytometry analysis or molecular characterization is comprehensive enough to allow subsequent detection of MRD". [Statement 12. Strong Recommendation] (Arber et al., 2017).

European Hematology Association (EHA)/European Society for Medical Oncology (ESMO)

In a 2021 guideline addressing the diagnosis, treatment and follow-up for multiple myeloma, EHA and ESMO (Dimopoulos et al.) made recommendations for both newly diagnosed individuals and also those with relapsed or refractory disease noting the introduction of the use of MRD in response criteria. The authors indicate that MRD may be used as a surrogate endpoint for progression free survival for individuals receiving first-line treatment and as an endpoint for speeding up drug development. The guideline indicates that cytogenetics including karyotype and FISH are necessary at diagnosis as well as BM cytology and biopsy and next-generation flow cytometry (NGF) or NGS.

European Society for Medical Oncology (ESMO)

In a 2021 clinical practice guideline, ESMO provided recommendations on the management of CLL (Eichhorst et al.) This guideline recommends cytogenetics and molecular genetics for TP53 mutation or del(17p)and indicates that bone marrow biopsy and MRD should be carried out to identify complete remission and MRD status within clinical trials. MRD assessment is generally not recommended for monitoring after therapy outside of clinical studies at this time.

ESMO also published a clinical practice guideline addressing myelodysplastic syndromes (MDS) in 2021 (Fenaux et al.), indicating that acquired molecular mutations are found in 80%-90% of individuals with MDS and 40% of individuals with MDS have more than one mutation. Established diagnostic methods for MDS include peripheral and differential blood counts, cytomorphology of peripheral blood and bone marrow smears and cytogenetics of bone marrow cells. Molecular profiling can be a valuable diagnostic tool if MDS is uncertain, but in most cases, mutations have limited impact on management of in the majority of cases.

Heuser et al. (2020) addressed diagnosis, treatment and follow up in an ESMO practice guideline focused on care of adults with AML. The guideline recommends prompt cytogenetic and molecular evaluation to assess risk and potential treatment options and assessment of MRD at diagnosis (to establish aberrant marker profile), after 2 cycles of chemotherapy and after treatment ends. Additionally MRD may be assessed approximately every 3 months (bone marrow) or every 4-6 weeks (peripheral blood) after the end of treatment for 24 months when individual has a molecular marker.

A clinical practice guideline from ESMO (Hoelzer et al., 2016) addressed diagnosis, treatment and follow-up of ALL in adult patients, noting mandatory use of cytogenetics for when diagnosing ALL. The use of MRD quantification and risk classification was also noted as a necessary step in diagnostic workup and response evaluation.

National Comprehensive Cancer Network (NCCN)

NCCN guidelines for ALL (v1.2022) recommend molecular characterization using FISH testing, reverse transcriptase-polymerase chain reaction testing and comprehensive testing via NGS for gene fusions and pathogenic mutations. Optional tests include CMA in cases of aneuploidy or failed karyotype. Regarding MRD, NCCN recommends sensitivity of 10^{-4} or better; NGS is listed as one the recommended methods for MRD assessment. For adult ALL, the NCCN guidelines describe the timing of MRD assessment to be upon completion of initial induction and additional time points should be guided by the treatment regimen used. In addition, for some techniques, a baseline MRD assessment may be helpful. Similarly, in pediatric populations for ALL, the timing of MRD assessment is upon completion of induction (de novo or relapse), at the end of consolidation, and additional time points are guided by the treatment regimen used (NCCN Pediatric Acute Lymphoblastic Leukemia, v1.2023).

The NCCN guidelines for AML indicate that multiplex gene panels and comprehensive NGS analysis are indicated for ongoing management of AML. Additionally, the guidelines state that the role of MRD is evolving in both prognosis and treatment and that clinical trial participation is encouraged. MRD is listed as a component in the course of sequential therapy and the most commonly used methods for MRD assessment include PCR, NGS assay, and flow cytometry. Timing of MRD assessment in AML is at completion of initial induction, before allogeneic transplants, and at additional time points as guided by the treatment path (NCCN Acute Myeloid Leukemia, v2.2022).

The use of gene panels including at least the 21 most frequently mutated MDS related genes to assess for MDS-associated mutations (either bone marrow or peripheral blood cells) is endorsed by the NCCN in the v1.2023 guideline for myelodysplastic syndromes. Because commercially available tests differ in specific genes analyzed, it is critical to consider the underlying indication and the area of expertise of the laboratory when selecting test panel/laboratory. Notably, genetic testing performed to identify somatic mutations in malignant cells is typically not designed to detect germline mutations, so may be inadequate to identify any underlying heritable hematologic malignancy predisposition syndrome.

The NCCN guideline for myeloproliferative neoplasms (v3.2022) recommends molecular testing via blood or bone marrow for specific gene mutations including *JAK2 V617F, CALR* and *MPL* and *JAK2* exon 12 mutations or a multigene panel including these genes during initial workup for individuals suspected of having a myeloproliferative neoplasm.

NCCN clinical practice guidelines for multiple myeloma state that single nucleotide polymorphism array or next generation sequencing panels on bone marrow have the potential to provide further risk categorization which may add prognostic value. No patient selection criteria were provided. The NCCN Multiple Myeloma Panel suggests baseline clone identification and consideration of MRD as indicated for prognostication (NCCN Multiple Myeloma, v2.2023).

Lung Cancer Tissue Testing

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

A comparison study by Yao et al. (2021) was performed to develop a quick gene testing procedure using fresh core needle biopsy samples from NSCLC patients. Thirty patients with NSCLC confirmed by frozen section examination were enrolled to compare the results of multi-gene mutation testing using fresh frozen (FF) tissues and paired formalin-fixed paraffin-embedded (FFPE) tissues. A total of 77 fresh NSCLC tissue samples obtained from core needle biopsy were evaluated by frozen section examination. The 77 patients consisted of 39 males (50.6%) and 38 females (49.4%) with a median age of 65 years (range, 42–85 years) of which 32 were smokers (41.6%) vs. 45 nonsmokers (58.4%). Frozen section examination revealed 70 (90.9%) AC, 6 (7.8%) SCC, and 1 (1.3%) adenosquamous carcinoma (ASC), which is consistent with the final pathological diagnosis using FFPE tissues. If the NSCLC diagnosis and adequate tumor cell counts were confirmed by histopathology, the fresh tissues were used to extract DNA and subsequent gene testing by ARMS-PCR. The paired FFPE core needle biopsy samples were from 30 NSCLC patients in stage IV, randomly selected for this study, who also underwent gene testing. The 77 fresh samples showed an EGFR mutation rate of 61.0%. The clinical treatment strategy for patients was optimized based on gene test results. Using this procedure of gene mutation testing, the time interval between physicians requesting and obtaining a test result has been shortened to fewer than 2 days. Following a comparison of gene testing results with fresh tissues and paired FFPE tissues from the 30 patients, no difference in the DNA concentration extracted from fresh tissues and FFPE tissues was found. DNA purity, however, was higher in fresh tissues than that in FFPE tissues. Gene testing detected the same gene mutations in 93.3% of cases in fresh tissues and paired FFPE tissues. The authors concluded that gene testing procedure using fresh biopsy samples greatly shortens the waiting time of patients. The multi-gene mutation testing using fresh core needle biopsy samples from NSCLC patients is a reasonable, achievable, and quick approach. The authors stated that fresh tissues may serve as a potential alternative to FFPE tissues for gene testing in NSCLC patients. Limitations to this study include a risk of misdiagnosis during frozen section examination and uncertain diagnosis of fresh tissues related to lack of pathologist experience. Additionally, the sensitivity and specificity of gene testing using FF tissues are 96 and 75% when compared with FFPE tissues. The high sensitivity and low specificity may be attributed to the selection of cases through frozen section examination. The sample size is too small to prove the usefulness of this test as a diagnostic tool. Further research with randomized controlled trials is needed to validate these findings.

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

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

Kris et al. (2014) reported on the Lung Cancer Mutation Consortium's study of the frequency of oncogenic drivers in patients with lung adenocarcinoma. These oncogenic drivers are then analyzed to determine if there is a way to guide treatment. Fourteen study sites from 2009 to 2012 enrolled patients with metastatic lung adenocarcinoma and used a multiplex assay to test for drivers in 10 genes (full genotyping). Tumors from 1007 patients were tested for at least 1 gene and 733 for 10 genes. Of the 733 patients, an oncogenic driver was found in 466 (64%) with 182 tumors (25%) had the KRAS driver; sensitizing EGFR, 122 (17%); ALK rearrangements, 57 (8%); other EGFR, 29 (4%); 2 or more genes, 24 (3%); ERBB2 (formerly HER2), 19 (3%); BRAF, 16 (2%); PIK3CA, 6 (< 1%); MET amplification, 5 (< 1%); NRAS, 5 (< 1%); MEK1, 1 (< 1%); AKT1, 0. Twenty-four of the 733 patient had two oncogenic drivers identified. Of the total 1007 patients, the results were used to select a targeted therapy or trial in 28%. Among the 1007 patients tested for at least 1 driver, 93% had sufficient information to be included in the survival analysis (456 were alive and 482 had died); among this group, median follow-up was 1.67 years (IQR, 0.9-2.69); range, 0-18.56. For the patients with an oncogenic driver and genotype directed therapy (n = 260), the median survival was 3.5 years (interquartile range [IQR], 1.96-7.70) compared with 2.4 years (IQR, 0.88-6.20) for the 318 patients with any oncogenic driver(s) who did not receive genotype-directed therapy (propensity score–adjusted hazard ratio, 0.69 [95% CI, 0.53-0.9], p = .006).

Clinical Practice Guidelines

American College of Chest Physicians (ACCP)

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

American Society of Clinical Oncology (ASCO)

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

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

National Comprehensive Cancer Network (NCCN)

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

- if a patient is medically unfit for invasive tissue sampling1.
- in the initial diagnostic setting, if following pathologic confirmation of a NSCLC diagnosis there is not sufficient material for molecular analysis, cell-free/circulating tumor DNA should be used only if follow up tissue based analysis is planned for all patients in which an oncogenic driver is not identified.
- In the setting of initial diagnosis, if tissue-based testing doesn't fully assess all recommended biomarkers due to tissue quantity or testing methods available, repeat biopsy or cell-free/circulating tumor DNA testing may be considered (NCCN Non-Small Cell Lung Cancer, v5.2022).

Melanoma

Cutaneous Melanoma

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

An Ontario Health Technology Assessment (2021) that evaluated the diagnostic accuracy, clinical utility and budget impact of pigmented lesion assays (PLA) for people with suspected melanoma skin lesions. The systematic review included seven studies consisting of six cohort studies (including three Ferris studies (2017, 2018 and 2019) that were previously discussed in this policy) and one survey that were conducted in dermatology offices, examining adults (> 18 years old) with suspected melanoma lesions using the DermTech pigmented lesion assay. The authors stated that the risk of bias in the included studies was generally moderate to high, and the quality of evidence was very low. Limitations noted in the review included the potential

bias from the industry sponsored studies, overestimation of the diagnostic accuracy of PLA, the diagnostic accuracy of visual assessment may have been underestimated when compared to published literature, and many parameters and assumptions used by the economic analysis were not reported in the study, which they stated had potentially serious limitations. They concluded that there was no evidence demonstrating the impact of PLA on patient outcomes and that the low-quality evidence for the diagnostic accuracy of PLA remains uncertain when compared to visual inspection alone. They also stated that the evidence is uncertain about whether PLA has an impact on clinical decision making and that the cost-effectiveness of this test compared with the standard care pathway is also uncertain.

Marchetti et al. (2020) completed a systematic review and meta-analysis to assess the performance of prognostic gene expression profile (GEP) tests in patients with American Joint Committee on Cancer (AJCC) stage I or stage II cutaneous melanoma. The review included seven studies with a total of 1450 participants. One study was determined to have a moderate risk of bias and the other six studies were determined to have a high risk of bias. There were 623 participants with stage I disease and 212 with stage II disease that were tested with DecisionDx-Melanoma. The authors found that DecisionDx-Melanoma correctly classified recurrence in 29% of the participants with stage I disease and 82% of those with stage II disease. It also found that the test correctly classified 90% with stage I disease and 44% with stage II disease among participants without recurrence. When they reviewed the data for MelaGenix, which had 88 participants with stage I disease and 245 with stage II disease, they found that the test correctly classified 32% with Stage I disease and 76% with stage II disease among those with recurrence. Among those participants tested with MelaGenix, the test correctly classified 77% with stage I disease and 43% with stage II disease. Limitations noted by the authors include the heterogeneity in study designs and data reporting, the lack of availability of individual participant data, short follow-up and significant censoring, the variability in the definitions used for melanoma recurrence, and the risk of bias and quality of the evidence. The authors concluded that the prognostic ability of DecisionDx-Melanoma and MelaGenix to predict recurrence among patients with localized melanoma varied by AJCC stage and appeared to be poor for patients with stage I disease. They recommend more rigorously structured studies be performed to better quantify the association of GEP tests with melanoma outcomes and to demonstrate clinical utility.

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

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

Zager et al. (2018, included in Hayes DecisionDx-Melanoma Molecular Test Assessment, above) conducted a multi-center trial of archived primary melanoma tumors from 523 patients, using a 31 gene expression classifier to classify patients as Class 1

(low risk) and Class 2 (high risk). The 5-year recurrence free survival (RFS) rates for Class 1 and Class 2 were 88% and 52%, respectively. Distant metastasis-free survival rates (DMFS) were 93% for Class 1 versus 60% for Class 2. The gene expression classifier was a significant predictor of RFS and DMFS in univariate analysis in addition to with Breslow thickness, ulceration, mitotic rate, and sentinel lymph node (SLN) status. GEP, tumor thickness and SLN status were significant predictors of RFS and DMFS in a multivariate model that also included ulceration and mitotic rate. The authors concluded that the 31 gene expression classifier provided value to prognostication, and more prospective studies are needed.

Ardakani et al. (2017) assessed the ability of CGH to differentiate between melanocytic naevi and melanoma in cases where the two-show overlapping histological features. Melanomas are characterized by CNVs, while naevi are normal. The team used 19 formalin fixed, paraffin embedded (FFPE) unambiguous naevi and 19 melanomas and tested them using a SurePrint G3 Human CGH 8x60K array. CGH was able to differentiate between the naevi and the melanoma in 95% of cases. One naevus showed two large CNV. The authors concluded that CGH may be a good adjunctive test to resolve histologically equivocal melanocytic samples.

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

Uveal Melanoma

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

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

In a 5-year clinical outcome report from a prospective registry of individuals tested with a prognostic 15-gene expression profile (15-GEP) test for UM and a meta-analysis with published cohorts, Aaberg et al. (2020) found that testing with the 15-GEP test guided management of individuals with UM. UM, a rare intraocular cancer, has a 30-50% risk of metastasis within 5 years of diagnosis. The prognostic 15-GEP was designed to predict 5-year metastatic risk using three risk categories indicating low, intermediate and high-risk groups. In this study, 89 patients who had undergone 15-GEP testing were prospectively enrolled at four separate locations. Clinical outcomes and management plans were tracked every six months. Eighty percent of class 1

(low-risk) participants received low-intensity management and all class 2 (high-risk) patients received high-intensity management ($p < 0.0001$). Five-year melanoma survival rates were 94% for class 1 and 63% for class 2. Five-year metastasis-free survival rates were 90% for class 1 and 41% for class 2. By meta-analysis performed on several prior studies to evaluate clinical outcomes of patients tested with15-GEP, class 2 was associated with an increased risk for both metastasis and mortality and was also the only independent predictor of metastasis.

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

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

Clinical Practice Guidelines

American Academy of Dermatology (AAD)

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

- Ancillary diagnostic molecular techniques (e.g., comparative genomic hybridization; fluorescence in situ hybridization, gene expression profiling [GEP]) may be used for equivocal melanocytic neoplasms.
- Routine molecular testing, including GEP, for prognostication is discouraged until better use criteria are defined. The application of molecular information for clinical management (e.g., sentinel lymph node eligibility, follow-up, and/or therapeutic choice) is not recommended outside of a clinical study or trial.
- Testing of the primary cutaneous melanoma for oncogenic mutations (e.g., *BRAF, NRAS*) is not recommended in the absence of metastatic disease.

National Comprehensive Cancer Network (NCCN)

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

The guideline further states the following:

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

- BRAF mutation testing is recommended for patients with stage III melanoma for whom future BRAF-directed therapy may be an option.

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

Cancers of Unknown Primary (CUP)

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

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

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

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

A systematic review conducted by Binder et al. (2018) to determine incidence and survival trends and to discuss the value of comprehensive genomic profiling (CGP) in cancer of unknown primary (CUP) patients. Age-standardized incidence rates (ASR) per 100,000 were calculated for 2,935 CUP patients from 1981 to 2014 using cancer registry data of the canton of Zurich, Switzerland. Kaplan-Meier survival curves were estimated for sex, age, and histological groups. Cox proportional hazards regression models were used to estimate adjusted hazard ratios (HR). A literature review was conducted to assess the current use of CGP in CUP patients. ASR of CUP increased from 10.3 to 17.6 between 1981 and 1997 and decreased to 5.8/100,000 in 2014. Mean overall survival remained stable. Mortality was lower for patients with squamous cell carcinoma (HR 0.48 [95% CI, 0.41-0.57]), neuroendocrine carcinoma (0.75 [0.63-0.88]), and higher for unclassified neoplasms (1.25 [1.13-1.66]) compared to adenocarcinomas. The literature review identified 10 studies using CGP of CUP tissue. Clinically relevant mutations were identified in up to 85% of CUP patients, of which 13%-64% may benefit from currently available drugs. The authors concluded that CUP incidence decreased most likely due to improved diagnostics, however, mortality did not improve over the last 34 years. CGP testing may help to identify molecular signatures in CUP patients and enable targeted treatment. Given poor prognosis and limited treatment options for patients with CUP, genomic profiling using NGS technologies may meet a clinical need. The findings of this study need to be validated by well-designed studies. Further investigation is needed before clinical usefulness of this procedure is proven.

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

Meleth et al. (2013) conducted a technology assessment on genetic testing or molecular pathology testing for cancer of unknown primary cancers with CancerTypeID, miRview, or PathworkDx to determine analytical validity, clinical validity, and clinical utility. The results showed that the clinical accuracy of all the three tests is similar, ranging from 85 percent to 88

percent. The evidence that the tests contribute to identifying a TOO is moderate; however, the researchers noted that they did not have sufficient evidence to assess the effect of the tests on treatment decision and outcomes.

Clinical Practice Guidelines

European Society for Medical Oncology (ESMO)

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

National Institute for Health and Care Excellence (NICE)

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

National Comprehensive Cancer Network (NCCN)

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

Colorectal Cancer (CRC)

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

Yothers et al. (2022) conducted a patient-specific meta-analysis of 12-gene colon cancer recurrence score validation studies for recurrence risk assessment after surgery with or without fluorouracil (5FU) and oxaliplatin. Three validation studies of the 12-gene colon recurrence score assay were used with pre-specified patient-specific meta-analysis (PSMA) methods to integrate the 12-gene Oncotype DX Colon Recurrence Score result (RS) with the clinical and pathology risk factors stage, T-stage, mis-match repair (MMR) status, and number of nodes examined to calculate individualized recurrence risk estimates. Baseline risk estimation used the most recent studies, so the risk estimates reflect current medical practice. The effect of 5FU was estimated with a meta-analysis of two studies. The effect of oxaliplatin was estimated using one of the RS assay validation studies, in

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

Daemen et al. (2021) conducted a retrospective study and review of randomized, open-label, prospective, parallel three-arm, phase 3 trial, sponsored by F. Hoffmann-La Roche, to improve high-risk classification by identifying biological pathways associated with outcome in adjuvant stage II/III colorectal cancer (CRC). A total of 1,062 patients with stage III or high-risk stage II colon carcinoma from the three-arm randomized phase 3 AVANT trial were included in this retrospective study. The authors performed expression profiling to identify a prognostic signature. Data from validation cohort GSE39582, The Cancer Genome Atlas, and cell lines were used to further validate the prognostic biology. Retrospective analysis of the adjuvant AVANT trial uncovered a prognostic signature capturing three biological functions-stromal, proliferative and immune-that outperformed the Consensus Molecular Subtypes (CMS) and recurrence prediction signatures like Oncotype Dx in an independent cohort. Importantly, within the immune component, high granzyme B (GZMB) expression had a significant prognostic impact while other individual T-effector genes were less or not prognostic. In addition, the authors found GZMB to be endogenously expressed in CMS2 tumor cells and to be prognostic in a T cell independent fashion. The authors concluded that this study furthers their understanding of the underlying biology that propagates stage II/III CRC disease progression and provides scientific rationale for future high-risk stratification and targeted treatment evaluation in biomarker defined subpopulations of resectable high-risk CRC. The results also shed light on an alternative GZMB source with context-specific implications on the disease's unique biology. A limitation to this study is that these results need to be clinically validated in a prospective study.

He et al. (2018) examined the clinicopathological features that could impact the sensitivity and specificity of SEPT9 analysis. A total of 1160 patients were included in the study from hospitals in China, which included 300 patients with colorectal cancer, 122 patients with adenoma, 103 patients with hyperplastic polyps, 568 normal participants (no evidence of disease), and 67 patients with other gastrointestinal diseases. Overall, the sensitivity and specificity of SEPT9 was impacted by cancer stage, size, invasion depth, classification, differentiation and metastasis. It was also noted that SEPT9 detected adenomas, hyperplastic polyps and other gastrointestinal diseases such as inflammatory bowel disease. When screening an average risk population, these non-colorectal cancer disorders are much more common and could lead to false positives and unnecessary intervention.

Molecular technologies are also under investigation to screen for colon cancer, such as the Epi proColon 2.0 assay that measures the methylated Septin9 (SEPT9), a circulating tumor cell marker. The premise of this test is that during colorectal cancer development, the tumor will release cell free DNA (cfDNA) into the bloodstream, and the ratio of SEPT9 DNA be detected through specialized techniques and can predict the presence of early colorectal cancer. A meta-analysis of one cohort study and thirteen case-controlled studies representing 9870 cases demonstrated a pooled sensitivity of 0.66 and specificity of 0.91. The authors compared this to data available for the gold standard test, fecal occult blood testing (FOBT) of a sensitivity of 0.60 and specificity of 0.91, equal to SEPT9. The authors combined the results of FOBT and SEPT9 and achieved a detection rate of colorectal cancer of 88.7% with a specificity of 78.8%. They concluded that FOBT and SEPT9 complement each other, but further studies are needed to determine the best screening tests and approaches (Yan et al., 2016).

Zhang et al. (2016) retrospectively reviewed the prognostic role of CDX2 expression in patients with stage 1 and stage III metastatic colorectal cancer (CRC) after complete surgical resection. The patient cohort (n = 145) included 66 patients with

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

To evaluate whether patients with CDX2-negative tumors might benefit from adjuvant chemotherapy, Dalerba et al. (2016) investigated the association between CDX2 status, and assessed at either the mRNA or protein level, the disease-free survival among patients who either did or did not receive adjuvant chemotherapy. Reviewing a database of 669 patients with stage II colon cancer and 1228 patients with stage III colon cancer, the authors reported that their results confirmed that treatment with CDX2 as a biomarker in colon cancer adjuvant chemotherapy was associated with a higher rate of disease-free survival in both the stage II subgroup (91% with chemotherapy vs. 56% with no chemotherapy, p = 0.006) and the stage III subgroup (74% with chemotherapy vs. 37% with no chemotherapy, p < 0.001) of the CDX2-negative patient population (Fig. 5). A test for the interaction between the biomarker and the treatment revealed that the benefit observed in CDX2-negative cohorts was superior to that observed in CDX2-positive cohorts in both the stage II subgroup (p = 0.02 for the interaction) and the stage III subgroup (p = 0.005 for the interaction). In the authors' opinion, their results indicate that patients with stage II or stage III CDX2-negative colon cancer might benefit from adjuvant chemotherapy and that adjuvant chemotherapy might be a treatment option for patients with stage II CDX2-negative disease, who are commonly treated with surgery alone. Given the exploratory and retrospective design of this study, these results will need to be further validated through randomized, clinical trials, in conjunction with genomic DNA sequencing studies.

Yamanaka et al. (2016) evaluated the 12-gene Recurrence Score assay for stage II and III colon cancer without chemotherapy to reveal the natural course of recurrence risk in stage III disease (the Sunrise Study). A cohort-sampling design was used. From 1,487 consecutive patients with stage II to III disease who had surgery alone, 630 patients were sampled for inclusion with a 1:2 ratio of recurrence and nonrecurrence. Sampling was stratified by stage (II v III). The assay was performed on formalin-fixed, paraffin-embedded primary cancer tissue. Association of the Recurrence Score result with recurrence-free interval (RFI) was assessed by using weighted Cox proportional hazards regression. With respect to prespecified subgroups, as defined by low (< 30), intermediate (30 to 40), and high (≥ 41) Recurrence Score risk groups, patients with stage II disease in the high-risk group had a 5-year risk of recurrence similar to patients with stage IIIA to IIIB disease in the low-risk group (19% v 20%), whereas patients with stage IIIA to IIIB disease in the high-risk group had a recurrence risk similar to that of patients with stage IIIC disease in the low-risk group (approximately 38%). The authors conclude that this validation study of the 12-gene Recurrence Score assay in stage III colon cancer without chemotherapy showed the heterogeneity of recurrence risks in stage III as well as in stage II colon cancer.

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

Clinical Practice Guidelines

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

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

National Comprehensive Cancer Network (NCCN)

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

Prostate Cancer

Decipher, Oncotype DX Prostate, Prolaris, and Promark

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

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

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

quality control requirements (22.4% of total trial samples did not pass quality control), as the median age of tissue samples was older than 20 years.

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

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

Hayes addresses the use of the Decipher GC for use with both biopsy and after RP. A Hayes Molecular Test assessment specific to use with prostate biopsy (Decipher Prostate Biopsy [Decipher Biosciences] 2019, updated 2022) found insufficient evidence to support the use of the Decipher test using needle biopsy specimens to prognosticate for individuals with localized prostate cancer. Though a limited number of studies suggested that Decipher may improve prediction of 5-year metastasis compared to clinical risk classifications alone, further study is needed to determine whether Decipher testing with prostate biopsy improves outcomes for individuals with prostate cancer. Additionally, Hayes indicates the evidence to support clinical validity and utility for use of the Decipher test with RP specimens for informing prognosis and treatment options for individuals with prostate cancer after RP is lacking as well (Decipher Prostate RP [Decipher Biosciences] 2019, updated 2022).

The Prolaris test for use with biopsy and post-prostatectomy underwent assessment by Hayes in 2019 as well. Hayes found insufficient evidence to support the use of Prolaris for risk determination in either situation (Hayes, Prolaris Biopsy Test [Myriad Genetic Laboratories Inc.], 2019 and Hayes, Prolaris Post-Prostatectomy [Myriad Genetic Laboratories Inc.], 2019, both updated 2022).

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

A Molecular Test Assessment produced by Hayes evaluated the Oncotype DX GPS for utility in clinical decision-making for individuals with newly diagnosed, localized prostate cancer who met NCCN criteria for very low, low, or favorable intermediate-risk prostate cancer and were eligible for active surveillance. In terms of clinical validity, the body of evidence consistently favors use of the GPS assay to assist with management strategies for such individuals, however more clinical utility studies

reporting on primary outcomes are recommended (Hayes, Oncotype DX Genomic Prostate Score [GPS] Assay [Genomic Health Inc.], 2018, updated 2022).

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

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

Oderda et al. (2016) assessed whether cell-cycle progression (CCP)-score (Prolaris) can improve the current risk assessment in newly diagnosed PCa patients. The CCP-score at biopsy was evaluated in 52 patients newly diagnosed with PCa who underwent radical prostatectomy. CCP-score was calculated as average RNA expression of 31 CCP genes, normalized to 15 housekeeping genes. The predictive ability of CCP-score was assessed in univariate and multivariate analyses and compared to that of Ki-67 levels and traditional clinical variables including prostate-specific antigen, Gleason score, stage, and percentage of positive cores at biopsy. The authors reported that despite an overall good accuracy in attributing the correct risk class, 7 high-risk and 13 intermediate-risk patients were misclassified by the Prolaris test, which is a limitation to this study. On analysis of variance, mean CCP-score significantly differed across different risk classes based on pathologic results (-1.2 in low risk, -0.444 in intermediate risk, 0.208 in high risk). CCP-score was a significant predictor of high-risk PCa both on univariate and multivariate analyses, after adjusting for clinical variables. Combining CCP-score and the European Association of Urology clinical risk assessment improved the accuracy of risk attribution by around 10%, up to 87.8%. CCP-score was a significant predictor of biochemical recurrence, but only on univariate analysis. The authors conclude that the CCP-score might provide important new information to risk assessment of newly diagnosed PCa in addition to traditional clinical variables. A correct risk attribution is essential to tailor the best treatment for each patient. Additional studies with larger patient sample sizes are needed to determine whether the use of this test in making treatment decisions improves patient outcomes.

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

Na et al. (2016) reviewed the literature on clinically available RNA profiling tests (Oncotype Dx, Prolaris, and Decipher) of prostate tumors. They concluded that these RNA profiling panels have shown promising results in regard to clinical utility, several limitations are worth noting: (1) the current studies are retrospective with relatively small sample sizes, so larger-scale prospective randomized trials are necessary for validation; (2) RNA quality varies among panels (e.g., microdissection is needed for Decipher [some medical center may not have the equipment], while for Prolaris, tissue extraction relies on the instruction from pathologist, which will lead to heterogeneity of the testing results); and (3) the relatively high prices limit potential use of the panels, will necessitate further evaluation of their cost-effective values.

Other Prostate Cancer Assays

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

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

A prospective, randomized, blinded two-armed clinical utility study was conducted by Tutrone et al. (2020) to evaluate the impact of the ExoDx Prostate (IntelliScore) test (EPI) on the decision whether to perform a biopsy in a real-world clinical setting. EPI is designed to assess risk for high grade prostate cancer. The study enrolled 1094 patients from 24 urology practices and a total of 72 urologists. All patients underwent EPI testing but were randomized into EPI vs. Control. Only the EPI arm received results for the biopsy. In the EPI group (458) of the participants received negative EPI scores. Of these, 63% were recommended to defer biopsy and 74% of those did indeed defer the biopsy. Of those with positive EPI scores, 87% were recommended by urologist to proceed with biopsy and 72% of participants complied with that recommendation. Ultimately, this led to detection of 305 more high grade prostate cancer in comparison with control group and the researchers estimated that 49% fewer high-grade cancers were missed due to deferred biopsy compared to standard of care. Sixty-eight percent of participating urologists indicated that the EPI influenced their decision regarding biopsy recommendation. The authors stated that this was the first report on a prostate cancer biomarker utility study with a blinded control group and felt that the study showed that the EPI test influenced decision making regarding prostate biopsy and patient stratification. Despite these positive outcomes, there were limitations. In the EPI group, there was a 5.7% assay failure, and in the entire group of participants, there was a failure rate of 7.1%. Data is lacking regarding long-term outcomes of the participants who deferred biopsy after using EPI, and the large number of testing sites and urologists involved required the use of streamlined questionnaires, limiting feedback. Lastly, a small number of participants (< 5%) had undergone pre-biopsy MRI, which can help refine biopsy accuracy and provide additional information related to EPI test performance. The researchers suggest that future studies could include a larger percentage of patients with MRI data available.

SelectMDx is an assay which measures urine mRNA biomarker levels and uses this information in conjunction with clinical risk factors to help determine risk in men with elevated PSA who have not previously been diagnosed with prostate cancer. A Hayes Molecular Test Assessment (SelectMDx for Prostate Cancer [MDxHealth Inc.] 2019, updated 2022) found the evidence supporting use of this test lacking. Additional investigation is required to determine clinical validity and utility and overall impact on patient outcomes.

Another molecular test used to assess risk for prostate cancer is ConfirmMDx. This test uses tissue from a negative prostate biopsy to identify genetic biomarkers which can then be used to help determine if an individual may be ruled out for repeat biopsy or to predict likelihood of Gleason score ≤ 6 or ≥ 7 prostate cancer on repeat biopsy when individuals have high-risk clinical pathological features associated with prostate cancer. In a Molecular Test Assessment (ConfirmMDx for Prostate

Cancer [MDxHealth Inc.], 2019, updated 2022), Hayes found positive but insufficient evidence to support use of ConfirmMDx for ruling out prostate cancer in repeat biopsy and insufficient evidence for prediction of Gleason score ≤ 6 or ≥ 7 prostate cancer on repeat biopsy. Additional studies are required to evaluate whether ConfirmMDx results in improved patient outcomes in individuals with high-risk clinical features of prostate cancer.

McKiernan et al. (2018) assessed the performance and utility of ExoDx Prostate IntelliScore (EPI) urine exosome gene expression assay versus SOC parameters for discriminating grades of prostate cancer from benign disease. This study compared EPI results with biopsy outcomes in men with age ≥ 50 yr. and prostate-specific antigen (PSA) 2–10 ng/ml, scheduled for initial prostate biopsy. The results were that in a total of 503 patients, with median age of 64 yr., median PSA 5.4 ng/ml, 14% African American, 70% Caucasian, 53% positive biopsy rate (22% GG1, 17% GG2, and 15% ≥ GG3), EPI was superior to SOC with an area under the curve (AUC) 0.70 versus 0.62, respectively, comparable with previously published results (n = 519 patients, EPI AUC 0.71). Using a validated cut-point 15.6 would have avoided 26% of unnecessary prostate biopsies and 20% of total biopsies, with negative predictive value (NPV) 89% and missing 7% of ≥ GG2 PCa. Setting a different cut-point 20 would avoid 40% of unnecessary biopsies and 31% of total biopsies, with NPV 89% and missing 11% of ≥ GG2 PCa. This study concluded that EPI has been validated in over 1000 patients across two prospective validation trials for risk stratification of high-grade and low-grade from benign disease. The use of test may improve identification of patients with higher grade disease and could reduce unnecessary biopsies, although 10% of prostate cancer cases would be missed based on the test characteristics.

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

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

Clinical Practice Guidelines

American Association of Clinical Urologists

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

American Society of Clinical Oncology (ASCO)

Eggener et al. (2020) published the recent ASCO guideline on molecular biomarkers in localized prostate cancer and summarized the evidence as follows:
"Few biomarkers had rigorous testing involving multiple cohorts and only 5 of these tests are commercially available currently: Onco*type* Dx Prostate, Prolaris, Decipher, Decipher PORTOS, and ProMark. With various degrees of value and validation, multiple biomarkers have been shown to refine risk stratification and can be considered for select men to improve management

decisions. There is a paucity of prospective studies assessing short- and long-term outcomes of patients when these markers are integrated into clinical decision making."

ASCO made four specific recommendations:

- Commercially available molecular biomarker tests (i.e., Oncotype Dx Prostate, Prolaris, Decipher, and ProMark) may be offered in situations in which the assay result, when considered as a whole with routine clinical factors, is likely to affect management. Routine ordering of molecular biomarkers is not recommended (Type: Evidence based; Evidence quality: Intermediate; Strength of recommendation: Moderate).
- Any additional molecular biomarkers evaluated do not have sufficient data to be clinically actionable or are not commercially available and thus should not be offered (Type: Evidence based; Evidence quality: Insufficient; Strength of recommendation: Moderate).
- Consideration of a commercially available molecular biomarker test (e.g., Decipher Genomic Classifier) is recommended in situations in which the assay result, when considered as a whole with routine clinical factors, is likely to affect management. In the absence of prospective clinical trial data, routine use of genomic biomarkers in the postprostatectomy setting to determine adjuvant versus salvage radiation or to initiate systemic therapies should not be offered (Type: Evidence based; Evidence quality: Intermediate; Strength of recommendation: Moderate).
- In men with newly diagnosed prostate cancer eligible for active surveillance, both magnetic resonance imaging and genomics intend to identify clinically significant cancers. The Expert Panel endorses their use only in situations in which the result, when considered with routine clinical factors, is likely to affect management. This may include, for instance, the initial management of men who are potentially eligible for active surveillance, where each of these approaches may provide clinically relevant and actionable information. These tests may provide information independent of routine clinical parameters and independent of one another (Type: Informal consensus; benefits/harms ratio unknown; Evidence quality: Low; Strength of recommendation: Weak).

American Urological Association (AUA)

In a clinical practice guideline on early detection of prostate cancer (Carter et al., 2013; reviewed and confirmed 2018) based on a systematic review and meta-analysis, the AUA notes that an improved understanding of the interaction between inherited risk alleles and the environment (lifestyle choices) could provide a potential means of prevention. Future studies of the genetic and epigenetic basis of disease development and progression may provide biomarkers and/or panels of biomarkers with improved specificity when compared to PSA. When available, risk assessment tools combining multiple predictors will need to be evaluated in carefully designed trials to be generalizable to the population in which they would be used.

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

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

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

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

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

In a 2020 guideline statement, Lowrance et al. addressed the use of predictive biomarkers to guide treatment of prostate cancer. They state that although there are several molecular approaches being investigated, at this time, there is no assay that has been prospectively demonstrated to lead to improvements in oncologic outcomes. They suggest that, moving forward, biologic make-up of tumors will be a focus to identify the best treatment options for patients.

Sanda et al. (2018) published the joint AUA/ASTRO/SUO guidelines for clinically localized prostate cancer. The guidelines stated that tissue based genomic biomarkers have not shown a clear role in active surveillance for localized prostate cancer and are not necessary for follow up.

In 2018, Bekelman et al. published the ASCO endorsement of the AUA/ASTRO/SUO guidelines, developed in 2017, for managing clinically localized prostate cancer (Sanda et al., 2018). This guideline stated that tissue based genomic biomarkers have not shown a clear role in active surveillance and not necessary for follow up.

In an endorsement of Cancer Care Ontario's guideline on active surveillance of localized prostate cancer, ASCO comments that ancillary radiologic and genomic tests are investigational but may have a role in patients with discordant clinical and/or pathologic findings. Prospective validation of these tests is needed to assess their impact on patient outcomes such as survival (Chen et al., 2016).

National Comprehensive Cancer Network (NCCN)

NCCN clinical practice guidelines for prostate cancer (NCCN Prostate Cancer, v1.2023) state that Decipher, Oncotype DX Prostate and Prolaris molecular assays may be considered in men with low or favorable intermediate risk prostate cancer and a life expectancy greater than or equal to ten years to help guide decision-making on treatment . Patients with unfavorable intermediate and high-risk disease may consider the use of Decipher and Prolaris molecular assays. Further, the Decipher test should be considered if not previously performed to inform adjuvant therapy when adverse features are found post prostatectomy and can be part of the discussion of risk stratification in patients with prostate specific antigen persistence/recurrence after radical prostatectomy (category 2B evidence.)

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

NCCN categorizes prostate cancer risk groups as follows:

Risk Group	Clinical/Pathological Features
Very low	Has all of the following: • cT1c • Grade Group 1 • PSA < 10 ng/mL • Fewer than 3 prostate biopsy fragments/cores positive, ≤ 50% cancer in each fragment/core • PSA density < 0.15 ng/mL/g
Low	Has all of the following but does not qualify for very-low risk: • cT1–cT2a • Grade Group 1 • PSA < 10 ng/mL

Risk Group	Clinical/Pathological Features		

Intermediate	Has all of the following:
	• No high-risk group features
	• No very-high-risk group features
	• Has one or more intermediate risk factors (IRFs):
	  ○ cT2b–cT2c
	  ○ Grade Group 2 or 3
	  ○ PSA 10–20 ng/mL
		Favorable intermediate	Has all of the following:
			• 1 IRF
			• Grade Group 1 or 2
			• < 50% biopsy cores positive (e.g., < 6 of 12 cores)

		Unfavorable intermediate	Has one or more of the following: • 2 or 3 IRFs • Grade Group 3 • ≥ 50% biopsy cores positive (e.g., ≥ 6 of 12 cores)
High	Has no very-high-risk features and has exactly one high-risk feature: • cT3a; or • Grade Group 4 or Grade Group 5; or • PSA > 20 ng/mL		
Very high	Has at least one of the following: • cT3b–cT4 • Primary Gleason pattern 5 • 2 or 3 high-risk features • > 4 cores with Grade Group 4 or 5		

Pancreatic Cancer and Ampullary Adenocarcinoma

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

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

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

A retrospective study was performed by Kandimalla et al. (2021) using a genome-wide DNA methylation analysis of multiple GI cancers to develop a pan-GI diagnostic assay and validate the tissue-specific differentially methylated regions (DMRs) in 300 cell-free DNA specimens for early detection and/or population screening of all GI cancers. The study design involved tissue discovery followed by plasma cell-free DNA (cfDNA) validation. Methylation data from 1,781 tumor and adjacent normal tissues and DMRs between individual GI cancers and adjacent normal were studied including colorectal cancer (CRC), hepatocellular carcinoma (HCC), esophageal squamous cell carcinoma (ESCC), gastric cancer (GC), esophageal adenocarcinoma (EAC), and pancreatic ductal adenocarcinoma (PDAC). By comparing data from tumor versus normal tissues within each GI cancer, as well as across all GI cancers, a total of 67,832 regions of interest (ROI) were identified based on differentially methylated probes with a $p < 0.001$ and an absolute delta beta of 0.20 across all the comparisons. Three distinct categories of DMR panels were developed to include (i) cancer-specific biomarker panels with AUC values of 0.98 (CRC), 0.98 (HCC), 0.94 (ESCC), 0.90 (GC), 0.90 (EAC), and 0.85 (PDAC); (ii) a pan-GI panel that detected all GI cancers with an AUC of 0.88; and (iii) a multi-cancer (tissue

of origin) prediction panel, EpiPanGI Dx, with a prediction accuracy of 0.85-0.95 for most GI cancers. The authors concluded that by using a novel biomarker discovery approach, they were able to provide the first evidence for a cfDNA methylation assay that offers strong diagnostic accuracy for multi-detection GI cancers in a non-invasive and cost-effective manner. This study is limited by its retrospective observations, limited sample size used to represent each stage, and lack of mutation profiles of cfDNA samples to be able to directly compare or combine the diagnostic performance of the methylation assay relative to genomic mutations. Further investigation with prospective evaluation is needed to determine the clinical relevance of these findings.

O'Kane et al. (2019) reported on the COMPASS trial for pancreatic ductal adenocarcinoma (PDAC). Patients were recruited before chemotherapy for whole genome sequencing (WGS) and RNA sequencing (RNASeq). The tumor tissue was analyzed, and tumor responses and clinical outcomes were correlated. Of the 157 patients that had a tumor biopsy, 141 genomes were reported. Twenty-five (21%) had a Moffitt basal-like RNA signature which is usually associated with chemotherapy resistance. GATA6 expression was able to separate the Moffitt subgroup from those with classical tumors. Also, 30% of patients had potentially actionable genetic alterations including BRAF variants (n = 4) and a *NTRK*3-EML4 fusion in *KRAS* WT tumors (8%). The researchers concluded that there are subsets of patients with advanced PDAC that have actionable variants.

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

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

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

Clinical Practice Guidelines

American Society of Clinical Oncology (ASCO)

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

In a guideline from ASCO in 2016, clinical decision support was outlined for metastatic pancreatic cancer. Sohal et al. (2018) published an update to this guideline that incorporated new evidence. The researchers conducted a literature review and found

two new studies to include. The recommendations included that select patients should be tested for mismatch repair deficiency or microsatellite instability, and pembrolizumab is recommended for patients with mismatch repair deficiency or high microsatellite instability tumors.

National Comprehensive Cancer Network (NCCN)

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

Comprehensive Genomic Profiling (CGP) and Tumor Mutational Burden (TMB) Testing

Solid Tumor Tissue Testing

In a 2022 bioinformatic analysis and meta-analysis, Cao et al. investigated the predictive efficacy of TMB testing when used as a biomarker for individuals with cancer that received treatment with immune checkpoint inhibitors (ICI). Outcomes included objective response rate (ORR), durable clinical benefit (DCB), overall survival (OS) and progress-free survival (PFS) in individuals with high TMB as compared to those with low TMB. Simple nucleotide variation (SNV) information from The Cancer Genome Atlas (TCGA) including 33 major cancer types was used for the non-ICI group; OS was compared between individuals with high TMB in the non-ICI group and the meta-analysis results. A total of 41 studies including 7,713 participants met inclusion criteria and were part of the evaluation. Individuals with high TMB results had a better ORR (RR = 2.73; 95% CI: 2.31–3.22; p = 0.043) and DCB (RR = 1.93; 95% CI: 1.64–2.28; p = 0.356) as well as a significantly higher OS (HR =0.24; 95% CI: 0.21–0.28; p < 0.001) and PFS (HR = 0.38; 95% CI: 0.34–0.42; p < 0.001) when compared with individuals with low TMB results. In addition, the study found that immunotherapy may improve OS in certain cancer types with high TMB and more positive prognosis when compared with non-ICI therapy group. These cancer types included colorectal cancer, lung cancer, melanoma, gastric cancer and pan-cancer. Based on the results of this analysis, the researchers concluded that TMB shows promise for use as a biomarker for immunotherapy treatment. They recommend establishing a standard for TMB assessment including cut-off values, to improve management of various cancer types.

In a retrospective evaluation, Cristescu et al. (2022) evaluated the association between TMB and treatment effectiveness in individuals with advanced solid tumors who were previously treated in the context of clinical trials for assessment of pembrolizumab monotherapy. This included 3 randomized trials comparing pembrolizumab with chemotherapy. The researchers defined high TMB as ≥ 175 mutations/exome and whole exome sequencing was used to determine microsatellite instability (MSI) phenotype. Immunohistochemistry was used to assess programmed death ligand 1 (PD-L1) expression. ORR was the primary endpoint of this evaluation and was assessed per Response Evaluation Criteria in Solid Tumors (RECIST) V1.1 via independent review. Additional end points included PFS and OS. Pembrolizumab monotherapy was used to treat 1,772 of the 2,234 individuals included in the study. The remaining 462 participants received chemotherapy. Of the individuals treated with pembrolizumab, ORR was 31.4% (95% CI 27.1 to 36.0) in participants with TMB ≥ 175 mutations/exome (n = 433) and 9.5% (95% CI 8.0 to 11.2) in the participants (n = 1,339) with TMB < 175 mutations/exome. Relationship between TMB and ORR was seen irrespective of PD-L1 expression and was not dependent on specific tumor types or participants with very high TMB or high MSI results. In the three randomized controlled trials, TMB was associated with ORR (p ≤ 0.016), PFS (p ≤ 0.005), and OS (p ≤ 0.029) specific to pembrolizumab but not chemotherapy (p ≥ 0.340, p ≥ 0.643, and p ≥ 0.174, respectively) and in participants with TMB ≥ 175 mutations/exome, pembrolizumab had greater efficacy compared to chemotherapy. Based on the results of this assessment, the authors concluded that a TMB of ≥ 175 mutations/exome is associated with clinically significant improvement in efficacy of pembrolizumab monotherapy and better outcomes for pembrolizumab versus chemotherapy in multiple types of previously treated advanced solid tumors, which implies that TMB has wide-ranging clinical utility regardless of tumor type, PD-L1 expression or MSI status. They advocate for use of TMB as a predictive biomarker for pembrolizumab monotherapy in individuals with previously treated advanced solid tumors.

A 2022 Hayes Precision Medicine Insight report found some support (based on review of 12 abstracts only) for comprehensive molecular profiling (CMP) of solid tumors when used to broadly profile tumor tissue and provide assistance with selection of matched therapy specific to the identified biomarkers. Hayes notes that support from professional guidelines for use of CMP in this manner is weak, citing one guideline indicating NGS may be used in some situations and two guidelines that address the need for appropriate infrastructure interpretation and implementation of test results as well as quality assurance. The report

specifically notes that the use of CMP to test for specific biomarkers with associated FDA-approved, cancer-specific therapies was not addressed in this report (Hayes, Comprehensive Molecular Profiling Test(s) for Solid Tumors Intended to be Used as Broad Molecular Profiling Tool to Assigned Matched Therapy, 2022).

In a comparative study, Ramos-Paradas et al. (2021) assessed two marketed NGS panels used for TMB evaluation in NSCLC. TruSight Oncology 500 (TSO500) and Oncomine Tumor Mutation Load (OTML) were compared to a reference assay (FoundationOne [FO]) in samples from 96 participants with NSCLC. Agreement in PD-L1 expression and level of various immune infiltrates compared to TMB were also assessed and an inter-laboratory reproducibility study was performed. Ultimately, determination was made regarding adjusted cut-off values to be used. Concordance correlation coefficients (CCC) were 0.933 (95% CI 0.908 to 0.959) for TSO500 and 0.881 (95% CI 0.840 to 0.922) for OTML, indicating strong agreement with FO. Corresponding CCCs in tumors with < 1% of cells expressing PD-L1 (PD-L1 < 1%; n = 55) were 0.951 (TSO500-FO) and 0.919 (OTML-FO). In tumors with PD-L1 ≥ 1% (n = 41), corresponding CCCs were 0.861 (TSO500-FO) and 0.722 (OTML-FO). TSO500 had higher reproducibility in the inter-laboratory reproducibility analyses and no significant differences were noted in immune infiltration compared to TMB. To guarantee sensitivity > 88%, adjusted cut-off values corresponding to 10 mut/Mb with FO needed to be lowered to 8.380 mut/Mb for OTML) and 7.847 mut/Mb for TSO500). Using these cutoff values, the positive predictive value (PPV) for TSO500 was 78.57% (95% CI 67.82 to 89.32) and the negative predictive value was 87.50% (95% CI 77.25 to 97.75) for TSO500 and the PPV for OTML was 73.33% (95% CI 62.14 to 84.52) and negative predictive 86.11% (95% CI 74.81 to 97.41). These study findings led to the conclusion that both TSO500 and OTML showed strong analytical performance for assessment of TMB. Concordance was stronger in those individuals with negative PD-L1 expression, and TSO500 demonstrated higher inter-laboratory reproducibility.

Marcus et al. (2021) summarized the FDA approval of pembrolizumab for treatment of adults and children with unresectable or metastatic TMB-high (defined as ≥ 10 mut/Mb) solid tumors. The approval specifies that TMB must be determined by an FDA-approved test and individuals must have progressed following prior treatment and have no satisfactory alternative treatment options available. The approval was based on findings from the KEYNOTE-158 multi-center single-arm trial, which showed a response rate of 29% (95% confidence interval: 21, 39) and 57% of those responses lasting ≥ 12 months in those individuals with TMB-high solid tumors (n = 102). Nine different tumor types were included. KEYNOTE-158 pre-specified ≥ 10 and ≥ 13 mut/Mb using the FoundationOne CDx assay (F1CDx) as cut-points to define the TMB-H population and TMB testing was blinded to clinical outcomes. At the same time as the approval of pembrolizumab for TMB-high indications, premarket approval was given for FoundationOne CDx to include companion diagnostic indication for TMB—high solid tumors using cut-point of 10 mut/Mb. Whole exome sequencing was used to analyze TMB in additional individuals enrolled in several different pembrolizumab clinical trials, which also supported efficacy of pembrolizumab along with comprehensive understanding of the impact of PD-1 inhibition. Adverse events were similar to those in prior trials that supported pembrolizumab approval for other indications.

Marabelle et al. (2020) published results from the KEYNOTE-158 study noted in above FDA summary by Marcus et al. KEYNOTE-158 evaluated anti-PD1 monoclonal antibody pembrolizumab in individuals with histologically or cytologically confirmed advanced and incurable solid tumor types including anal, biliary, cervical, endometrial, mesothelioma, neuroendocrine, salivary, small-cell lung, thyroid and vulvar. Participants must have either progressed on or been intolerant to one or more standard therapies, showed measurable disease as per RECIST v1.1, had Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and had adequate organ function, available tumor sample and life expectancy of at least 3 months. TMB was assessed using FoundationOne CDx with prespecified definition of TMB-high of at least 10 mut/Mb and participants received pembrolizumab 200 mg intravenously every 3 weeks for a maximum of 35 cycles. The primary outcome was proportion of participants with a complete or partial response per RECIST v1.1. Objective responses were recorded in 29% (95% CI 21–39) of 102 participants in the TMB-high group and 6% of 688 participants in the non TMB-high group. The researchers concluded that TMB-high status can help identify individuals who may have a strong response to treatment with pembrolizumab as monotherapy and TMB may thus be a helpful predictive biomarker for response in individuals with previously treated recurrent or metastatic advanced solid tumors.

The TRITON2 trial was an international open-label phase II study assessing the use of rucaparib in individuals diagnosed with metastatic castration-resistant prostate cancer (mCRPC) associated with a mutation in BRCA or another homologous recombination-directed DNA damage repair (DDR) gene who had progressed subsequent to treatment with next-generation androgen receptor (AR)-directed therapy and taxane-based chemotherapy. Abida et al. (2020) reported on results of this study related to mCRPC associated with a BRCA mutation that was treated with rucaparib twice daily as part of the TRITON2 study. Key outcomes included ORR per RECIST as determined by blinded, independent radiology reviewers and investigators and

locally assessed PSA response rate. The population under review was comprised of 115 individuals with a BRCA gene alteration that did or did not have measurable disease. Confirmed ORRs were 43.5% (95% CI, 31.0% to 56.7%; 27 of 62 participants) for those with measurable disease and 50.8% (95% CI, 38.1% to 63.4%; 33 of 65 participants) for those without measurable disease. PSA response rate was 54% (95% CI, 45.2% to 64.1%; 63 of 115 participants). Consistent ORRs were seen in individuals with germline or somatic BRCA alterations and for those individuals with a BRCA1 or BRCA2 alteration. A higher PSA response rate was seen, however, in those individuals with BRCA2 alterations. The authors concluded that data from the TRITON2 study highlight the importance of use of genomics in the identification of individuals that may benefit from treatment with a PARP inhibitor and are consistent with results of other studies on PARP inhibitors and their association with mCRPC and BRCA alterations. Although no control arm was present in this study and OS data is limited so far, the researchers assert that the TRITON2 study results support the importance of the antitumor impact of rucaparib in individuals with mCRPC and a detrimental BRCA mutation while maintaining a manageable safety profile.

CANCERPLEX (KEW Inc.) is a test that uses a solid tumor tissue sample for NGS to provide a personalized report for individuals with malignant solid tumors. The intent of the test is to help identify individuals most likely to respond to ICI therapy as well as identify presence of human papilloma virus/Epstein-Barr virus viral integration which could impact treatment decisions. A Hayes Molecular Test Assessment identified five studies addressing analytical and clinical validity of CANCERPLEX, but evidence addressing clinical validity did not provide any direct support for this test and no peer-reviewed studies addressing clinical utility were identified. Thus, evidence to support the use of CANCERPLEX to detect HPV/EB viral integration and identify individuals likely to respond to treatment with ICI is insufficient at this time (Hayes CANCERPLEX [KEW Inc], 2019, updated 2022).

FoundationOne CDx

FoundationOne CDx (F1CDx) is an FDA-approved panel that is used as a companion diagnostic test to help identify individuals who might benefit from treatment in accordance with FDA product labeling for 28 unique drug therapies. NGS-based CGP methodology is used in F1CDx to analyze 324 genes associated with cancer in solid tumor tissue. Known and likely pathogenic short variants, copy number alterations and select rearrangements as well as biomarkers including tumor mutational burden (TMB) and microsatellite instability (MSI), and in ovarian cancer, genomic loss of heterozygosity (gLOH) are reported with F1CDx. Included in a 2022 clinical and analytical validation were multiple comprehensive evaluations of F1CDx including limit of detection, limit of blank, precision and orthogonal concordance for short variants, copy number alterations, genomic rearrangements and select biomarkers. This assay validation including over 30,000 test results added to the growing body of evidence supporting clinical utility of F1CDx for matching individuals with solid tumors to targeted treatments based on their tumor's genomic variations and biomarkers. (Milbury et al., 2022)

In a prospective cohort study evaluating the role of CGP with F1CDx, Takeda et al. (2021) performed genomic testing on 181 tumor tissue samples from individuals with cytologically or histologically confirmed advanced or recurrent solid tumor cancers. Of the total samples, data was successfully obtained for 175 samples. Known and likely pathogenic actionable variations were found in 174 individuals (99%) and 24 of those (14%) received matched targeted therapy. TP53 (n = 113), PIK3CA (n = 33), APC (n = 32), and KRAS mutations (n = 29) were the most common known/likely pathogenic variants found. Of 153 individuals evaluated for TMB, median TMB was 4 mutations/Mb. Tumors with high TMP defined as ≥ 10 mutations/Mb were more likely to be lung cancer (11/32) than other solid tumor types (9/121). The authors concluded that F1CDx assay testing had an overall success rate of > 95% and may assist with matching individual tumors with targeted therapy.

Hayes addressed the use of FoundationOne CDx for use as a broad molecular profiling tool in a 2022 Molecular Test Assessment. The evidence base for this indication consisted of three clinical utility studies which reported no difference in outcomes between treatment directed by FoundationOne CDx results and treatment not directed by use of FoundationOne CDx. As such, the evidence was determined to be insufficient for this indication. The Hayes report did not assess the use of FoundationOne CDx for the primary purpose of evaluating predetermined biomarkers that are associated with at least one FDA-approved therapy for the individual's specific cancer type, nor did it address clinical or analytical validity, which would require focused review of individual biomarkers (Hayes, FoundationOne CDx [Foundation Medicine Inc.] for the Intended Use as a Broad Molecular Profiling Tool, 2022).

Trédan et al. (2019) studied the impact of molecular profiling on adult and pediatric patients with solid or hematological advanced cancer that was previously treated in advanced/metastatic settings. The profile was performed on tumors, relapse or biopsies and then reviewed by a molecular tumor board to determine if any molecular-based therapies were available. At four different institutions, 2,579 patients were enrolled, and the tumor board reviewed 1,980 patient molecular profiles. There were some genes determined to be most frequently altered and those included: CDKN2A (n = 181, 7%), KRAS (n = 177, 7%), PIK3CA

(n = 185, 7%), and CCND1 (n = 104, 4%). A molecular-based therapy was recommended for 699/2579 patients (27%), however only 163/2579 patients (6%) received at least one MBRT. Likewise, out of the 182 lines of therapy initiated, 23 (13%) partial responses were observed. Overall, only 0.9% of the whole cohort experienced an objective response. The researchers concluded that molecular screening should not be used at present to guide clinical decision-making outside of a clinical trial.

Hirshfield et al. (2016) conducted a prospective clinical study on 100 patients with diverse-histology, rare, or poor-prognosis cancers to evaluate the clinical implications of a comprehensive genomic profiling assay (FoundationOne), using formalin-fixed, paraffin-embedded tumors. The primary objectives were to assess utility, feasibility, and limitations of genomic sequencing for genomically guided therapy or other clinical purpose in the setting of a multidisciplinary molecular tumor board. Of the tumors from the 92 patients with sufficient tissue, 88 (96%) had at least one genomic alteration (average 3.6, range 0–10). Use of comprehensive profiling led to implementable clinical action in 35% of tumors with genomic alterations, including genomically guided therapy, diagnostic modification, and trigger for germline genetic testing. Although use of targeted next-generation sequencing in the setting of an institutional molecular tumor board led to implementable clinical action in more than one third of patients with rare and poor-prognosis cancers, major barriers to implementation of genomically guided therapy were clinical status of the patient and drug access. Early and serial sequencing in the clinical course and expanded access to genomically guided early-phase clinical trials and targeted agents may increase clinical application.

Kato et al. (2015) investigated the clinical correlates of CDK4/6 and CDKN2A/B abnormalities in diverse malignancies. Patients with various cancers who underwent molecular profiling by targeted next generation sequencing (Foundation Medicine; 182 or 236 cancer-related genes) were reviewed. Of 347 patients analyzed, 79 (22.8%) had aberrant CDK 4/6 or CDKN2A/B. Only TP53 mutations occurred more frequently than those in CDK elements. Aberrations were most frequent in glioblastomas (21/26 patients, 81%) and least frequent in colorectal cancers (0/26 patients). Aberrant CDK elements were independently associated with EGFR and ARID1A gene abnormalities. CDK aberrations were associated with poor overall survival. In multivariate analysis, PTEN and TP53 aberrations were independently associated with poorer survival; CDK aberrations showed a trend toward worse survival. There was also a trend toward worse progression-free survival (PFS) with platinum-containing regimens in patients with abnormal CDK elements (3.5 versus 5.0 months). In conclusion, aberrations in the CDK pathway were some of the most common in cancer and independently associated with EGFR and ARID1A alterations. Patients with abnormal CDK pathway genes showed a trend toward poorer survival, as well as worse PFS on platinum-containing regimens. According to the authors, further investigation of the prognostic and predictive impact of CDK alterations across cancers is warranted. This study was limited due to it being performed retrospectively in a single institution with a relatively limited number of patients.

Johnson et al. (2014) retrospectively assessed demographics, next-generation sequencing (NGS) results, and therapies received for patients undergoing targeted NGS using the FoundationOne test. Co-primary endpoints were the percentage of patients with targeted therapy options uncovered by mutational profiling and the percentage who received genotype-directed therapy. Samples from 103 patients were tested; most frequently found were breast carcinoma (26%), head and neck cancers (23%), and melanoma (10%). Most patients (83%) were found to harbor potentially actionable genetic alterations, involving cell-cycle regulation (44%), phosphatidylinositol 3-kinase-AKT (31%), and mitogen-activated protein kinase (19%) pathways. With median follow-up of 4.1 months, 21% received genotype-directed treatments, most in clinical trials (61%), leading to significant benefit in several cases. The most common reasons for not receiving genotype-directed therapy were selection of standard therapy (35%) and clinical deterioration (13%). The authors concluded that mutational profiling using a targeted NGS panel identified potentially actionable alterations in a majority of advanced cancer patients. The assay identified additional therapeutic options and facilitated clinical trial enrollment. According to the authors, there are many unanswered questions regarding implementation of this technology. First, based on this study, some patients with potentially actionable alterations did not respond to genotype-directed therapy, highlighting the still underdeveloped understanding of the pathophysiologic implications of many genetic alterations. Second, the most appropriate indications for obtaining targeted NGS are not yet clear. Third, randomized studies in the future will need to assess whether targeted NGS improves overall outcomes.

Frampton and colleagues (2013) conducted an analytical and clinical validation study to evaluate massively parallel DNA sequencing using the FoundationOne assay to characterize base substitutions, indels, copy number alterations, and selected fusions across 287 cancer-related genes from routine formalin-fixed and paraffin-embedded (FFPE) clinical specimens. The authors implemented a validation strategy with reference samples of pooled cell lines that modeled key drivers of test accuracy, including mutant allele frequency, indel length and amplitude of copy change. Test sensitivity achieved was 95% to 99% across alteration types, with high specificity (positive predictive value [PPV] > 99%). The authors confirmed accuracy using 249 FFPE cancer specimens characterized by established assays. Application of the test to 2,221 clinical cases revealed clinically actionable alterations in 76% of tumors, three times the number of actionable alterations detected by current diagnostic tests.

This study did not evaluate the clinical utility of such findings in improving care and outcome of patients by tailoring treatments or predicting response to treatment. Hence, it is important to note that the clinical utility of genomic profiling using massively parallel DNA sequencing remains unknown. In addition, study authors colleagues did not categorize the data regarding sensitivity, specificity, and positive predictive value (PPV) by cancer type.

FoundationOne Heme

FoundationOne Heme analyzes sequence information for gene variations in human hematological malignancies and sarcomas. Included genes code for known or likely targets for treatments or known drivers of oncogenesis. Analysis of complete coding DNA sequences of 406 genes as well as selected introns of 31 genes associated with rearrangements is included, as well as RNA sequences of 265 commonly rearranged genes so that gene fusions can be more clearly identified. Foundation One Heme was evaluated for characterization of 81 histologically confirmed localized soft tissue sarcomas (STS) from a single institution (Department of Othopaedics and Trauma, Medical University of Graz) in a 2021 retrospective study. All sarcomas were diagnosed as per WHO Classification of Tumours of Soft Tissue and Bone and were graded per the French Federation of Cancer Centres Sarcoma Group or by tumor entity. Five or more genetic variations (average of 12 variations) were detected per individual, which suggested the assay's coverage is broad. However, sensitivity for fusion detection was low (42%.4) and will require further evaluation in larger cohorts. Overall, the authors concluded that the molecular findings in this small cohort support existing evidence for potential therapeutic targets for the treatment of STS. Additional high-quality studies with larger and more diverse populations are required. (Scheipl et al., 2021)

In a 2018 Molecular Test Assessment, Hayes found insufficient published evidence to support genomic profiling using FoundationOne Heme for hematologic malignancies and sarcomas. Further study is required to establish clinical validity and utility for this test (Hayes, FoundationOne Heme [Foundation Medicine Inc.], 2018, updated 2022).

MI Profile and MI Tumor Seek (Caris Life Sciences)

In 2022, Hayes published a Molecular Test Assessment on the MI Profile (Caris Life Sciences) for the proposed use as a broad molecular profiling tool to detect tumor biomarkers and allocate matched therapy specific to those biomarkers for individuals with solid tumors. The MI Profile performs multiplatform solid tumor biomarker analysis by using DNA (NGS-based WES), RNA (NGS-based whole transcriptome sequencing) and proteins from solid tumor tissue samples to report on biomarker variation results, therapeutic agents associated with biomarker results and finally, applicable open clinical trials the individual may be eligible for, to assist oncologists with treatment decisions. The review uncovered no peer-reviewed studies meeting the inclusion criteria for evaluation of clinical utility; as such, overall quality of evidence was not rated and at this time and Hayes concluded that there is insufficient data to support clinical utility of the MI Profile for use as a broad molecular profiling tool at this time. The Hayes report did not address the use of this test for the primary purpose of testing limited biomarkers that have one or more associated FDA-approved therapies for the specific cancer types, or the analytical or clinical validity of the test (Hayes, MI Profile [Caris Life Sciences] for the Intended Use as a Broad Molecular Profiling Tool, 2022).

Hayes also published a Molecular Test Assessment evaluating analytical validity, clinical validity, and clinical utility the MI Tumor Seek test by Caris Life Sciences, which uses an NGS platform to seek DNA point mutations, copy number alterations, insertions/deletions, genomic signatures (MSI and TMB) and RNA whole transcriptome sequencing to ostensibly provide clinically actionable information to support personalized therapies for individuals with cancer. The review found insufficient evidence to support the use of the Tumor Seek test to assist physicians with clinically actionable data to provide tailored cancer therapies. One study assessing analytical validity on MI Tumor Seek suggested that this platform is capable of detecting MSI, but no analytical validity studies were found that included the entire Tumor Seek test and the existing published studies pertinent to this technology addressed molecular landscape only (Hayes, MI TumorSeek (Caris Life Sciences), 2019, updated 2020).

Liquid Biopsy

Liquid biopsy is a non-invasive technique of obtaining bodily fluids, such as blood, urine, cerebrospinal fluid, saliva, and other aspirates, to analyze different types of biomolecules including circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and exosomes. Liquid biopsies have been investigated for a number of cancer types; however, this testing has not been widely accepted yet. Research continues to study this technique for non-invasive methods that may assist in therapeutic decisions without traditional biopsy.

In a systematic review and meta-analysis in 2022, Palmieri et al. evaluated the diagnostic performance of circulating free DNA (cfDNA) compared to tissue testing for KRAS mutations. Forty studies including 2,805 individuals with non-small cell lung cancer NSCLC were identified and values were extracted concerning the number of true-positive, false-positive, false-negative, and true-negative. Overall diagnostic performance was assessed and pooled sensitivity for cfDNA was 0.71 (95% CI 0.68–0.74), and specificity was 0.93 (95% CI 0.92–0.94). Also, the meta-analysis showed high specificity and area under curve (AUC) > 0.9, standing for a general high diagnostic efficacy in the exposure of KRAS mutations by cfDNA investigation. The values of the likelihood ratios (PLR and NLR) showed the informativeness of the test on cfDNA. Limitations included high variability among clinical stages, the small size of some studies, and the risk of bias. The authors concluded that the outcomes offer evidence that identifying KRAS mutation via cfDNA testing is of reasonable diagnostic accuracy and offers promise as a screening test for individuals with NSCLC. Authors Thompson et al. (2016), Sacher et al.(2016), and Leighl et al.(2019), previously cited in this policy, were included in the Palmieri systematic review.

Hayes Precision Medicine Insights reports addressed comprehensive molecular profiling (CMP) of circulating solid tumor DNA when used as a broad molecular profiling tool to assist with both treatment selection and monitoring. According to Hayes, minimal support and very minimal support, respectively, was found for these indications in the peer-reviewed literature, with no clear evidence of clinical utility for either selection of treatments or monitoring. In applicable professional guidelines, weak support was found for use of CMP to assist with clinical decision-making for biomarker-matched treatment and to aid in monitoring treatment response or failure. The majority of guidelines addressing CMP of circulating solid tumor DNA were disease specific (most often for NSCLC or GI tract cancers.) In addition, recommendations focused on individuals with metastatic/advanced disease and some guidelines recommended use only when tissue biopsy is not possible (Hayes, Comprehensive Molecular Profiling of Circulating Solid Tumor DNA for the Intended Use as a Broad Molecular Profiling Tool to Aid Treatment Selection, 2022; Hayes, Comprehensive Molecular Profiling of Circulating Solid Tumor DNA for the Intended Use as a Broad Molecular Profiling Tool for Monitoring, 2022).

In a 2021 systematic review and meta-analysis, Zhang et al. studied the predictive value of TMB in the blood (bTMB) using studies evaluating bTMB use in ICIs or the efficacy of ICIs compared with chemotherapy. A total of seven trials including 2,610 individuals with NSCLC were included in the systematic review. No significant differences between high and low bTMB groups in the ICI cohort were found with regard to OS (HR = 1.09; 95% CI: 0.62–1.91, p = 0.774) or PFS (HR = 0.73; 95% CI: 0.20–2.65, p = 0.629). In the comparisons of ICI to chemotherapy, ICIs showed improvement in OS (HR = 0.74; 95% CI: 0.59–0.92, p = 0.006), but improvement in PFS and ORR was attributable to a mathematical trend only (PFS: HR = 0.83; 95% CI: 0.63–1.09, P = 0.173; ORR: RR = 0.92, 95% CI: 0.77–1.10, p = 0.372). Participants treated with ICIs in the high bTMB group had greater survival benefits than individuals receiving chemotherapy in terms of OS (HR = 0.63; 95% CI: 0.51–0.76, p < 0.001), PFS (HR = 0.63; 95% CI: 0.52–0.76, p < 0.001), and ORR (RR = 1.86; 95% CI: 1.32–2.62, p < 0.001). In the low TMB group, there was either no change in the outcome or a reversal of the findings in the high bTMB group (OS: HR = 0.89; 95% CI: 0.64–1.24, p = 0.485; PFS: HR =1.21, 95% CI: 0.93–1.58, p = 0.154; ORR: RR = 0.68, 95% CI: 0.54–0.85, p = 0.001). Limitations included the heterogeneity of the studies, the risk of bias, and the retrospective nature of the studies reviewed. The authors concluded that TMB has been shown to be a reliable biomarker for identifying individuals with NSCLC who may benefit from ICI. The role of bTMB remains limited at this time, and more prospective data are needed.

In an effort to analyze the incidence and varying aspects of circulating tumor DNA (ctDNA) and evaluate its association with metastatic disease recurrence after longer than 5 years in individuals diagnosed with high-risk, early stage hormone receptor positive (HR+) breast cancer, Lipsyc-Sharf et al. (2022) conducted a prospective study enrolling 103 individuals. Participants had no evidence of recurrence at enrollment. WES was performed on archived tumor tissue from initial breast cancer surgery and detection of somatic mutations was then leveraged to personalize a ctDNA RaDaR assay, which was applied every 6-12 months at routine follow up visits via plasma collection. Of the initial 103 individuals enrolled, 85 had sufficient tumor tissue available for sequencing (at least 20% of tumor present). Of those, WES was successfully performed for 83 tumor samples. Median age at time of initial diagnosis was 53 years and all were female. A median of 26 variants were targeted to test 219 total plasma samples (median number of plasma samples per individual was two). Eight individuals in the group had positive MRD testing at any point in time, and six of these developed distant metastatic recurrence, with median ctDNA lead time of 12.4 months. MRD was not identified in one individual with a localized recurrence. The final two of the eight individuals with positive MRD had not had clinical recurrence at their last follow-up visit. For individuals with high-risk HR+ breast cancer greater than 5 years from initial diagnosis, the researchers found that ctDNA was identified approximately one year before all cases of distant metastasis in this study. Further high-quality studies are needed to determine if ctDNA-guided interventions will ultimately impact clinical outcomes for individuals with cancer.

A Hayes Clinical Utility Evaluation indicates that evidence documenting the ability of liquid biopsy testing to identify early-stage colorectal cancer and high-risk adenoma accurately in an unselected, prospective population is insufficient to support conclusions regarding clinical utility at this time. Per the Hayes report, evidence for other types of liquid biopsy screening tests for CRC are lacking as well (Hayes, Liquid Biopsy Tests for Colorectal Cancer Screening, 2020, updated 2022).

Petit et al. (2019) performed a systematic review to determine the evidence available regarding ctDNA as a screening tool for colorectal cancer. After review, 69 studies were included and 17 studies reviewed total cell free DNA, six studies looked at the DNA integrity index and 15 focused on ctDNA. While the researchers concluded that ctDNA is a promising candidate for colorectal cancer screening, further researcher is required.

A study on renal cell carcinoma by Yamamoto et al. (2019) evaluated circulating tumor DNA for clinical utility. Fifty-three patients histologically diagnosed with clear cell RCC were enrolled and sequencing was performed on plasma cell-free DNA (cfDNA) and tumor DNA. A total of 38 mutations across 16 (30%) patients were identified from cfDNA, including mutations in TP53 (n = 6) and VHL (n = 5), and median mutant allele frequency of ctDNA was 10%. The researchers concluded that this study shows the clinical utility of ctDNA for prognosis and disease monitoring in RCC.

A study by Lam et al. (2019, included in Hayes Guardant360 [Guardant Health Inc.], 2018) studied lung squamous-cell carcinoma (LUSC) and cfDNA. The researchers retrospectively evaluated 492 LUSC patients: 410 patients (stage 3B or 4 LUSC) were tested with a targeted cell-free circulating DNA NGS assay and 82 patients (any stage) were tested with a tissue NGS cancer panel. Overall, 467 patients (95%) had a diagnosis of LUSC, and 25 patients (5%) had mixed histology. Of the LUSC subgroup, a total of 11% had somatic alterations with therapeutic relevance in the cfDNA testing, including in EGFR (3%), ALK/ROS1 (1%), BRAF (2%), and MET amplification or exon 14 skipping (5%). Three of these patients were treated with targeted therapy and all experienced a partial response. Of the group with mixed histology, 16% had an actionable alteration. The researchers found actionable alterations in genes that were clinically significant through this testing; however, they state that further evaluation is needed.

InVisionFirst is a liquid biopsy test that analyzes the presence of relevant genetic variants in the ALK, BRAF, EGFR, ERBB2, KRAS, MET, ROS1 and STK11 and 26 other genes in patients with non-small cell lung cancer. Plagnol et al. (2018) reported on the analytical validation of the TAm-Seq technology utilized in InVisionFirst Lung. At least two 10ml tubes of blood were collected from each donor into Streck Cell Free DNA Blood Collection tubes (BCT) and EDTA tubes. Ninety-five samples from healthy donors were analyzed for gene fusions, and no genetic variants were found. One hundred and nine samples from healthy donors were analyzed for SNVs, indels and amplifications, and no copy number variants were found. Three splice site variants were found. Digital PCR (dPCR) was performed on these three and a TP53 mutation was confirmed, but not the other two. A further 92 samples from healthy donors and 242 samples from untreated NSCLC patients were tested, and these three variants were not seen. In the affected group, twenty NSCLC patients were tested by both InVisionFirst and dPCR at two separate labs, who were blinded to each other's results. In this cohort, 40% of patients had a genetic variant. dPCR detected 19 of 20 expected changes. InVisionFirst identified a mutation in one sample not seen with dPCR, and the sample had a very low cfDNA fraction. It cannot be determined if this was a true positive undetectable by dPCR or a false positive. In addition, contrived samples using various seeded cell lines and reference material were used to simulate a wide array of copy number and other genetic variations were tested in the same way. Overall, in the donor samples and contrived materials, the concordance rate between InVisionFirst and dPCR was high. InVisionFirst demonstrated a > 99% sensitivity for SNVs and > 92% for indels.

Sun et al. (2018) published a study examining liquid biopsies in colorectal cancer (CRC). The researchers analyzed blood from 140 CRC patients with matched tumor samples. Both the circulating tumor cells (CTC) and tumor DNA (ctDNA) were extracted before surgery and treatment. The samples were quantified and tested for mutations in KRAS, NRAS and BRAF. Within this sample cohort, there was good agreement between the CTC and the ctDNA (97% concordance). The researchers also determined that patients who were refractory to specific medications showed molecular profile changes and were positive for KRAS, NRAS or BRAF. This was noteworthy as the changes were detected in the circulating tumor cells first. The study concluded that using CTC and ctDNA for monitoring CRC patients molecular profile changes to treatment may be useful.

A study from Dieffenbacher et al. (2018) evaluated tumor tissue and liquid biopsies in metastatic clear cell renal cell cancer patients in the MORE-TRIAL. Samples were performed at baseline and first and second progression under treatment. The study stated that this relatively new technique may help to avoid the necessity for invasive biopsies in the future and a further aim of MORE is to study the reliability and relevance of ctDNA in RCC patients.

Cohen et al. (2017) conducted a cohort study to develop a noninvasive test for detection of pancreatic ductal adenocarcinoma. They combined blood tests for KRAS gene mutations with protein biomarkers as a testing method. They tested this assay on a cohort of 221 patients with resectable pancreatic ductal adenocarcinomas and 182 control patients without known cancer. In the plasma samples of 66 patients (30%), KRAS mutations were detected, and every mutation found in the plasma was also detected in the primary tumor (100% concordance). This combination of tests increased the sensitivity to 64%. Only one of the control samples was positive for any of the DNA or protein biomarkers (99.5% specificity). The researchers concluded that this approach may prove useful for early cancer detection.

Kim et al. (2017, included in the 2018 Hayes Guardant360 [Guardant Health Inc.] Molecular Test Assessment) performed a prospective study on solid tumor cancers and ctDNA guided matched therapy. The testing identified point mutations in 70 genes and indels, fusions, and copy number amplifications in selected genes. Alterations in somatic genes was detected in 59 patients with gastric cancer (78%), and 25 patients (33%) had targetable alterations (ERBB2, n = 11; MET, n = 5; FGFR2, n = 3; PIK3CA, n = 6). In NSCLC, 62 patients (85%) had somatic alterations, and 34 (47%) had targetable alterations (EGFR, n = 29; ALK, n = 2; RET, n = 1; ERBB2, n = 2). In a small subgroup of patients that had tissue available for confirmation (10 with gastric cancer and 17 with NSCLC), molecularly matched therapy was initiated. The response rate and disease control rate in this group was 67% and 100%, respectively, in gastric cancer and 87% and 100%, respectively, in NSCLC. Response was independent of targeted alteration variant allele fraction in NSCLC (p = .63). The researchers concluded that response rates in this analysis were similar to tissue-based targeted therapy studies.

Oxnard et al. (2016) studied whether noninvasive genotyping of cell-free plasma DNA (cfDNA) is a useful biomarker for prediction of outcome from a third-generation *EGFR*-TKI, osimertinib. All patients had plasma collected and genotyping was performed by using BEAMing. The use of plasma genotyping for detection of T790M had a sensitivity of 70%. Of 58 patients with T790M-negative tumors, T790M was detected in plasma of 18 (31%). This study suggested that the use of plasma T790M assays could help certain patients avoid a tumor biopsy for T790M genotyping. However due to the 30% false-negative rate of plasma genotyping, patients with T790M-negative plasma results still need a tumor biopsy to determine presence or absence of T790M.

FoundationOne Liquid CDx

FoundationOne Liquid CDx (Foundation Medicine, Cambridge, MA) is an FDA-approved test that can detect gene variations (> 300 genes tested) in circulating cfDNA that has been isolated from whole blood plasma samples (also referred to as “liquid biopsy”). Results can help providers identify individuals that might benefit from certain cancer drugs. Details on indications, biomarkers and FDA-approved treatments associated with those biomarkers are noted in the table below.

Indications	Biomarkers Detected	FDA-approved treatment
Non-small cell lung cancer	ALK rearrangements, EGFR exon 19 deletions and EGFR exon 21 L858R substitution, MET single nucleotide variants (SNVs) and indels that lead to MET exon 14 skipping, ROS1 mutations	Alecensa® (alectinib) Iressa® (gefitinib) Tagrisso® (osimertinib) Tarceva® (erlotinib) Tabrecta® (capmatinib) Rozlytrek® (entrektinib)
Prostate cancer	BRCA1, BRCA2, ATM alterations	Lynparza® (olaparib) Rubraca® (rucaparib)
Ovarian cancer	BRCA1, BRCA2 alterations	Rubraca® (rucaparib)
Breast cancer	PIK3CA mutations	Piqray® (alpelisib)

Caputo et al. (2022) used the FoundationOne Liquid Analysis (either FoundationOne Liquid [70 genes] or FoundationOne CDx [324 genes]) to evaluate clinical impact and viability of these tests across different tumor types. In all, 398 samples from various tumor types were evaluated with an overall success rate of 92% (97% success rate in FoundationOne Liquid CDx individually). The most common molecular alterations were *TP53* (74), *APC* (40), *DNMT3A* (39) and *KRAS* (23). Overall clinical impact of FoundationOne Liquid Analysis use compared to standard diagnostic testing was 64.7% vs 22.1% [risk ratio (RR) = 2.94; p < 0.001] and potential clinical impact was 58.6% compared to 11.0% (RR = 5.32; p < 0.001). Also noted is that FoundationOne Liquid Analysis detected actionable alterations that offered an unexpected therapeutic choice. The authors assert that NGS

using FoundationOne Liquid Analysis is a helpful assay to guide treatment decisions in oncology, but comment that more study is needed in terms of selection criteria for affected individuals to avoid over-diagnosis.

Dziadziuszko et al. (2021) reported on the ongoing Blood First Assay Screening Trial (BFAST) in a 2021 publication. BFAST is an open-label, multi-cohort study which is prospectively analyzing the association between blood-based NGS detection of actionable genetic alterations and the activity of targeted treatments including therapy/immunotherapy in individuals with advanced or metastatic NSCLC who have not yet received treatment. The trial includes adults (18 years or older) with stage IIIB or IV NSCLC and *ALK* rearrangements detected by blood-based NGS (Foundation ACT). These individuals received alectinib 600 mg twice daily. In this trial, asymptomatic or treated central nervous systema metastases were permitted. Primary outcome was investigator-assessed objective response rate (ORR); secondary outcomes included independent review facility-assessed ORR, duration of response, progression-free survival (PFS), overall survival (OS) and safety. A total of 2,219 individuals were screened and of those, 98.6% produced results from blood-based NGS. *ALK*-positive disease was found in 119 individuals (5.4%) and of these, 87 were enrolled and treated with alectinib. Confirmed ORR by investigator was 87.4% (95% confidence interval [CI]: 78.5–93.5) and 92% (95% CI: 84.1–96.7) by independent review facility. The investigator-confirmed 12- month duration of response was 75.9% (95% CI: 63.6–88.2). Of the 35 (40%) individuals with baseline CNS disease, investigator-assessed ORR was 91.4% (95% CI: 76.9–98.2). The 12-month investigator-assessed PFS was 78.4% (95% CI: 69.1–87.7) and median PFS was not reached due to the limited follow-up time and number of events. The safety findings were consistent with the known tolerability of alectinib. Based on these findings, the researchers concluded that the clinical application of blood-based NGS, a less invasive diagnostic tool, predicts for high ORR and substantial clinical benefit and may be used as a method to assist with clinical decision-making in individuals with *ALK*-positive NSCLC.

In a clinical and analytical validation of FoundationOne Liquid CDx, Woodhouse et al. (2020) published data to support the use of this test across multiple types of cancer. Validation studies for FoundationOne Liquid CDx included over 7,500 tests and more than 30,000 individual variants over more than 300 genes and > 30 types of cancer. The results of this analysis show a 95% limit of detection of 0.40% variant allele fraction for select substitutions and insertions or deletions, 0.37% variant allele fraction for select rearrangements, 21.7% tumor fraction for copy number amplifications and 30.4% TF for copy number losses. The false positive variant rate was 0.013% or 1 in 8,0000. Reproducibility of variant identification was 99.59%. Overall positive percent agreement and negative percent agreement of 96.3% and > 99.9%, respectively, was observed. The authors concluded that FoundationOne Liquid CDx is accurate with reproducible results can reliably detect the main types of genomic alterations as well as complex biomarkers (e.g., microsatellite instability, blood tumor mutational burden, and tumor fraction).

Galleri

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

Klein et al. (2021) documented the results of an observational study to validate a multi-cancer early detection test designed to complement existing screening methods and potentially increase the number of cancers found through population screening, potentially impacting and improving clinical outcomes. Including 4077 participants in an independent validation set (cancer n = 2823, non-cancer n = 1254), sensitivity, specificity and cancer signal origin (CSO) were measured. This population was a pre-specified sub-study of the Circulating Cell-free Genome Atlas Study, a prospective, multi-center, observational study designed to collect biological samples (blood and tumor tissue) both from participants with newly diagnosed cancer and from participants without a diagnosis of cancer to characterize population heterogenicity in cancer and cancer-free participants so that models for distinguishing between cancer and non-cancer could be developed. According to the authors, the Atlas study demonstrated that MCED testing using cfDNA in combination with machine learning could detect cancer signals across various cancer types and predict cancer signal origin with high accuracy. The objective of the current study is to further validate an MCED test that has been refined for use as a screening tool. Overall sensitivity for cancer signal detection was 51.5% and showed increasing sensitivity with stage of cancer. Cancer signal detection specificity was 99.5% (95% confidence interval). Cancer signals were detected across more than 50 cancer types. CSO prediction in true positives was 88.7% overall. The researchers concluded that the MCED test showed high specificity and accuracy in prediction of CSO and detected signals across multiple cancer types. A noted limitation is that blood sample collection from participants with cancer done after biopsies had been performed could increase the possibility that tumor cfDNA fraction could also increase relative to pre-biopsy.

In addition, CCGA is a case-control study, so would not reflect performance in a screening population. Further studies evaluating test performance and clinical utility in target-use population are needed.

In a prospective case-control sub-study of the Atlas and STRIVE studies (NCT02889978 and NCT03085888), the performance of targeted methylation analysis of cfDNA in detecting and localizing multiple cancer types across all stages, with high specificity, was assessed. A total of 6689 participants (2482 with cancer [over 50 types), 4207 without cancer] were grouped into training or validation sets. Cell-free DNA was sequenced, targeting a panel of over 100,000 informative methylation areas. From this, a classifier was developed and validated for detection of cancer and localization of tissue of origin. The publication (Liu et al., 2020) documented consistent performance in both the training and validation sets. In the validation set, specificity was 99.3%. Stage I-III sensitivity was 67.3% in a pre-selected set of 12 cancer types (head and neck, esophagus, liver/bile-duct, anus, colon/rectum, bladder, plasma cell neoplasm, stomach, pancreas, ovary, lung, and lymphoma), which make up approximately 63% of annual cancer deaths in the US. Stage I-III sensitivity was 43.9% in all cancer types, with increase in detection as cancer stage increased. Tissue of original was predicted in 96% of samples with cancer-like signals and of that group, the tissue of origin localization was accurate in 93%. In conclusion, the researchers indicate that cfDNA sequencing using informative methylation patterns warrants further evaluation in prospective, population-level studies.

Guardant 360 CDx

Guardant 360 CDx (Guardant Health, Redwood City, CA) is an FDA-approved liquid biopsy for advanced solid tumors, intended to be used as a companion diagnostic to identify patients with non-small cell lung cancer (NSCLC) who might benefit from targeted therapies. This test uses circulating cell-free DNA (cfDNA) from the plasma of peripheral whole blood and high throughput hybridization-based capture technology to detect single nucleotide variants (SNVs), insertions and deletions in 55 genes, fusions in 4 genes and copy number amplifications (CNAs) in 2 genes.

Olsen et al. (2022, included in Hayes Comprehensive Molecular Profiling of Circulating Solid Tumor DNA for the Intended Use as a Broad Molecular Profiling Tool to Aid Treatment Selection, 2022) evaluated data from 3,084 individuals with advanced NSCLC who had been registered in a real-world healthcare claims database and had undergone NGS-based circulating tumor DNA (ctDNA) testing with Guardant360 after first-line treatment. In 89.9% of the samples, ctDNA was detected and 41.9% of those samples showed actionable variations (most commonly EGFR – 29.7%).Of individuals previously treated with non-targeted drugs, actionable alterations were found in 26.7% of individuals and emerging and potentially targetable mutations were found in 40.1%. In patients whose ctDNA testing showed qualifying alterations, time to discontinuation of therapy and overall survival were longer in individuals who received matched second-line treatment versus unmatched second-line treatment. The authors concluded that use of blood-based NGS assays before second-line treatment helps to inform treatment-making decisions that may improve clinical outcomes in individuals with advanced NSCLC in a real world practice situation. Of note, this study was limited to biomarker testing using only the Guardant Health testing platform and Guardant Health funded this study.

In 2022, Bauml (included in the Palmieri systematic review above) assessed the clinical validation of Guardant 360 CDx as a blood-based companion diagnostic for sotorasib to detect KRAS p. G12C (an oncogenetic non-small cell lung cancer driver mutation). The primary aim of the current analysis was to evaluate the clinical validity of Guardant360 CDx via data and samples from the CodeBreaK100 (NCT03600883) study. The secondary purposes were to evaluate the concordance among KRAS p.G12C mutation status decided by the therascreen® KRAS RGQ PCR kit and Guardant360 CDx in individuals with NSCLC; to assess the representativeness of the Guardant360 CDx–positive cohort related to the entire analysis group. And to consider DOR, DCR, and time to response (TTR) in individuals with KRASp.G12C–mutant NSCLC as detected by Guardant360 CDx comparative to the whole analysis group. The ORR (95% CI; individuals with objective response/all individuals in the dataset) for all individuals was 37.1% (28.6%, 46.2%; n = 46/124) in the Full Analysis Set, 36.4% (25.7%, 48.1%; n = 28/77) in the Guardant360 positive cohort, and 46.7% (28.3%, 65.7%; n = 14/30) in the Guardant360 negative cohort. Rates of PD, SD, and PR were similar among the cohorts, with SD being the most common outcome (Full Analysis Set, n = 54/124 [43.5%]; Guardant360 Evaluable, n = 46/107 [43.0%]; Guardant360 positive, n = 32/77 [41.6%]; Guardant360 negative, n = 14/30 [46.7%]). DCR (95% CI; individuals with disease control/all those in the dataset) was 80.6% (72.6%, 87.2%; n = 100/124) in the Full Analysis Set and 77.9% (67.0%, 86.6%; n = 60/77) in the Guardant360 positive cohort. Among responders, DOR was ≥ 3 months in 38/46 (82.6%) those in the Full Analysis Set and 24/28 (85.7%) individuals in the Guardant360 positive cohort; DOR was ≥ 6 months in 28/46 (60.9%) and 15/28 (53.6%) those in the Full Analysis Set and Guardant360 positive cohort, respectively. Of the four cohorts, DOR ≥ three months among responders was numerically highest in the Guardant360 positive cohort (n = 24/28 [85.7%]), while DOR ≥ 6 months was mathematically highest in the Guardant360 negative (n = 9/14 [64.3%]) cohort. The average time to objective response was comparable between all cohorts. The authors concluded that liquid biopsy

using Guardant360 CDx has clinical validity for the identification of individuals with KRASp.G12C–mutant NSCLC and, amplified by tissue testing methodologies, will identify individuals for treatment with sotorasib.

Dagogo-Jack et al. (2019) performed a study on ROS1 fusions in NSCLC with the Guardant360 NGS assay and the Guardant Health plasma dataset (n = 56). The assay part of the study aimed to detect potential genetic mediators of resistance in the plasma of patients with ROS-1 positive NSCLC who were relapsing on crizotinib. The researchers found that the sensitivity for detection of ROS1 fusions in plasma at relapse on crizotinib therapy was 50%. Of 18 post-crizotinib plasma specimens, six (33%) had ROS1 kinase domain mutations (five were ROS1 G2032R). Two (11%) post-crizotinib plasma specimens had genetic alterations (n = 1 each BRAF V600E and PIK3CA E545K). Additionally, the plasma dataset provided by Guardant Health was compared to institutional tissue data. There was 100% concordance between the specific tissue- and plasma-detected ROS1 fusion for seven patients genotyped with both methods.

In a 2019 publication, Aggarwal et al. (included in Hayes Guardant360 Molecular Test Assessment, 2018) reported the results of their prospective cohort study designed to determine whether plasma next-generation sequencing (NGS) was associated with increased detection of mutations and better delivery of targeted therapy for NSCLC in a “real-world” setting. A total of 323 individuals with metastatic NSCLC were enrolled from April 1, 2016, to January 2, 2018. For these individuals, plasma testing had been ordered as part of standard clinical management. Plasma NGS was performed using the 73-gene platform (Guardant Health). Therapeutically targetable mutations in EGFR, ALK, MET, BRCA1, ROS1, RET, ERBB2 or BRAF were detected for 113 individuals (35.0%). Of the 323 patients tested, 94 had only plasma testing at the discretion of the treating physician or related to patient preference. Of those, 31 (33.0%) had a therapeutically targetable mutation detected (eliminating the need for invasive biopsy). In the remaining 229 participants who had undergone both plasma and tissue NGS (or were unable to have tissue NGS) a therapeutically targetable mutation was found in tissue alone for 47 individuals (20.5%); the addition of plasma testing increased this number to 82 (35.8%).Forty-two participants received a targeted therapy based on the plasma result, and of those, 36 achieved a complete or partial response, or had stable disease. The authors concluded that the integration of plasma NGS testing into standard management of metastatic NSCLC leads to a substantial increase of the detection of therapeutically targetable mutations, and thus improvement of delivery of molecularly guided treatment. Of note, the study only looked at plasm NGS testing at a single point; additional study on longitudinal plasma NGS-based monitoring is an active area of study.

A Hayes Molecular Test Assessment (2018, updated 2021) found evidence supporting both analytical and clinical validity of Guardant360, however evidence supporting clinical utility was not clear regarding overall improved outcomes when the results were used to inform clinical decision-making (Hayes, Guardant360 [Guardant Health Inc.], 2018, updated 2021).

McCoach et al. (2018) evaluated patients with advanced NSCLC and with tumors that carried ALK gene fusions. The researchers sought to analyze the cfDNA to find a non-invasive way to identify these gene fusions. The study used the Guardant360 database of NSCLC cases to identity patients. Eighty-eight patients with 96 plasma-detected ALK fusions were determined. The fusion partners identified included EML4 (85.4%), STRN (6%), and KCNQ, KLC1, KIF5B, PPM1B, and TGF (totaling 8.3%). The study concluded that in this cohort, cfDNA was acceptable at detecting targetable alterations.

The majority of studies with Guardant360 have focused on NSCLC; however, more research is being performed with other tumor types. A study by Yang, et al. (2017) evaluated lung cancer and other solid tumors. Plasma from patients with lung cancer (n = 103) and other solid tumors (n = 74) was analyzed for ctDNA using the Guardant360 test. In this cohort, mutations in TP53, EGFR, and KRAS genes were most often determined. Mutations in BRCA1, BRCA2, and ATM were found in 18.1% (32/177) of cases. Also, the researchers compared the ctDNA and tumor tissue of 37 lung cancer cases. This analysis found that key mutations could be found in plasma even if they were minor in the tumor tissue.

Villaflor et al. (2016) reported on patients with NSCLC undergoing analysis of ctDNA using Guardant360. As part of clinical care, 90 patients submitted for ctDNA testing, but only 68 provided consent. These patients had lung adenocarcinoma (n = 55, 81%), lung squamous cell carcinoma (n = 12, 17.7%) and other lung cancers (n = 1, 1.3%). Of these 68, 38 were tested using the 54-gene ctDNA panel and 31 were analyzed on the 68-gene ctDNA panel. Tissue-based testing was performed on 44 subjects using 9 different testing platforms. The researchers found that 83% of subjects had at least one genomic alteration and the most commonly mutated genes were TP53, KRAS and EGFR. Only 31 patients had matched tissue and blood samples, and, in those patients, an EGFR activating was found in both tissue and blood in 5 paired samples, and in tissue only in 2 samples (71% concordance). In 9 subjects with paired tissue and blood samples, an EGFR driver mutation was identified in plasma and tissue (n = 5), plasma only (n = 1) or tissue only (n = 3). Overall, the investigators concluded that in this limited cohort, ctDNA is an option when tissue is unavailable.

Signatera

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

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

The use of ctDNA as a prognostic biomarker for relapse of metastatic colorectal cancer (mCRC) was the subject of a cohort study by Loupakis et al. (2021). In this study, 112 individuals with mCRC were evaluated. These participants were part of the PREDATOR clinical trial and had undergone resection of metastases with curative intent. In this study, evaluation of the prognostic value of ctDNA was performed by correlating clinical outcomes with molecular residual disease (MRD) status after surgery using a tumor-informed, personalized ctDNA assay (Signatera). MRD positive results were found in 54.4% of the participants after surgery. Of those, 96.7% progressed at the time data collection ended. Positive results were also associated with lower overall survival. At the time of data analysis, 96% of all study participants in the MRD-negative arm of the study were living, compared with only 52.4% in the MRD-positive arm. For individuals who were MRD-negative in the combined ctDNA analysis at both points in time and did not receive systemic therapy, overall survival rate was 100%. When multivariate analysis was performed, the most significant prognostic factor associated with disease -free survival was ctDNA based MRD status. The researchers concluded that post-operative MRD evaluation is a strong biomarker in individuals with mCRC undergoing metastatic resection and feel that it has potential use in clinical decision-making. Further clinical studies will be needed to support use of this technology in the future.

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

Reinert et al. (2019) reported results of a prospective, multi-center cohort study designed to analyze how ctDNA is associated with CRC recurrence. Ultradeep sequencing of plasma cell-free DNA was performed in study participants with CRC before pre- and post-surgery, during and after adjuvant chemotherapy (ACT), and during the surveillance period. The study took place in

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

Clinical Practice Guidelines

American Society of Clinical Oncology (ASCO)

In a 2022 Provisional Opinion, the ASCO (Chakravarty et al.) addressed the use of somatic tumor genomic testing in individuals with advanced or metastatic solid tumors. ASCO provides the following opinions:

- Individuals who have been diagnosed with advanced or metastatic cancer and adequate performance status should be tested with genomic sequencing when:
  - Genomic biomarker-associated therapies exist which have been approved by regulatory agencies for the individual's cancer.
  - Treatment for which there are specific biomarker-based contraindications or exclusions exist (strength of recommendation: strong).
- Multigene panel tests should be performed when individual has metastatic or advanced solid tumor and is eligible for genomic biomarker-linked, approved therapy (strength of recommendation: moderate).
- Multigene panel tests should be performed when individual has more than one genomic biomarker associated with an approved therapy (strength of recommendation: strong).
- Testing used to inform clinical care must be done in an appropriately certified laboratory (strength of recommendation: strong).
- Clinical decision making should include:
  - Known or predicted impact of genomic alteration on protein expression/function.
  - Clinical data on efficacy of targeting the genomic alteration with a specific treatment agent (strength of recommendation: strong).
- Individuals with advanced or metastatic solid tumors should undergo germline testing for genetic alterations that have been linked to approved therapies under consideration. This should not be limited by clinical criteria for familial risk or family history reports. In addition, individuals with pathogenic or likely pathogenic (P/LP) variations should be referred for genetic counseling (strength of recommendation: strong).
- Evaluation of mismatch repair deficiency status (dMMR) should be performed for individuals with advanced or metastatic solid tumors who are under consideration for use of immunotherapy (strength of recommendation: strong).
- Testing with either large multigene panels including validated TMB testing or whole exome analysis should be performed when TMB may influence decision-making regarding use of immunotherapy (strength of recommendation: strong).
- Individuals with advanced or metastatic solid tumors should undergo fusion testing if there are fusion-targeted therapies approved for their specific disease (strength of recommendation: strong).
- In individuals with advanced or metastatic solid tumors who may be considered for TRK-inhibitor therapy, NTRK fusion testing should be performed (strength of recommendation: strong).
- Individuals with advanced or metastatic solid tumors may be tested for other fusions if no oncogenic driver alterations have been identified on large panel DNA sequencing (strength of recommendation: moderate).
- MET exon 14 skipping testing is recommended for individuals diagnosed with any type of non-small-cell lung cancer (strength of recommendation: strong).
- In individuals with advanced or metastatic solid tumors, genomic testing should be considered in order to determine whether individuals is an appropriate candidate for tumor-agnostic therapies without genomic biomarker-linked therapies (strength of recommendation: moderate).

- When no genomic biomarker-linked targeted therapies exist for potentially actionable genomic alterations, individual participation in clinical trials is encouraged (after considering efficacy of available standard-of-care treatments) (strength of recommendation: strong).
- The use of off-label and off-study biomarker-linked treatments which have been approved for other diseases is not recommended when clinical trial participation is an option or when there is no clinical evidence of meaningful efficacy (strength of recommendation: strong).

ASCO also addresses rationale for repeat genomic testing indicating that this testing may be justified when individuals were initially sequenced with a limited NGS panel, however there is limited evidence to support the utility of repeat testing for individuals who underwent large panel testing or whole exome/whole genome sequencing when no treatment was provided that could change tumor genomics. The document further states that the body of evidence on cfDNA/liquid biopsy is growing with studies to date showing "substantial concordance" between tumor testing and cfDNA testing, however copy-number changes may be harder to assess in cfDNA and fusion testing may be for limited in the cfDNA tests being used today. Genomic testing using cfDNA is most useful when genomic testing is indicated for an individual, archival tissue is not available and new tumor biopsies aren't feasible. Studies are ongoing regarding the clinical utility of serial liquid biopsy.

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

The European Society for Medical Oncology (ESMO)

In a 2020 report (Mosele et al.) the ESMO Precision Medicine Working Group recommended use of NGS on tumor samples of individuals presenting with advanced non-squamous NSCLC, prostate, ovarian cancers, and cholangiocarcinoma. For these tumor types, large multigene panels are proposed as a possibility based on cost effectiveness in comparison with small panels and for colon cancers, NGS could be used instead of PCR. ESMO further recommends testing TMB in cervical cancer, well- and moderately- differentiated neuroendocrine tumors, salivary cancer, thyroid cancer and vulvar cancers, since data from the KEYNOTE-158 trial showed that TMB-high results predicted response to pembrolizumab specific to these cancer types. ESMO points out that the use of large multigene panels may lead to few meaningful responders and if such panels are used, the individual undergoing the testing must be informed of the low likelihood of benefit. Lastly, ESMO encourages clinical research centers to develop multigene sequencing as a screening tool for individuals under consideration for clinical trials and to support further drug development. They assert that clinical trials as well as economic study should be pursued to enhance the body of evidence in this area.

International Association for the Study of Lung Cancer (IASLC)

In a 2021 Consensus Statement from the IASLC, Rolfo et al. acknowledge the dramatic advances in precision medicine on the clinical management of non-small cell lung cancer (NSCLC) and advanced staged cancers overall. The authors note that while the data are most robust for NSCLC, there may well be benefit shown for other cancer types as well, impacting selection of targeted therapy types, as research progresses. Recommendations from this group now include using a clinically validated NGS platform rather than single gene, PCR-based approaches, considering plasma ctDNA a valid tool for genotyping advanced NSCLC in newly diagnosed patients, and the use of liquid biopsy either as complementary to tissue-based analysis or as the initial approach to biomarker evaluation in oncogene-addicted NSCLC and for monitoring efficacy of therapies. The authors anticipate continued growth of the role of liquid biopsy in both the near and long-term future.

National Institute for Health and Care Excellence (NICE)

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

In 2017, NICE conducted a Medtech innovation briefing on the Caris Molecular Intelligence (CMI) for guiding future management of locally advanced or metastatic cancer treatment. The evidence collected was from 5 observational studies, mainly showing that CMI-guided treatment is associated with better progression-free survival vs. clinical decisions alone. Additionally, some evidence uncovered demonstrated that CMI may lead to improved overall survival. However, no randomized controlled studies compared CMI-guided treatment to non-CMI-guided treatment, there was limited evidence on CMI-guided treatment for site-specific cancers and metastatic cancer of unknown primary origin, and no evidence of its use in children.

National Comprehensive Cancer Network (NCCN)

NCCN guidelines for Treatment by Cancer Type address the use of individual tumor markers for specific cancer types as well as the use of multigene panels and molecular profiling. NCCN specifically mentions liquid biopsy (plasma) testing in certain clinical scenarios as well. Studies have demonstrated cell-free tumor DNA generally has very high specificity, but significantly compromised sensitivity, with up to a 30% false-negative rate. In spite of this, evidence supports complementary testing to reduce turnaround time and increase yield of targetable alteration detection.

Ampullary Adenocarcinoma

For ampullary adenocarcinoma, tumor/somatic molecular profiling to identify uncommon mutations is recommended for those individuals with locally advanced/metastatic disease who are candidates for treatment with anti-cancer therapy. Specifically, testing for potentially actionable somatic findings to include fusions (ALK, NRG1, NTRK, ROS1, FGFR2, RET), mutations (BRAF, BRCA1/2, KRAS, PALB2), amplifications (HER2), microsatellite instability (MSI) and/or mismatch repair (MMR) deficiency. Testing on tumor tissue is preferable, but cfDNA testing can be considered if tumor tissue testing is not feasible. (NCCN Ampullary Adenocarcinoma, v1.2022)

Bladder Cancer

For bladder cancer, NCCN recommends molecular/genomic testing (in a CLIA-approved laboratory) for stages IVA and IVB bladder cancer and consideration of this testing for stage IIIB bladder cancer. Recommendation is for early testing, ideally at diagnosis of advanced bladder cancer, to assist with decision-making. NCCN notes that genetic variations are common in bladder cancer, citing data as the third highest mutated cancer. (NCCN Bladder Cancer, v2.2022)

Bone Cancer

NCCN Bone Cancer guidelines recommends consideration of CGP via validated/FDA-approved assay for individuals with metastatic chondrosarcoma, recurrent chordoma, metastatic Ewing sarcoma and metastatic osteosarcoma to identify potential targeted treatment opportunities and encourages impacted individuals to participate in well-designed clinical trials to further advance study. (NCCN Bone Cancer, v2.2023)

Breast Cancer

The NCCN guideline for Breast Cancer indicates that genomic profiling may be performed for use in determining appropriate treatment for breast cancer. In the setting of recurrent unresectable or stage IV breast cancer, testing for biomarkers associated with FDA-approved therapies is recommended. *PIK3CA* mutations may be assessed with tumor or liquid biopsy to identify candidates for alpelisib plus fulvestrant in individuals with HR-positive/HER2-negative cancer of the breast. *PIK3CA* mutation testing may be carried out on tumor tissue or ctDNA in peripheral blood (liquid biopsy). If liquid biopsy is negative, tumor tissue testing is recommended. (NCCN Breast Cancer, v4.2022)

Cervical Cancer

For persistent or recurrent cervical cancer, NCCN indicates

- CGP via a validated and/or FDA approved assay should be considered.
- If tissue biopsy of metastatic site is not feasible or tissue isn't available, CGP via a validated plasma ctDNA assay may be considered. (NCCN Cervical Cancer, v1.2022)

Colorectal Cancer (CRC)

The NCCN guidelines for Colon Cancer and Rectal Cancer indicate that targeted treatment for advanced/metastatic CRC is becoming more common and as such, NCCN has expanded recommendations for biomarker testing. For individuals with metastatic CRC, recommended workup should include determination of tumor gene status for *RAS* and *BRAF* mutations, HER2

amplifications, and MSI/MMR status (if not previously done) either individually or as part of tissue- or blood-based NGS panel test. NGS panels have the advantage of the ability to detect rare and actionable gene alterations such as *NTRK* fusions. The guideline further notes that molecular testing on tissue samples is preferred, but blood-based assays are also an option. Both tissue- and blood-based NGS panels have the ability to pick up rare and actionable mutations and fusions. (NCCN Colon Cancer, v2.2022, NCCN Rectal Cancer, v3.2022)

Histiocytic Neoplasms

In individuals suspected of having Rosai-Dorfman disease or histiocytosis and biopsy is not possible due to location or other risk factors, liquid biopsy for analysis of variants in the peripheral blood is an option. (NCCN Histiocytic Neoplasms, v1.2022)

Gastric and Esophageal/Esophagogastric Junction Cancers

Several target agents have been approved by the FDA for use in gastric, esophageal, and esophagogastric junction cancers. In solid tumor cancers, genomic alterations can be identified via ctDNA in the blood. Such testing is becoming more common in individuals with advanced disease; specifically those individuals who are not able to undergo clinical biopsy for disease surveillance and management. For individuals with metastatic or advanced gastric cancer that cannot undergo traditional biopsy, or in the setting of disease progression monitoring, testing with a validated NGS-based CGP profile may be considered. NCCN cautions that negative results must be interpreted carefully, as this does not necessarily exclude tumor mutations or amplifications. (NCCN Gastric Cancer, v2.2022, NCCN Esophageal and Esophagogastric Junction Cancers, v4.2022)

Melanoma: Cutaneous

Per the NCCN Cutaneous Melanoma guideline, NGS, including various sequencing technologies, allows DNA and RNA sequencing to be performed more quickly and is less costly than Sanger sequencing. Single gene or small multi-gene panels can be used in some cases to test a single gene (e.g., *BRAF*) or a limited number of genes. Tumor tissue is preferred for molecular testing, however liquid biopsy may be performed if tumor tissue is not available. (NCCN Melanoma: Cutaneous, v3.2022)

Non-Small Cell Lung Cancer

Per NCCN, the use of cell-free/circulating tumor DNA testing should not be used in lieu of a histologic tissue diagnosis but can be considered in specific clinical circumstances, including the following examples:

- Patient is not medically fit for invasive tissue sampling.
- In the setting of initial diagnosis, if following pathologic confirmation of a NSCLC diagnosis there is insufficient material for molecular analysis, cell-free/circulating tumor DNA should be used only if follow-up tissue-based analysis is planned for any patient in which an oncogenic driver is not identified.
- In the setting if initial diagnosis, if tissue-based testing does not fully assess all recommended biomarkers due to quantity of tissue available or testing methodologies available, consider repeat biopsy and/or cell-free/circulating tumor DNA testing.
- In advanced or metastatic disease, If there is not enough available tissue to allow testing for genes including EGFR, KRAS, ALK, ROS1, BRAF, NTRK1/2/3, MET, RET, and ERBB2 (HER2) repeat biopsy and/or plasma testing should be done.

Data suggest that plasma cfDNA testing can be used to identify EGFR, ALK, and other oncogenic biomarkers that would not otherwise be identified in patients with metastatic NSCLC. (NCCN Non-Small Cell Lung Cancer, v5.2022)

Occult Primary

NCCN indicates that NGS should be considered based on individual clinicopathologic features where clinical decision-making is impacted. Tumor tissue is preferred for molecular testing but cell-free DNA may be considered if tumor tissue testing is not feasible. (NCCN Occult Primary, v2.2023)

Ovarian Cancer/Fallopian Tube Cancer/Primary Peritoneal Cancer

NCCN recommends tumor molecular evaluation via validated test(s) in a CLIA-certified laboratory to identify, at a minimum, the potential benefit of targeted therapeutic agents with tumor specific or tumor agnostic benefit. These include (but are not limited to) BRCA 1/2, HR status, MSI, TMB, and NTRK, if any prior testing performed did not include these markers. Further testing with more comprehensive panels may be of specific importance in less common ovarian cancers with limited approved options for therapy. Also recommended is molecular testing prior to start of therapy for persistent or recurrent disease if such testing was not already performed. (NCCN Ovarian Cancer/Fallopian Tube Cancer/Primary Peritoneal Cancer, v5.2022)

Pancreatic Adenocarcinoma

NCCN recommends tumor/somatic molecular profiling for individuals with locally advanced/metastatic disease who are candidates for anti-cancer therapy for identification of uncommon mutations. Testing for potentially actionable somatic findings including, but not limited to: fusions (ALK, NRG1, NTRK, ROS1, FGFR2, RET), mutations (BRAF, BRCA1/2, KRAS, PALB2), amplifications (HER2), microsatellite instability (MSI), and/or mismatch repair (MMR) deficiency should be considered. Of note, testing on tumor tissue is preferred; however, cell-free DNA testing can be considered if tumor tissue testing is not feasible. (NCCN Pancreatic Adenocarcinoma, v1.2022)

Prostate Cancer

Metastatic biopsy for histologic and molecular evaluation is strongly recommended by NCCN for prostate cancer. When this is unsafe or not feasible, plasma ctDNA is an option, with preference for collection during a biochemical and/or radiographic progression to maximize diagnostic yield. The NCCN panel urges caution when interpreting ctDNA only evaluations due to the potential for interference from clonal hematopoiesis of indeterminate potential (CHIP), which could result in a false-positive. (NCCN Prostate Cancer, v1.2023)

U.S. Food and Drug Administration (FDA)

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

Laboratories that perform genetic tests are regulated under the Clinical Laboratory Improvement Amendments (CLIA) Act of 1988. More information is available at: https://www.fda.gov/medicaldevices/deviceregulationandguidance/ivdregulatoryassistance/ucm124105.htm. (Accessed December 8, 2022)

References

Aaberg TM, Covington KR, Tsai T, et al. Gene expression profiling in uveal melanoma: Five-year prospective outcomes and meta-analysis. Ocul Oncol Pathol. 2020 Oct;6(5):360-367.

Abida W, Patnaik A, Campbell D, et al.; TRITON2 investigators. Rucaparib in men With metastatic castration-resistant prostate cancer harboring a BRCA1 or BRCA2 gene alteration. J Clin Oncol. 2020 Nov 10;38(32):3763-3772.

Adalsteinsson VA, Ha G, Freeman SS, et al. Scalable whole-exome sequencing of cell-free DNA reveals high concordance with metastatic tumors. Nature Communications. 2017;8:1324.

Aggarwal C, Thompson JC, Black TA, et al. Clinical implications of plasma-based genotyping with the delivery of personalized therapy in metastatic non-small cell lung cancer. JAMA Oncol. 2019 Feb 1;5(2):173-180.

Alexander EK, Kennedy GC, Baloch ZW, et al. Preoperative diagnosis of benign thyroid nodules with indeterminate cytology. N Engl J Med. 2012;367(8):705–715.

Altman AM, Marmor S, Tuttle TM, Hui JYC. 21-gene recurrence score testing in HER2-positive patients. Clin Breast Cancer. 2018 Nov 27.

American Association of Clinical Urologists. Position statement: genomic testing in prostate cancer. AACU website. Position Statements - AACU (aacuweb.org). Accessed December 8, 2022.

Andre F, Ismaila N, Henry NL, et al. Use of biomarkers to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer: ASCO Clinical Practice Guideline update-integration of results from TAILORx. J Clin Oncol. 2019;37(22):1956-1964.

Angell TE, Wirth LJ, Cabanillas ME, et al. Analytical and clinical validation of expressed variants and fusions from the whole transcriptome of thyroid FNA samples. Front Endocrinol (Lausanne). 2019;10:612.

Arber DA, Borowitz, MJ, Cessna M, et al. Initial diagnostic workup of acute leukemia: Guideline from the College of American Pathologists and the American Society of Hematology. Archives of Pathology & Laboratory Medicine: October 2017, Vol. 141, No. 10, pp. 1342-1393.

Ardakani MN, Thomas C, Robinson C, et al. Detection of copy number variations in melanocytic lesions utilising array based comparative genomic hybridisation. Pathology. 2017 Apr;49(3):285-291.

Avet-Loiseau H, Corre L, Lauwers-Cances V, et al. Evaluation of minimal residual disease (MRD) by next generation sequencing (NGS) is highly predictive of PFS in the IFM/DFCI 2009 trial. Blood. 2015;126:191.

Azeez HJ, Neri F, Hosseinpour Feizi MA, et al. Transcriptome profiling of HCT-116 colorectal cancer cells with RNA sequencing reveals novel targets for polyphenol nano curcumin. Molecules. 2022 May 27;27(11):3470.

Babazadeh NT, Sinclair TJ, Krishnamurthy V, et al. Thyroid nodule molecular profiling: the clinical utility of Afirma Xpression Atlas for nodules with Afirma Genomic Sequencing Classifier-suspicious results. Surgery. 2022 Jan;171(1):155-159.

Bartlett JMS, Sgroi DC, Treuner K, et al. Breast cancer index and prediction of benefit from extended endocrine therapy in breast cancer patients treated in the Adjuvant Tamoxifen-To Offer More? (aTTom) trial. Ann Oncol. 2019;30(11):1776-1783.

Bauml JM, Li BT, Velcheti V, et al. Clinical validation of guardant360 cdx as a blood-based companion diagnostic for sotorasib. Lung Cancer. 2022 Apr;166:270-278.

Beaudenon-Huibregtse S, Alexander EK, Guttler RB, et al. Centralized molecular testing for oncogenic gene mutations complements the local cytopathologic diagnosis of thyroid nodules. Thyroid. 2014;24(10):1479–1487.

Bekelman JE, Rumble RB, Freedland SJ. Clinically localized prostate cancer: ASCO Clinical Practice Guideline endorsement of an AUA/ASTRO/SUO Guideline Summary. J Oncol Pract. 2018 Oct;14(10):618-624.

Beltran H, Yelensky R, Frampton GM, et al. Targeted next-generation sequencing of advanced prostate cancer identifies potential therapeutic targets and disease heterogeneity. Eur Urol. 2013 May;63(5):920-6.

Berger AC, Davidson RS, Poitras JK, et al. Clinical impact of a 31-gene expression profile test for cutaneous melanoma in 156 prospectively and consecutively tested patients. Curr Med Res Opin. 2016 Sep;32(9):1599-604.

Berlin A, Murgic J, Hosni A, et al. Genomic classifier for guiding treatment of intermediate-risk prostate cancers to dose-escalated image guided radiation therapy without hormone therapy. Int J Radiat Oncol Biol Phys. 2019;103(1):84-91.

Binder C, Matthes KL, Korol D, et al. Cancer of unknown primary-epidemiological trends and relevance of comprehensive genomic profiling. Cancer Med. 2018 Sep;7(9):4814-4824.

Brand TC, Zhang N, Crager MR, et al. Patient-specific meta-analysis of 2 clinical validation studies to predict pathologic outcomes in prostate cancer using the 17-gene genomic prostate score. Urology. 2016 Mar;89:69-75.

Brauner E, Holmes BJ, Krane JF, et al. Performance of the Afirma gene expression classifier in Hurthle cell thyroid nodules differs from other indeterminate thyroid nodules. Thyroid. 2015;25(7):789–796.

Bremer T, Whitworth PW, Patel R, et al. A biological signature for breast ductal carcinoma *in situ* to predict radiotherapy Benefit and assess recurrence risk. Clin Cancer Res. 2018 Dec 1;24(23):5895-5901.

Brooks MA, Thomas L, Magi-Galluzzi C, et al. GPS assay association with long-term cancer outcomes: twenty-year risk of distant metastasis and prostate cancer-specific mortality. JCO Precis Oncol. 2021 Feb 24;5:PO.20.00325.

Cao J, Yang X, Chen S, et al. The predictive efficacy of tumor mutation burden in immunotherapy across multiple cancer types: A meta-analysis and bioinformatics analysis. Transl Oncol. 2022 Jun;20:101375.

Caputo V, De Falco V, Ventriglia A, et al. Comprehensive genome profiling by next generation sequencing of circulating tumor DNA in solid tumors: a single academic institution experience. Ther Adv Med Oncol. 2022 May 7;14:17588359221096878.

Cardoso F, Kyriakides S, Ohno S, et al. Early breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2019;30:1194-1220.

Cardoso F, van't Veer LJ, Bogaerts J, et al. 70-gene signature as an aid to treatment decisions in early-stage breast cancer. N Engl J Med. 2016 Aug 25;375(8):717-29.

Carter HB, Albertsen PC, Barry MJ, et al. American Urological Association (AUA). Early detection of prostate cancer. Published 2013; reviewed and validity confirmed 2018.

Chakravarty D, Johnson A, Sklar J, et al. Somatic Genomic Testing in Patients With Metastatic or Advanced Cancer: ASCO Provisional Clinical Opinion. J Clin Oncol. 2022 Apr 10;40(11):1231-1258.

Chantrill LA, Nagrial AM, Watson C, et al. Australian Pancreatic Cancer Genome Initiative (APGI) and the Individualized Molecular Pancreatic Cancer Therapy (IMPaCT) Trial Management Committee of the Australasian Gastrointestinal Trials Group

(AGITG). Precision medicine for advanced pancreas cancer: the Individualized Molecular Pancreatic Cancer Therapy (IMPaCT) Trial. Clin Cancer Res. 2015 May 1;21(9):2029-37.

Chen RC, Rumble RB, Loblaw DA, et al. Active surveillance for the management of localized prostate cancer (Cancer Care Ontario guideline): American Society of Clinical Oncology Clinical practice guideline endorsement. J Clin Oncol 2016 34:2182-2190.

Chua MLK, Lo W, Pintilie M, et al. Prostate cancer "nimbosus": genomic instability and schlap1 dysregulation underpin aggression of intraductal and cribriform subpathologies. Eur Urol. 2017 May 13. [Epub ahead of print].

Cohen JD, Javed AA, Thoburn C, et al. Combined circulating tumor DNA and protein biomarker-based liquid biopsy for the earlier detection of pancreatic cancers. Proc Natl Acad Sci U S A. 2017 Sep 19;114(38):10202-10207.

Cooley LD, Lebo M, Li MM, et al. Working Group of the American College of Medical Genetics and Genomics (ACMG) Laboratory Quality Assurance Committee. American College of Medical Genetics and Genomics technical standards and guidelines: microarray analysis for chromosome abnormalities in neoplastic disorders. (2013) Genet Med ;15:484–494.

Covas Moschovas M, Chew C, Bhat S, et al. Association between Oncotype DX Genomic Prostate Score and adverse tumor pathology after radical prostatectomy. Eur Urol Focus. 2022 Mar;8(2):418-424.

Cristescu R, Aurora-Garg D, Albright A, et al. Tumor mutational burden predicts the efficacy of pembrolizumab monotherapy: a pan-tumor retrospective analysis of participants with advanced solid tumors. J Immunother Cancer. 2022 Jan;10(1):e003091.

Crozier JA, Barone J, Whitworth P, et al. High concordance of 70-gene recurrence risk signature and 80-gene molecular subtyping signature between core needle biopsy and surgical resection specimens in early-stage breast cancer. J Surg Oncol. 2022 Mar;125(4):596-602.

Daemen A, Udyavar AR, Sandmann T, et al. Transcriptomic profiling of adjuvant colorectal cancer identifies three key prognostic biological processes and a disease specific role for granzyme B. PLoS One. 2021 Dec 31;16(12):e0262198.

Dagogo-Jack I, Rooney M, Nagy RJ, et al. Molecular analysis of plasma from patients with ROS1-Positive NSCLC. J Thorac Oncol. 2019 Jan 18. pii: S1556-0864(19)30027-9.

Dalerba P, Sahoo D, Paik S, et al. CDX2 as a prognostic biomarker in stage II and stage III colon cancer. N Engl J Med 2016;374:211-22.

Davey MG, Davey CM, Bouz L, et al. Relevance of the 21-gene expression assay in male breast cancer: a systematic review and meta-analysis. Breast. 2022 Aug;64:41-46.

Den RR, Santiago-Jimenez M, Alter J, et al. Decipher correlation patterns post prostatectomy: initial experience from 2342 prospective patients. Prostate Cancer Prostatic Dis. 2016 Dec; 19(4): 374–379.

Deaver KE, Haugen BR, Pozdeyev N, Marshall CB. Outcomes of Bethesda categories III and IV thyroid nodules over 5 years and performance of the Afirma gene expression classifier: a single-institution study. Clin Endocrinol (Oxf). 2018 May 23.

Detterbeck FC, Lewis SZ, Diekemper R, et al. Executive Summary: Diagnosis and management of lung cancer, 3rd Ed: American College of Chest Physicians evidence-based clinical practice guidelines. Chest. 2013 May;143(5 Suppl):7S-37S.

Dieffenbacher S, Zschäbitz S, Hofer L, et al. Prospective single center trial of next-generation sequencing analysis in metastatic renal cell cancer: the MORE-TRIAL. Future Sci OA. 2018;4(5):FSO299. Published 2018 Mar 14.

Dimopoulos MA, Moreau P, Terpos E, et al. Multiple myeloma: EHA-ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2021 Mar;32(3):309-322.Ding Y, Jiang J, Xu J, et al. Site-specific therapy in cancers of unknown primary site: a systematic review and meta-analysis. ESMO Open. 2022 Apr;7(2):100407.

Drilon A, Wang L, Arcila ME, et al. Hybrid capture–based next-generation sequencing identifies actionable genomic alterations in lung adenocarcinomas otherwise negative for such alterations by other genomic testing approaches. Clin Cancer Res. 2015 Aug 15;21(16):3631-9.

Dubsky PC, Singer CF, Egle D, et al. The endopredict score predicts response to neoadjuvant chemotherapy and neoendocrine therapy in hormone receptor-positive, human epidermal growth factor receptor 2-negative breast cancer patients from the abcsg-34 trial. Eur J Cancer. 2020 Jul;134:99-106.

Dummer R, Hauschild A, Lindenblatt N, et al. ESMO Guidelines Committee. Cutaneous melanoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2015 Sep;26 Suppl 5:v126-32.

Dziadziuszko R, Mok T, Peters S, et al. Blood First Assay Screening Trial (BFAST) in treatment-naive advanced or metastatic NSCLC: initial results of the phase 2 ALK-positive cohort. J Thorac Oncol. 2021 Dec;16(12):2040-2050.

Eastham JA, Auffenberg GB, Barocas DA, et al. Clinically localized prostate cancer: AUA/ASTRO guideline, part I: introduction, risk assessment, staging, and risk-based management. J Urol. 2022 Jul;208(1):10-18.

Eastham JA, Auffenberg GB, Barocas DA, et al. Clinically localized prostate cancer: AUA/ASTRO guideline, part II: principles of active surveillance, principles of surgery, and follow-up. J Urol. 2022 Jul;208(1):19-25.

Eastham JA, Auffenberg GB, Barocas DA, et al. Clinically localized prostate cancer: AUA/ASTRO guideline. part III: principles of radiation and future directions. J Urol. 2022 Jul;208(1):26-33.

Eggener SE, Rumble RB, Armstrong AJ, et al. Molecular biomarkers in localized prostate cancer: ASCO Guideline. J Clin Oncol. 2020;38(13):1474-1494.

Eichhorst B, Robak T, Montserrat E, et al.; ESMO Guidelines Committee. Chronic lymphocytic leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2021 Jan;32(1):23-33.

Endo M, Nabhan F, Porter K, et al. Afirma gene sequencing classifier compared with gene expression classifier in indeterminate thyroid nodules. Thyroid. 2019;29(8):1115-1124.

Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group. Recommendations from the EGAPP Working Group: does the use of Oncotype DX tumor gene expression profiling to guide treatment decisions improve outcomes in patients with breast cancer? Genet Med. 2016 Aug;18(8):770-9.

Evans AG, Ahmad A, Burack WR, et al. Combined comparative genomic hybridization and single-nucleotide polymorphism array detects cryptic chromosomal lesions in both myelodysplastic syndromes and cytopenias of undetermined significance. Mod Pathol. 2016 Oct;29(10):1183-99.

Fakih M, Sandhu J, Wang C, et al. Evaluation of comparative surveillance strategies of circulating tumor DNA, imaging, and carcinoembryonic antigen levels in patients with resected colorectal cancer. JAMA Netw Open. 2022 Mar 1;5(3):e221093.

Fenaux P, Haase D, Santini V, et al.; ESMO Guidelines Committee. Myelodysplastic syndromes: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2021 Feb;32(2):142-156.

Feng FY, Huang HC, Spratt DE, et al. Validation of a 22-gene genomic classifier in patients with recurrent prostate cancer: an ancillary study of the NRG/RTOG 9601 randomized clinical trial. JAMA Oncol. 2021 Apr 1;7(4):544-552.

Ferris LK, Jansen B, Ho J, et al. Utility of a noninvasive 2-gene molecular assay for cutaneous melanoma and effect on the decision to biopsy. JAMA Dermatol. 2017;153(7):675–680.

Ferris LK, Gerami P, Skelsey MK,et al. Real-world performance and utility of a noninvasive gene expression assay to evaluate melanoma risk in pigmented lesions. Melanoma Res. 2018 Oct;28(5):478-482.

Ferris LK, Rigel DS, Siegel DM, et al. Impact on clinical practice of a non-invasive gene expression melanoma rule-out test: 12-month follow-up of negative test results and utility data from a large US registry study. Dermatol Online J. 2019 May 15;25(5):13030/qt61w6h7mn.

Fitzal F, Filipits M, Fesl C, et al. Austrian breast and colorectal cancer study group (abcsg). pam-50 predicts local recurrence after breast cancer surgery in postmenopausal patients with er + /her2- disease: results from 1204 patients in the randomized abcsg-8 trial. Br J Surg. 2021 Apr 5;108(3):308-314.

Fizazi K, Greco FA, Pavlidis N, et al. Cancers of unknown primary site: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol (2015) 26 (suppl 5): v133-v138.

Food and Drug Administration (FDA) FDA authorizes first next generation sequencing-based test to detect very low levels of remaining cancer cells in patients with acute lymphoblastic leukemia or multiple myeloma. Available at: https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm622004.htm. Accessed December 8, 2022.

Frampton GM, Fichtenholtz A, Otto GA, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol. 2013 Nov;31(11):1023-31.

Ganesan P, Moulder S, Lee JJ, et al. Triple-negative breast cancer patients treated at MD Anderson Cancer Center in phase I trials: improved outcomes with combination chemotherapy and targeted agents. Mol Cancer Ther. 2014 Dec;13(12):3175-84.

Gharib H, Papini E, Garber JR, et al. American Association Of Clinical Endocrinologists, American College Of Endocrinology, And Associazione Medici Endocrinologi Medical Guidelines For Clinical Practice For The Diagnosis And Management Of Thyroid Nodules – 2016 Update. Endocrine Practice May 2016 Vol 22 (Suppl 1).

Glass AG, Leo MC, Haddad Z., et al. Validation of a genomic classifier for predicting post-prostatectomy recurrence in a community-based health care setting. J Urol. 2016 Jun;195(6):1748-53.

Gnant M, Filipits M, Dubsky P, et al. Predicting risk for late metastasis: The PAM50 risk of recurrence (ROR) score after 5 years of endocrine therapy in postmenopausal women with HR+early breast cancer: a study on 1,478 patients for the ABCSG-8 trial. Ann Oncol. 2013;24(3):iii29-iii37.

Gorringe KL, Fox SB. Ductal carcinoma in situ biology, biomarkers, and diagnosis. Frontiers in Oncology. 2017;7:248.

Grail, LLC. Galleri multi-cancer early detection test. Available at: https://www.galleri.com/hcp/the-galleri-test. Accessed December 8, 2022.

Greenhaw BN, Covington KR, Kurley SJ, et al. Molecular risk prediction in cutaneous melanoma: A meta-analysis of the 31-gene expression profile prognostic test in 1,479 patients. J Am Acad Dermatol. 2020 Sep;83(3):745-753.

Göker E, Hendriks MP, van Tilburg M, et al. Treatment response and 5-year distant metastasis-free survival outcome in breast cancer patients after the use of Mammaprint and Blueprint to guide preoperative systemic treatment decisions. Eur J Cancer. 2022 May;167:92-102.

Griguolo G, Bottosso M, Vernaci G, et al. Gene-expression signatures to inform neoadjuvant treatment decision in hr+/her2- breast cancer: available evidence and clinical implications. Cancer Treat Rev. 2022 Jan;102:102323.

Grinfeld J, Nangalia J, Baxter EJ, et al. Classification and Personalized Prognosis in Myeloproliferative Neoplasms. N Engl J Med. 2018 Oct 11;379(15):1416-1430.Han M, Liew CT, Zhang HW, et al. Novel, blood-based five-gene panel biomarker set for the detection of colorectal cancer. Clin Cancer Res. 2008;14:455–60.

Harnan S, Tappenden P, Cooper K, et al. Tumour profiling tests to guide adjuvant chemotherapy decisions in early: a breast cancer systematic review and economic analysis. Health Technol Assess. 2019 Jun;23(30):1-328.

Harrell RM, Bimston DN. Surgical utility of Afirma: effects of high cancer prevalence and oncocytic cell types in patients with indeterminate thyroid cytology. Endocr Pract. 2014;20(4):364–369.

Harris LN, Ismaila N, McShane LM, et al. Use of biomarkers to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer: American Society of Clinical Oncology Clinical Practice Guideline. Oncol Pract. 2016a Apr;12(4):384-9.

Harris LN, Ismaila N, McShane LM, et al. Use of biomarkers to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer: American Society of Clinical Oncology Clinical practice guideline. J Clin Oncol 2016b;34:1134-1150.

Hassett MJ, Somerfield MR, Baker ER, et al. Management of male breast cancer: ASCO Guideline. J Clin Oncol. 2020 Jun 1;38(16):1849-1863. Available at: https://ascopubs.org/doi/full/10.1200/JCO.19.03120. Accessed November 11, 2022.

Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. 2016;26(1):1-133.

Hayes, Inc. Clinical Utility Evaluation. Liquid biopsy tests for colorectal cancer screening. Hayes Inc.; March 26, 2020, updated May 17, 2022.

Hayes, Inc. Molecular Test Assessment. Afirma genomic sequencing classifier (Veracyte Inc). Hayes Inc.; April 7, 2021, updated March 28, 2022.

Hayes, Inc. Molecular Test Assessment. Breast Cancer Index (BioTheranostics Inc.) for lymph node–negative patients. Hayes Inc.; October 29, 2020, updated May 11, 2022.

Hayes, Inc. Molecular Test Assessment. Breast Cancer Index (BioTheranostics Inc.) for lymph node–positive (1-3) patients. Hayes Inc.; October 29, 2020, updated June 15, 2022.

Hayes, Inc. Molecular Test Assessment. CANCERPLEX (KEW Inc.). Hayes Inc.; August 26, 2019, updated March 25, 2022.

Hayes, Inc. Molecular Test Assessment. CancerTYPE ID (bioTheranostics Inc.). Hayes, Inc.; July 30, 2018, updated March 25, 2022.

Hayes, Inc. Molecular Test Assessment. clonoSEQ (Adaptive Biotechnologies). Hayes Inc.; June 22, 2022.

Hayes, Inc. Molecular Test Assessment. ConfirmMDx for prostate cancer (MDxHealth Inc.). Hayes Inc.; February 27, 2019, updated March 16, 2022.

Hayes, Inc. Molecular Test Assessment. DCISionRT (Prelude Corp). Hayes Inc.; June 29, 2022.

Hayes, Inc. Molecular Test Assessment. Decipher Prostate Biopsy (Decipher Biosciences). Hayes Inc.; May 21, 2019, updated March 21, 2022.

Hayes, Inc. Molecular Test Assessment. Decipher RP (Decipher Biosciences). Hayes Inc.; May 21, 2019, updated March 21, 2022.

Hayes, Inc. Molecular Test Assessment. DecisionDx-Melanoma. Hayes Inc.; March 29, 2022.

Hayes, Inc. Molecular Test Assessment. DecisionDx-UM (Castle Biosciences Inc.). Hayes Inc.; June 17, 2020, updated May 11, 2022.

Hayes, Inc. Molecular Test Assessment. EndoPredict (Myriad Genetics Laboratories Inc.). Hayes Inc.; December 10, 2020, updated October 21, 2022.

Hayes, Inc. Molecular Test Assessment. FoundationOne CDx (Foundation Medicine Inc.) for the intended use as a broad molecular profiling tool. Hayes Inc.; April 26, 2022.

Hayes, Inc. Molecular Test Assessment. Guardant360 (Guardant Health Inc.). Hayes Inc.; December 11, 2018, updated November 19, 2021.

Hayes, Inc. Molecular Test Assessment. MI Profile (Caris Life Sciences) for the intended use as a broad molecular profiling tool. Hayes Inc.; May 3, 2022.

Hayes, Inc. Molecular Test Assessment. MI TumorSeek (Caris Life Sciences). Hayes Inc.; September 17, 2019, updated August 25, 2020.

Hayes, Inc. Molecular Test Assessment. myPath Melanoma (Myriad Genetics). Hayes Inc.; May 14, 2018, updated June 1, 2022.

Hayes, Inc. Molecular Test Assessment. Oncotype DX Breast DCIS Score (Genomic Health Inc.). Hayes Inc.; November 8, 2018, updated October 21, 2022.

Hayes, Inc. Molecular Test Assessment. Oncotype DX Breast Recurrence Score for lymph node–negative patients (Genomic Health Inc.). Hayes Inc.; April 23, 2020, updated March 23, 2022.

Hayes, Inc. Molecular Test Assessment. Oncotype DX Breast Recurrence Score (Genomic Health Inc.) for lymph node–positive patients. Hayes Inc.; May 6, 2020, updated March 23, 2022.

Hayes, Inc. Molecular Test Assessment. Oncotype DX Genomic Prostate Score (GPS) assay (Genomic Health Inc.). Hayes Inc.; Nov 12, 2018, updated June 17, 2022.

Hayes, Inc. Molecular Test Assessment. PancraGEN (Interpace Diagnostics). Hayes, Inc.; Aug 29, 2022.

Hayes, Inc. Molecular Test Assessment. Prolaris biopsy test (Myriad Genetic Laboratories, Inc.). Hayes Inc.; March 29, 2019, updated February 14, 2022.

Hayes, Inc. Molecular Test Assessment. Prolaris post-prostatectomy (Myriad Genetic Laboratories, Inc.). Hayes Inc.; April 19, 2019, updated February 14, 2022.

Hayes, Inc. Molecular Test Assessment. SelectMDx for prostate cancer (MDxHealth Inc.). Hayes Inc.; March 21, 2019.

Hayes, Inc. Molecular Test Assessment. ThyGeNEXT and ThyraMIR (Interpace Diagnostics Group Inc). Hayes Inc.; July 10, 2019, updated October 21, 2022.

Hayes, Inc. Molecular Test Assessment. ThyroSeq v3 Genomic Classifier (GC) (University of Pittsburgh Medical Center, CBLPath Inc). Hayes Inc.; May 9, 2019, updated October 19, 2021.

Hayes, Inc. Precision Medicine Insights. Comprehensive molecular profiling of circulating solid tumor DNA for the intended use as a broad molecular profiling tool to aid treatment selection. Hayes Inc.; October 18, 2022.

Hayes, Inc. Precision Medicine Insights. Comprehensive molecular profiling of circulating solid tumor DNA for the intended use as a broad molecular profiling tool for monitoring. Hayes Inc.; October 18, 2022.

Hayes, Inc. Precision Medicine Insights. Comprehensive molecular profiling tests for solid tumors intended to be used as a broad molecular profiling tool to assign matched therapy. Hayes Inc.; January 11, 2022.

Hayes, Inc. Precision Medicine Research Brief. PancreaSeq genomic classifier (University of Pittsburgh medical center MGP laboratory). Hayes, Inc.; November 14, 2022.

He N, Song L, Kang Q, et al. The pathological features of colorectal cancer determine the detection performance on blood ctDNA. Technol Cancer Res Treat. 2018;17:1533033818791794.

Heichman K. Blood-based testing for colorectal cancer screening. Molecular Diagnosis & Therapy; Volume 18, Issue 2; p:127-135.

Heuser M, Ofran Y, Boissel N, et al.; ESMO Guidelines Committee. Acute myeloid leukaemia in adult patients: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2020 Jun;31(6):697-712.

Hirshfield KM, Tolkunov D, Zhong H, et al. Clinical actionability of comprehensive genomic profiling for management of rare or refractory cancers. Oncologist. 2016 Nov;21(11):1315-1325.

Hoelzer D, Bassan R, Dombret H, et al.; ESMO Guidelines Committee. Acute lymphoblastic leukaemia in adult patients: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2016 Sep;27(suppl 5):v69-v82.

Hu MI, Waguespack SG, Dosiou C, et al. Afirma Genomic Sequencing Classifier and Xpression Atlas molecular findings in consecutive Bethesda III-VI thyroid nodules. J Clin Endocrinol Metab. 2021 Jul 13;106(8):2198-2207.

Hutchinson KE, Lipson D, Stephens PJ, et al. BRAF fusions define a distinct molecular subset of melanomas with potential sensitivity to MEK inhibition. Clin Cancer Res. 2013 Dec 15;19(24):6696-702.

Jairath NK, Dal Pra A, Vince R Jr, et al. A systematic review of the evidence for the Decipher genomic classifier in prostate cancer. Eur Urol. 2021 Mar;79(3):374-383.

Johnson DB, Dahlman KH, Knol J, et al. Enabling a genetically informed approach to cancer medicine: a retrospective evaluation of the impact of comprehensive tumor profiling using a targeted next-generation sequencing panel. Oncologist. 2014 Jun;19(6):616-22.

Jongen-Lavrencic M, Grob T, Hanekamp D, et al. Molecular minimal residual disease in acute myeloid leukemia. N Engl J Med 378:1189-1199, 2018.

Kalemkerian GP, Narula N, Kennedy EB et al. Molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: American Society of Clinical Oncology Endorsement Summary of the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology Clinical Practice Guideline Update. J Oncol Pract. 2018 Mar 28:JOP1800035.

Kalinsky K, Barlow WE, Gralow JR, et al. 21-Gene Assay to Inform Chemotherapy Benefit in Node-Positive Breast Cancer. N Engl J Med. 2021 Dec 16;385(25):2336-2347.

Kamps R, Brandão RD, van den Bosch BJ, et al. Next-generation sequencing in oncology: Genetic diagnosis, risk prediction and cancer classification. Cho WC, ed. International Journal of Molecular Sciences. 2017;18(2):308.

Kandimalla R, Xu J, Link A, et al. EpiPanGI Dx: a cell-free DNA methylation fingerprint for the early detection of gastrointestinal cancers. Clin Cancer Res. 2021 Nov 15;27(22):6135-6144.

Kato S, Schwaederle M, Daniels GA, et al. Cyclin-dependent kinase pathway aberrations in diverse malignancies: clinical and molecular characteristics. Cell Cycle. 2015 Apr 18;14(8):1252-9.

Kaufman SA, Harris EER, Bailey L, et al. Expert panel on radiation oncology–breast. ACR Appropriateness Criteria® ductal carcinoma in situ [online publication]. Reston (VA): American College of Radiology (ACR); 2014.

Kim HL, Li P, Huang HC, et al. Validation of the Decipher Test for predicting adverse pathology in candidates for prostate cancer active surveillance. Prostate Cancer Prostatic Dis. 2019;22(3):399-405.

Kim K, Zakharkin SO, Allison DB. Expectations, validity, and reality in gene expression profiling. Clin Epidemiol. 2010 Sep;63(9):950-9.

Kim ST, Banks KC, Lee SH, et al. Prospective feasibility study for using cell-free circulating tumor DNA–guided therapy in refractory metastatic solid cancers: an interim analysis. JCO Precision Oncology 2017:1, 1-15.

Klein EA, Richards D, Cohn A, et al. Clinical validation of a targeted methylation-based multi-cancer early detection test using an independent validation set. Ann Oncol. 2021 Sep;32(9):1167-1177.

Klein EA, Santiago-Jiménez M, Yousefi K, et al. Molecular analysis of low-grade prostate cancer using a genomic classifier of metastatic potential. J Urol. 2016 Jan;197(1):122-128.

Klufas MA, Richter E, Itty S, et al. Comparison of gene expression profiling and chromosome 3 analysis by fluorescent in situ hybridization and multiplex ligation probe amplification in fine-needle aspiration biopsy specimens of uveal melanoma. Ocul Oncol Pathol. 2017 Dec;4(1):16-20.

Kolquist KA, Schultz RA, Furrow A, et al. Microarray-based comparative genomic hybridization of cancer targets reveals novel, recurrent genetic aberrations in the myelodysplastic syndromes. Cancer Genet 2011;204(11):603-628.

Kornberg Z, Cooperberg MR, Cowan JE, et al. A 17-gene genomic prostate score as a predictor of adverse pathology in men on active surveillance. J Urol. 2019;202(4):702-709.

Kris MG, Johnson BE, Berry LD, et al. Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. JAMA. 2014;311(19):1998–2006.

Krop I, Ismaila N, Andre F, et al. Use of biomarkers to guide decisions on adjuvant systemic therapy for women with early-stage invasive breast cancer: American Society of Clinical Oncology clinical practice guideline focused update. J Clin Oncol. 2017 Aug 20;35(24):2838-47.

Lam VK, Tran HT, Banks KC, et al. Targeted tissue and cell-free tumor DNA sequencing of advanced lung squamous-cell carcinoma reveals clinically significant prevalence of actionable alterations. Clin Lung Cancer. 2019 Jan;20(1):30-36.e3.

Lastra RR, Pramick MR, Crammer CJ, et al. Implications of a suspicious Afirma test result in thyroid fine-needle aspiration cytology: an institutional experience. Cancer Cytopathol. 2014;122(10):737–744.

Laurie CC, Laurie CA, Smoley SA, et al. Acquired chromosomal anomalies in chronic lymphocytic leukemia (CLL) patients compared to > 50,000 quasi-normal subjects. Cancer Genetics. 2014;207(0):19-30.

Lee E, Terhaar S, McDaniel L, et al. Diagnostic performance of the second-generation molecular tests in the assessment of indeterminate thyroid nodules: A systematic review and meta-analysis. Am J Otolaryngol. 2022 May-Jun;43(3):103394.

Leighl NB, Page RD, Raymond VM, et al. Clinical utility of comprehensive cell-free DNA analysis to identify genomic biomarkers in patients with newly diagnosed metastatic non-small cell lung cancer. Clin Cancer Res. 2019 Aug 1;25(15):4691-4700.

Lipsyc-Sharf M, de Bruin EC, Santos K, et al. Circulating tumor DNA and late recurrence in high-risk hormone receptor-positive, human epidermal growth factor receptor 2-negative breast cancer. J Clin Oncol. 2022 Aug 1;40(22):2408-2419.

Liu MC, Oxnard GR, Klein EA, Swanton C, Seiden MV; CCGA Consortium. Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA. Ann Oncol. 2020 Jun;31(6):745-759.

Livhits MJ, Zhu CY, Kuo EJ, et al. Effectiveness of Molecular Testing Techniques for Diagnosis of Indeterminate Thyroid Nodules: A Randomized Clinical Trial. JAMA Oncol. 2021 Jan 1;7(1):70-77.

Lombardo R, Tosi F, Nocerino A, et al. The quest for Improving treatment of cancer of unknown primary (CUP) through molecularly-driven treatments: a systematic review. Front Oncol. 2020 May 8;10:533.

Loupakis F, Sharma S, Derouazi M, et al. Detection of molecular residual disease using personalized circulating tumor DNA assay in patients With colorectal cancer undergoing resection of metastases. JCO Precis Oncol. 2021 Jul 21;5:PO.21.00101.

Lowery MA, Wong W, Jordan EJ, et al. Prospective evaluation of germline alterations in patients with exocrine pancreatic neoplasms. J Natl Cancer Inst. 2018 Oct 1;110(10):1067-1074.

Lowrance WT, Breau RH, Chou R et al. Advanced Prostate Cancer: AUA/ASTRO/SUO Guideline PART I. J Urol 2021; 205: 14.

Lowrance WT, Breau RH, Chou R et al. Advanced Prostate Cancer: AUA/ASTRO/SUO Guideline PART II. J Urol 2021; 205: 22.

Magbanua MJM, Swigart LB, Wu HT, et al. Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival. Ann Oncol. 2021 Feb;32(2):229-239.

Marabelle A, Fakih M, Lopez J, et al. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. Lancet Oncol. 2020 Oct;21(10):1353-1365.

Marascio J, Spratt DE, Zhang J, et al. Prospective study to define the clinical utility and benefit of Decipher testing in men following prostatectomy. Prostate Cancer Prostatic Dis. 2020 Jun;23(2):295-302.

Marchetti MA, Coit DG, Dusza SW, et al. Performance of gene expression profile tests for prognosis in patients with localized cutaneous melanoma: A systematic review and meta-analysis. JAMA Dermatol. 2020 Sep 1;156(9):953-962.

Marcus L, Fashoyin-Aje LA, Donoghue M, et al. FDA approval summary: pembrolizumab for the treatment of tumor mutational burden-high solid tumors. Clin Cancer Res. 2021 Sep 1;27(17):4685-4689.

Marrone M, Potosky AL, Penson D, Freedman AN. A 22 gene-expression assay, Decipher® (GenomeDx Biosciences) to predict five-year risk of metastatic prostate cancer in men treated with radical prostatectomy. PLoS Currents. 2015;7.

Marshall KW, Mohr S, El Khettabi F, et al. A blood-based biomarker panel for stratifying current risk for colorectal cancer. Int J Cancer. 2010;126:1177–86.

Marti JL, Avadhani V, Donatelli LA, et al. Wide inter-institutional variation in performance of a molecular classifier for indeterminate thyroid nodules. Ann Surg Oncol. 2015;22(12):3996–400.

McCoach CE, Blakely CM, Banks KC, et al. Clinical utility of cell-free DNA for the detection of ALK fusions and genomic mechanisms of ALK inhibitor resistance in non-small cell lung cancer. Clin Cancer Res. 2018 Jun 15;24(12):2758-2770.

McKiernan J, Donovan MJ, O'Neill V, et al. A novel urine exosome gene expression assay to predict high-grade prostate cancer at initial biopsy. JAMA Oncol. 2016 Jul 1;2(7):882-9.

McKiernan J, Donovan M, Margolis E, et al. A prospective adaptive utility trial to validate performance of a novel urine exosome gene expression assay to predict high-grade prostate cancer in patients with prostate-specific Antigen 2–10 ng/ml at initial biopsy. European Urology 2018; 74(6):731-738.

MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US) [updated 2020 Jun 24]. What are whole exome sequencing and whole genome sequencing? [updated 2021 July 28]. Available at: https://medlineplus.gov/genetics/understanding/testing/sequencing/. Accessed December 8, 2022.

Mehta S, Shelling A, Muthukaruppan A, et al. Predictive and prognostic molecular markers for cancer medicine. Ther Adv Med Oncol. 2010 Mar;2(2):125-48.

Meleth S, Whitehead N, Swinson T, et al. Technology assessment on genetic testing or molecular pathology testing of cancers with unknown primary site to determine origin. Technology Assessment Report. Rockville, MA: Agency for Healthcare Research and Quality. February 2013.

Merker JD, Oxnard GR, Compton C, et al. Circulating tumor DNA analysis in patients with cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review. J Clin Oncol. 2018 Jun 1;36(16):1631-1641.

Meti N, Kelly D, Allen MJ, et al. Genomic sequencing to inform therapy in advanced pancreatic cancer: A systematic review and meta-analysis of prospective studies. Cancer Treat Rev. 2021 Dec;101:102310. Epub 2021 Oct 21.

Mikhael J, Ismaila N, Cheung MC, et al. Treatment of multiple myeloma: ASCO and CCO joint clinical practice guideline. J Clin Oncol. 2019 May 10;37(14):1228-1263.

Milbury CA, Creeden J, Yip WK, et al. Clinical and analytical validation of FoundationOne®CDx, a comprehensive genomic profiling assay for solid tumors. PLoS One. 2022 Mar 16;17(3):e0264138.

Moschini M, Spahn M, Mattei A, et al. Incorporation of tissue-based genomic biomarkers into localized prostate cancer clinics. BMC Med. 2016; 14: 67.

Mosele F, Remon J, Mateo Jet al. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. Ann Oncol. 2020 Nov;31(11):1491-1505.

Na R, Wu Y, Ding Q, et al. Clinically available RNA profiling tests of prostate tumors: utility and comparison. Asian J Androl. 2016 Jul-Aug; 18(4): 575–579.

National Cancer Institute (NCI). Liquid biopsy. Available at: https://www.cancer.gov/publications/dictionaries/cancer-terms/def/liquid-biopsy. Accessed November 23, 2022.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Acute lymphoblastic leukemia. Version 1.2022.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Acute myeloid leukemia. Version 3.2022.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Ampullary Adenocarcinoma. Version 1.2022.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Bladder cancer. Version 2.2022.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Breast cancer. Version 4.2022.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Bone Cancer. Version 2.2023.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Cervical Cancer. Version 1.2022.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Colon cancer. Version 2.2022.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Esophageal and esophagogastric junction cancers. Version 4.2022.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Gastric Cancer. Version 2.2022.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Histiocytic Neoplasms. Version 1. 2022.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Melanoma: Cutaneous. Version 3. 2022.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Melanoma: Uveal. Version 2.2022.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Multiple myeloma. Version 3.2023.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Myelodysplastic syndromes. Version 1.2023.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Myeloproliferative neoplasms. Version 3.2022.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Non-small cell lung cancer. Version 5.2022.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Occult primary (cancer of unknown primary [CUP]). Version 2.2023.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Ovarian cancer including fallopian tube cancer and primary peritoneal cancer. Version 5.2022.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Pancreatic adenocarcinoma. Version1.2022.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Pediatric acute lymphoblastic Leukemia. Version 1.2022.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Prostate cancer. Version 1.2023.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Rectal cancer. Version 3.2022.

National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology. Thyroid Carcinoma. Version 3.2022.

National Institute for Health and Care Excellence (NICE). Caris Molecular Intelligence for guiding cancer treatment. Medtech innovation briefing. September 2017.

National Institute for Health and Care Excellence (NICE). clonoSEQ for minimal residual disease assessment in multiple myeloma, acute lymphoblastic leukaemia and chronic lymphocytic leukaemia. Medtech innovation briefing [MIB278]. November 2021.

National Institute for Health and Care Excellence (NICE). Signatera for detecting molecular residual disease from solid tumour cancers. Medtech innovation briefing. October 2022.

National Institute for Health and Care Excellence (NICE). Metastatic malignant disease of unknown primary origin in adults: diagnosis and management. Clinical Guideline CG04. July 2010.

National Institute for Health and Care Excellence (NICE). Tumor profiling tests for guiding adjuvant chemotherapy choice in early breast cancer. Diagnostics guidance [DG34]. December 2018.

Nikiforova MN, Mercurio S, Wald AI, et al. Analytical performance of the ThyroSeq v3 genomic classifier for cancer diagnosis in thyroid nodules. Cancer. 2018 Apr 15;124(8):1682-1690.

Nishino M and Nikiforova M. Update on molecular testing for cytologically indeterminate thyroid nodules. Archives of Pathology & Laboratory Medicine: April 2018, Vol. 142, No. 4, pp. 446-457.

Noordhoek I, Treuner K, Putter H et al. Breast Cancer Index predicts extended endocrine benefit to individualize selection of patients with HR+ early-stage breast cancer for 10 years of endocrine therapy. Clin Cancer Res. 2021 Jan 1;27(1):311-319.

Oderda M, Cozzi G, Daniele L, et al. Cell-cycle progression-score might improve the current risk assessment in newly diagnosed prostate cancer patients. Urology. 2016 Nov 25.

Olsen S, Liao J, Hayashi H. Real-world clinical outcomes after genomic profiling of circulating tumor DNA in patients with previously treated advanced non-small cell lung cancer. Curr Oncol. 2022;29(7):4811-4826.

Ontario Health (Quality). Pigmented Lesion Assay for Suspected Melanoma Lesions: A Health Technology Assessment. Ont Health Technol Assess Ser. 2021 Jun 4;21(5):1-81.

Oxnard GR, Thress KS, Alden RS, et al. Association between plasma genotyping and outcomes of treatment with osimertinib (AZD9291) in advanced non-small-cell lung cancer. J Clin Oncol. 2016 Oct 1;34(28):3375-82.

Pagan M, Kloos RT, Lin C-F, et al. The diagnostic application of RNA sequencing in patients with thyroid cancer: an analysis of 851 variants and 133 fusions in 524 genes. BMC Bioinformatics. 2016;17(Suppl 1):6.

Palmetto GBA. Local Coverage Article for MolDx: AFIRMA™ Assay by Veracyte Update (Article ID Number A54356). January 5, 2018.

Palmieri M, Zulato E, Wahl SGF, et al. Diagnostic accuracy of circulating free DNA testing for the detection of kras mutations in non-small cell lung cancer: A systematic review and meta-analysis. Front Genet. 2022 Oct 25;13:1015161.

Parker JS, Mullins M, Cheang MCU, et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Onc. 2009;27(8):1160-1167.

Patel KN, Yip L, Lubitz CC, et al. The American Association of Endocrine Surgeons Guidelines for the definitive surgical management of thyroid disease in adults. Ann Surg. 2020 Mar;271(3):e21-e93.

Penault-Llorca F, Kwiatkowski F, Arnaud A, et al. Decision of adjuvant chemotherapy in intermediate risk luminal breast cancer patients: a prospective multicenter trial assessing the clinical and psychological impact of Endopredict® (epclin) use (ucbg 2-14). Breast. 2020 Feb;49:132-140.

Peterson JF, Aggarwal N, Smith CA, et al. Integration of microarray analysis into the clinical diagnosis of hematological malignancies: How much can we improve cytogenetic testing? Oncotarget. 2015;6(22):18845-18862.

Peterson JF, Van Dyke DL, Hoppman NL, et al. The utilization of chromosomal microarray technologies chromosomal microarray technologies for hematologic neoplasms: Hematologic Neoplasms: An ACLPS critical review. Am J Clin Pathol. 2018 Oct 1;150(5):375-384.

Petit J, Carroll G, Gould T, et al. Cell-Free DNA as a diagnostic blood-based biomarker for colorectal cancer: a systematic review. J Surg Res. 2019;236:184-197.

Piccart M, van 't Veer LJ, Poncet C, et al. 70-gene signature as an aid for treatment decisions in early breast cancer: updated results of the phase 3 randomised MINDACT trial with an exploratory analysis by age. Lancet Oncol. 2021 Apr;22(4):476-488.

Plagnol V, Woodhouse S, Howarth K, et al. Analytical validation of a next generation sequencing liquid biopsy assay for high sensitivity broad molecular profiling. PLoS One. 2018;13(3):e0193802. Published 2018 Mar 15.

Plasseraud KM, Cook RW, Tsai T, et al. Clinical performance and management outcomes with the DecisionDx-UM gene expression profile test in a prospective multicenter study. J Oncol. 2016;2016:5325762.

Poorvu PD, Gelber SI, Rosenberg SM, et al. Prognostic impact of the 21-gene recurrence score assay among young women with node-negative and node-positive ER-positive/HER2-negative breast cancer. J Clin Oncol. 2020;38(7):725-733.

Prelude Corporation. DCISionRT [patient brochure]. Available at: https://preludedx.com/wp-content/uploads/2020/02/Patient-Brochure-English.pdf. Accessed December 8, 2022.

Ramos-Paradas J, Hernández-Prieto S, Lora D, et al. Tumor mutational burden assessment in non-small-cell lung cancer samples: results from the TMB2 harmonization project comparing three NGS panels. J Immunother Cancer. 2021 May;9(5):e001904.

Reinert T, Henriksen TV, Christensen E, et al., Analysis of plasma cell-free DNA by ultradeep sequencing in patients with stages I to III colorectal cancer. JAMA Oncol. 2019 Aug 1;5(8):1124-1131.

Riediger AL, Dietz S, Schirmer U, et al. Mutation analysis of circulating plasma DNA to determine response to EGFR tyrosine kinase inhibitor therapy of lung adenocarcinoma patients. Sci Rep. 2016 Sep 19;6:33505.

Rolfo C, Mack P, Scagliotti GV, et al. Liquid biopsy for advanced NSCLC: A consensus statement from the International Association for the Study of Lung Cancer. J Thorac Oncol. 2021 Oct;16(10):1647-1662.

Ross JS, Ali SM, Wang K, et al. Comprehensive genomic profiling of epithelial ovarian cancer by next generation sequencing-based diagnostic assay reveals new routes to targeted therapies. Gynecol Oncol. 2013 Sep;130(3):554-9.

Ross JS, Sokol ES, Moch H, et al. Comprehensive genomic profiling of carcinoma of unknown primary origin: retrospective molecular classification considering the CUPISCO study design. Oncologist. 2021 Mar;26(3):e394-e402.

Sacher AG, Paweletz C, Dahlberg SE, et al. Prospective validation of rapid plasma genotyping for the detection of EGFR and KRAS mutations in advanced lung cancer. JAMA Oncol. 2016 Aug 1;2(8):1014-22.

Sakata S, Otsubo K, Yoshida H, et al. Real-world data on NGS using the Oncomine DxTT for detecting genetic alterations in non-small-cell lung cancer: WJOG13019L. Cancer Sci. 2022 Jan;113(1):221-228.

Sanda MG, Cadeddu JA, Kirkby E, et al. Clinically localized prostate cancer: AUA/ASTRO/SUO Guideline. Part I: risk stratification, shared decision making, and care options. Care Options. J Urol. 2018 Mar;199(3):683-690.

Santhanam P, Khthir R, Gress T, et al. Gene expression classifier for the diagnosis of indeterminate thyroid nodules: a meta-analysis. Med Oncol. 2016 Feb;33(2):14.

Scheipl S, Brcic I, Moser T, et al. Molecular profiling of soft-tissue sarcomas with FoundationOne® Heme identifies potential targets for sarcoma therapy: a single-centre experience. Ther Adv Med Oncol. 2021 Jul 25;13:17588359211029125.

Sepulveda AR, Hamilton SR, Allegra CJ, et al. Molecular biomarkers for the evaluation of colorectal cancer: guideline from the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology. J Mol Diagn. 2017 Mar;19(2):187-225.

Sestak I, Buus R, Cuzick J, et al. Comparison of the performance of 6 prognostic signatures for estrogen receptor-positive breast cancer: a secondary analysis of a randomized clinical trial. JAMA Oncol. 2018 Apr 1;4(4):545-553.

Sestak I, Filipits M, Buus R, et al. Prognostic value of EndoPredict in women with hormone receptor positive, HER2-negative invasive lobular breast cancer. Clin Cancer Res. 2020;26(17):4682-4687.

Sestak I, Zhang Y, Schroeder BE, et al. Cross-stratification and differential risk by Breast Cancer Index and Recurrence Score in women with hormone receptor-positive lymph node-negative early-stage breast cancer. Clin Cancer Res. 2016 Oct 15;22(20):5043-5048.

Shah C, Bremer T, Cox C, et al. The clinical utility of DCISionRT® on radiation therapy decision making in patients with ductal carcinoma in situ following breast-conserving surgery. Ann Surg Oncol. 2021 Oct;28(11):5974-5984.

Short NJ, Fu C, Berry DA, et al. Association of hematologic response and assay sensitivity on the prognostic impact of measurable residual disease in acute myeloid leukemia: a systematic review and meta-analysis. Leukemia. 2022 Oct 19.

Short NJ, Jabbour E, Albitar M, et al. Recommendations for the assessment and management of measurable residual disease in adults with acute lymphoblastic leukemia: A consensus of North American experts. Am J Hematol. 2019 Feb;94(2):257-265.

Singh AD, Binkley EM, Wrenn JM, et al. Predicted vs observed metastasis-free survival in individuals with uveal melanoma. JAMA Ophthalmol. 2022 Sep 1;140(9):847-854.

Singh AP, Shum E, Rajdev L, et al. Impact and diagnostic gaps of comprehensive genomic profiling in real-world clinical practice. Cancers (Basel). 2020 May 4;12(5):1156.

Singhi AD, McGrath K, Brand RE, et al. Preoperative next-generation sequencing of pancreatic cyst fluid is highly accurate in cyst classification and detection of advanced neoplasia. Gut. 2018 Dec;67(12):2131-2141.

Sipos JA, Blevins TC, Shea HC, et al. Long-term nonoperative rate of thyroid nodules with benign results on the Afirma gene expression classifier. Endocr Pract. 2016 Jun;22(6):666-72.

Sohal DPS, Kennedy EB, Cinar P, et al. Metastatic pancreatic cancer: ASCO guideline update. J Clin Oncol. 2020 Aug 5:JCO2001364.

Sohal DPS, Kennedy EB, Khorana A, et al. Metastatic pancreatic cancer: ASCO Clinical Practice Guideline Update. Journal of Clinical Oncology, 2018 36:24,2545-2556.

Soliman H, Shah V, Srkalovic G, et al. MammaPrint guides treatment decisions in breast cancer: results of the impact trial. BMC Cancer. 2020 Jan 31;20(1):81.

Song Q, Peng M, Chu Y, Huang S. Techniques for detecting chromosomal aberrations in myelodysplastic syndromes. Oncotarget. 2017a;8(37):62716-62729.

Spratt DE, Yousefi K, Deheshi S, et al. Individual patient-level meta-analysis of the performance of the Decipher genomic classifier in high-risk men after prostatectomy to predict development of metastatic disease. J Clin Oncol 2017;35:1991-1998.

Steward DL, Carty SE, Sippel RS, et al. Performance of a multigene genomic classifier in thyroid nodules With indeterminate cytology: a prospective blinded multicenter study. JAMA Oncol. 2019 Feb 1;5(2):204-212.

Sun Q, Liu Y, Liu B, Liu Y. Use of liquid biopsy in monitoring colorectal cancer progression shows strong clinical correlation. Am J Med Sci. 2018 Mar;355(3):220-227.

Swetter SM, Tsao H, Bichakjian CK, et al. Guidelines of care for the management of primary cutaneous melanoma. J Am Acad Dermatol. 2019 Jan. 80 (1):208-250.

Takeda M, Takahama T, Sakai K, et al. Clinical application of the FoundationOne CDx Assay to therapeutic decision-making for patients with advanced solid tumors. Oncologist. 2021 Apr;26(4):e588-e596.

Thompson JC, Yee SS, Troxel AB, et al. Detection of therapeutically targetable driver and resistance mutations in lung cancer patients by next-generation sequencing of cell-free circulating tumor DNA. Clin Cancer Res. 2016;22(23):5772-5782.

Tosoian JJ, Trock BJ, Morgan TM, et al. Use of the MyProstateScore test to rule out clinically significant cancer: validation of a straightforward clinical testing approach. J Urol. 2021 Mar;205(3):732-739.

Trédan O, Wang Q, Pissaloux D, et al. Molecular screening program to select molecular-based recommended therapies for metastatic cancer patients: analysis from the ProfiLER trial. Ann Oncol. 2019;30(5):757-765.

Trikalinos TA, Terasawa T, Raman G, et al. A systematic review of loss-of-heterozygosity based topographic genotyping with PathfinderTG. Technology Assessment Report GEND0308. Prepared by the Tufts Evidence-based Practice Center for the Agency for Healthcare Research and Quality AHRQ under Contract No HHSA 290 10055 I AHRQ March. 2010.

Tutrone R, Donovan MJ, Torkler P, et al. Clinical utility of the exosome based ExoDx Prostate(IntelliScore) EPI test in men presenting for initial biopsy with a PSA 2-10 ng/mL. Prostate Cancer Prostatic Dis. 2020 Dec;23(4):607-614.

van Steenhoven JEC, Kuijer A, van Diest PJ, et al. Conventional pathology versus gene signatures for assessing luminal A and B type breast cancers: results of a prospective cohort study. Genes (Basel). 2018;9(5):261.

Varadhachary GR, Raber MN. Cancer of unknown primary site. N Engl J Med 2014;371:757-65.

Venook AP, Niedzwiecki D, Lopatin M, et al. Biologic determinants of tumor recurrence in stage II colon cancer: validation study of the 12-gene recurrence score in cancer and leukemia group B (CALGB) 9581. J Clin Oncol. 2013;31(14):1775-1781.

Villaflor V, Won B, Nagy R, et al. Biopsy-free circulating tumor DNA assay identifies actionable mutations in lung cancer. Oncotarget. 2016;7(41):66880-66891.

Vince RA Jr, Jiang R, Qi J, et al. Impact of Decipher Biopsy testing on clinical outcomes in localized prostate cancer in a prospective statewide collaborative. Prostate Cancer Prostatic Dis. 2021 Jul 20.

Vliek SB, Hilbers FS, Jager A, et al. Ten-year follow-up of the observational raster study, prospective evaluation of the 70-gene signature in er-positive, her2-negative, node-negative, early breast cancer. Eur J Cancer. 2022 Nov;175:169-179.

Wang M, Wu K, Zhang P, Zhang M, et al. The prognostic significance of the Oncotype DX Recurrence Score in T1-2N1M0 estrogen receptor-positive HER2-negative breast cancer based on the prognostic stage in the updated AJCC 8th edition. Ann Surg Oncol. 2019;26(5):1227-1235.

Wang S, Qu X, Cao L, et al. Assessment of nine driver gene mutations in surgically resected samples from patients with non-small-cell lung cancer. Cancer Manag Res. 2020 May 28;12:4029-4038.

Weinhold N, Heuck C, Rosenthal A, et al. The clinical value of molecular subtyping multiple myeloma using gene expression profiling. Leukemia. 2016 February; 30(2): 423–430.

Weinmann S, Leo MC, Francisco M, J, et al. Validation of a ductal carcinoma *in situ* biomarker profile for risk of recurrence after breast-conserving surgery with and without radiotherapy. Clin Cancer Res. 2020 Aug 1;26(15):4054-4063.

Wheler JJ, Parker BA, Lee JJ, et al. Unique molecular signatures as a hallmark of patients with metastatic breast cancer: implications for current treatment paradigms. Oncotarget. 2014 May 15;5(9):2349-54.

Wheler J, Yelensky R, Falchook G, et al. Next generation sequencing of exceptional responders with BRAF-mutant melanoma: implications for sensitivity and resistance. BMC Cancer. 2015 Feb 18;15:61.

Wierda WG, Rawstron A, Cymbalista F, et al. Measurable residual disease in chronic lymphocytic leukemia: expert review and consensus recommendations. Leukemia. 2021 Nov;35(11):3059-3072.

Wolmark N, Mamounas EP, Baehner FL et al. Prognostic impact of the combination of recurrence score and quantitative estrogen receptor expression (ESR1) on predicting late distant recurrence risk in estrogen receptor–positive breast cancer after 5 years of tamoxifen: Results from NRG Oncology/National Surgical Adjuvant Breast and Bowel Project B-28 and B-14 Journal of Clinical Oncology 34, no. 20 (July 2016) 2350-2358.

Wong W, Lowery MA, Berger MF, et al. Ampullary cancer: evaluation of somatic and germline genetic alterations and association with clinical outcomes. Cancer. 2019 Jan 8.

Wood B, Wu D, Crossley B, et.al. Measurable residual disease detection by high-throughput sequencing improves risk stratification for pediatric B-ALL. Blood. 2018 Mar 22;131(12):1350-1359.

Woodhouse R, Li M, Hughes J, et al. Clinical and analytical validation of FoundationOne Liquid CDx, a novel 324-Gene cfDNA-based comprehensive genomic profiling assay for cancers of solid tumor origin. PLoS One. 2020 Sep 25;15(9):e0237802.

Wuerstlein R, Kates R, Gluz O, et al. Strong impact of Mammaprint and Blueprint on treatment decisions in luminal early breast cancer: results of the wsg-prime study. Breast Cancer Res Treat. 2019 Jun;175(2):389-399.

Wylie D, Beaudenon-Huibregtse S, Haynes B, et al. Molecular classification of thyroid lesions by combined testing for miRNA gene expression and somatic gene alterations. The Journal of Pathology: Clinical Research. 2016;2(2):93-103.

Yamamoto Y, Uemura M, Fujita M, et al. Clinical significance of the mutational landscape and fragmentation of circulating tumor DNA in renal cell carcinoma. Cancer Sci. 2019;110(2):617-628.

Yamanaka T, Oki E, Yamazaki K, et al. 12-gene recurrence score assay stratifies the recurrence risk in stage II/III colon cancer with surgery alone: the SUNRISE study. J Clin Oncol. 2016 Aug 20;34(24):2906-13.

Yan S, Liu Z, Yu S, Bao Y. Diagnostic value of methylated septin9 for colorectal cancer screening: a meta-analysis. Med Sci Monit. 2016;22:3409–3418.

Yang M, Topaloglu U, Petty WJ, et al. Circulating mutational portrait of cancer: manifestation of aggressive clonal events in both early and late stages. J Hematol Oncol. 2017 May 4;10(1):100.

Yang SE, Sullivan PS, Zhang J et al. Has Afirma gene expression classifier testing refined the indeterminate thyroid category in cytology? Cancer Cytopathol. 2016 Feb;124(2):100-9.

Yao ZG, Wei ZG, Cheng XK, et al. Comparison of multi-gene testing data between fresh and formalin-fixed specimens from core needle biopsy in patients with NSCLC. Pathol Oncol Res. 2021 Dec 13;27:1609931.

Yothers G, O'Connell MJ, Lee M, et al. Validation of the 12-gene colon cancer recurrence score in NSABP C-07 as a predictor of recurrence in patients with stage II and III colon cancer treated with fluorouracil and leucovorin (FU/LV) and FU/LV plus oxaliplatin. J Clin Oncol. 2013 Dec 20;31(36):4512-9.

Yothers G, Venook AP, Oki E, et al. Patient-specific meta-analysis of 12-gene colon cancer recurrence score validation studies for recurrence risk assessment after surgery with or without 5FU and oxaliplatin. J Gastrointest Oncol. 2022 Feb;13(1):126-136.

Zager JS, Gastman BR, Leachman S, et al. Performance of a prognostic 31-gene expression profile in an independent cohort of 523 cutaneous melanoma patients. BMC Cancer. 2018;18:130.

Zhang BY, Jones JC, Briggler, et al. Lack of caudal-type homeobox transcription factor 2 expression as a prognostic biomarker in metastatic colorectal cancer. Clin Colorectal Cancer. 2016 Sep 17.

Zhang M and Lin O. Molecular testing of thyroid nodules: a review of current available tests for fine-needle aspiration specimens. Archives of Pathology & Laboratory Medicine: December 2016, Vol. 140, No. 12, pp. 1338-1344.

Zhang N, Zhang J, Wang G, et al. Predictive efficacy of blood-based tumor mutation burden assay for immune checkpoint inhibitors therapy in non-small cell lung cancer: a systematic review and meta-analysis. Front Oncol. 2022 Feb 9;12:795933.

Zhang Y, Schnabel CA, Schroeder BE, et al. Breast cancer index identifies early-stage estrogen receptor-positive breast cancer patients at risk for early- and late-distant recurrence. Clin Cancer Res. 2013 Aug 1;19(15):4196-205.

Zhang Y, Schroeder BE, Jerevall PL,et al. A novel breast cancer index for prediction of distant recurrence in HR(+)early-stage breast cancer with one to three positive nodes. Clin Cancer Res. 2017 Dec 1;23(23):7217-7224.

Policy History/Revision Information

Date	Summary of Changes

10/01/2023	**Application**
	Individual Exchange Plans
	• Removed language indicating this Medical Policy does not apply to Individual Exchange benefit plans in the states of Massachusetts, Nevada, and New York
	Applicable Codes
	• Updated list of applicable CPT codes to reflect quarterly edits:
	  ○ Added 0409U
	  ○ Removed 0386U and 0397U
	  ○ Revised description for 0362U
	Supporting Information
	• Archived previous policy version 2023T0588AA

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, please 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.