Molecular Oncology Testing for Cancer Diagnosis, Prognosis, and Treatment Decisions (for New Jersey Only)

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➲ Instructions for Use

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Related Policies
None

Application

This Medical Policy only applies to the state of New Jersey.

Coverage Rationale

Breast Cancer

The use of one of the following Gene Expression Tests – MammaPrint, Oncotype Dx Breast, Prosigna PAM-50 Breast Cancer Prognostic Gene Signature Assay, Breast Cancer Index (BCI) and EndoPredict – is proven and medically necessary to make a treatment decision regarding adjuvant chemotherapy in females or males with invasive breast cancer in the following situations:

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

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

Use of more than one predictive Gene Expression Test 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.

Gene Expression Tests for breast cancer are unproven and not medically necessary for all other indications, including ductal carcinoma in situ (DCIS), due to insufficient evidence of efficacy.

Due to insufficient evidence of efficacy, gene expression profiling assays for breast cancer treatment other than those previously described as covered are unproven and not medically necessary, including but not limited to:
- BluePrint (also referred to as "80-gene profile")
- Breast Cancer Gene Expression Ratio (also known as Theros H/I)
- DCISionRT
- Oncotype DX DCIS
- The 41-gene signature assay
- The 76-gene "Rotterdam signature" assay

Thyroid Cancer
Molecular profiling of thyroid nodules with indeterminate cytology (e.g., Afirma GSC, ThyroSeq V3, ThyGeNEXT/ThyraMIR is proven and medically necessary when all the following criteria are met:
- Follicular pathology on fine needle aspiration is indeterminate (Bethesda III/IV)
- The results of the test will be used for making decisions about further surgery

Molecular profiling of confirmed thyroid cancer (except anaplastic thyroid cancer) with genes or gene panels (NTRK, ALK, MMR, MSI, RAS, HRAS, NRAS, RET/PTC1, RET/PTC3, PAX8/PPARγ) is unproven and not medically necessary for all indications due to insufficient evidence of efficacy.

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

Hematological Cancer
Molecular profiling using chromosomal microarray Analysis (e.g., Oncoscan, Reveal SNP-Oncology, CGH or SNP array) is proven and medically necessary for individuals with acute leukemia.

Use of a Next Generation Sequencing profile test to assess minimal residual disease (e.g., ClonoSeq, MyMRD) is proven and medically necessary when the following criteria are met:
- Individual has acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL) and testing is being performed within 3 months of completing a course of therapy and there is no clinical evidence of disease; or
- Individual has multiple myeloma and testing is being performed within three months of an allogenic or autologous bone marrow transplant; and there is no clinical evidence of disease

All other multigene, gene expression or microarray molecular profiling for hematological malignancies is unproven and not medically necessary due to insufficient evidence of efficacy.
This includes, but is not limited to the following:
- Assessment of minimal residual disease by Next Generation Sequencing for acute myeloid leukemia
- Use of multi-gene Next Generation Sequencing gene panels for predicting prognosis

Lung Cancer
Multigene molecular profiling of metastatic non-small cell lung cancer is proven and medically necessary when all of the following criteria are met:
- The panel selected has no more than 50 genes; and
- No prior molecular profiling has been performed on the same tumor

Liquid biopsy (circulating tumor cell free DNA) molecular profiling tests of non-small cell lung cancer are proven and medically necessary when the following criteria is met:
- The test selected has no more than 50 genes; and
- No prior molecular profiling has been performed on the same tumor; and

- The individual is not medically fit for invasive biopsy; or
- Non-small cell lung cancer has been pathologically confirmed, but there is insufficient material available for molecular testing; 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 therapy

Uveal Melanoma

Gene expression profile testing (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
- Has not previously had DecisionDx-UM testing for current diagnosis

Liquid biopsy (circulating tumor cell free DNA or circulating tumor cells) for any other tumor genetic analysis or tumor screening (e.g., Guardant360, ColoSentry, epi ProColon, OncoCEE CTC, Foundation One Liquid CDx) or multi-cancer early detection tests (e.g., Galleri) are unproven and not medically necessary due to insufficient evidence of efficacy.

Due to insufficient evidence of efficacy, molecular testing such as gene expression profiling, Chromosome Microarray Analysis and multi-gene cancer panels are unproven and not medically necessary for all other indications, including but not limited to:
- Bladder Cancer (e.g., Decipher Bladder) (NCCN, Bladder 2021)
- Cancers of unknown primary site (e.g., Response Dx, CancerTYPE ID, Rosetta Cancer Origin, ProOnc, SourceDX,)
- Pancreatic Cancer (e.g., PancraGen)
- Colorectal Cancer (e.g., Oncotype DX® Colon Cancer Assay, Colorectal Cancer DSA™, Genefx Colon® (also known as ColDx), OncoDefender™, CRC, ColoPrint®, ColDx)
- Gene panels of >50 genes
- Leukemia other than Chromosome Microarray Analysis (e.g., FoundationOne® Heme)
- Melanoma (e.g., DecisionDx–Melanoma, DermTech PLA)
- Multiple myeloma (e.g., MyPRS/MyPRS Plus)
- Prostate cancer [e.g., Oncotype DX Prostate Cancer Assay, TMPRSS2 fusion gene, Prolaris Prostate Cancer Test, Decipher Prostate Cancer Classifier, ExoDX Prostate IntelliScore (EPI)]
- Tumor-informed assays (Signatera)
- Whole Exome Sequencing (WES) and Whole Genome Sequencing (WGS) of tumors

Definitions

Comparative Genome Hybridization (CGH): CGH is a technology that can be used for the detection of 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 labelled 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: 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).

Gene Expression Testing: A laboratory test that analyzes mRNA patterns to determine gene activity (Kim et al. 2010).

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).

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 (U.S. National Library of Medicine, 2017A).

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 (U.S. National Library of Medicine, 2017B).

Applicable Codes

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

CPT Code	Description
0005U	Oncology (prostate) gene expression profile by real-time RT-PCR of 3 genes (ERG, PCA3, and SPDEF), urine, algorithm reported as risk score
0011M	Oncology, prostate cancer, mRNA expression assay of 12 genes (10 content and 2 housekeeping), RT-PCR test utilizing blood plasma and urine, algorithms to predict high-grade prostate cancer risk
0012M	Oncology (urothelial), mRNA, gene expression profiling by real-time quantitative PCR of five genes (MDK, HOXA13, CDC2 [CDK1], IGFBP5, and CXCR2), utilizing urine, algorithm reported as a risk score for having urothelial carcinoma
0013M	Oncology (urothelial), mRNA, gene expression profiling by real-time quantitative PCR of five genes (MDK, HOXA13, CDC2 [CDK1], IGFBP5, and CXCR2), utilizing urine, algorithm reported as a risk score for having recurrent urothelial carcinoma
0013U	Oncology (solid organ neoplasia), gene rearrangement detection by whole genome next-generation sequencing, DNA, fresh or frozen tissue or cells, report of specific gene rearrangement(s)
0014U	Hematology (hematolymphoid neoplasia), gene rearrangement detection by whole genome next-generation sequencing, DNA, whole blood or bone marrow, report of specific gene rearrangement(s)
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
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, cholangiocarcinoma and non-small cell lung neoplasia, DNA and RNA analysis, 1-23 genes, interrogation for sequence variants and rearrangements, reported as presence/absence of variants and associated therapy(ies) to consider

CPT Code	Description
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
0056U	Hematology (acute myelogenous leukemia), DNA, whole genome next-generation sequencing to detect gene rearrangement(s), blood or bone marrow, report of specific gene rearrangement(s)
0069U	Oncology (colorectal), microRNA, RT-PCR expression profiling of miR-31-3p, formalin-fixed paraffin-embedded tissue, algorithm reported as an expression score
0089U	Oncology (melanoma) gene expression profiling by RTqPCR PRAME and LINC00518 superficial collection using adhesive patch(es)
0090U	Oncology (cutaneous melanoma), mRNA gene expression profiling by RT-PCR of 23 genes (14 content and 9 housekeeping), utilizing formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as a categorical result (i.e., benign, intermediate, malignant)
0091U	Oncology (colorectal) screening cell enumeration of circulating tumor cells utilizing whole blood algorithm for the presence of adenoma or cancer reported as a positive or negative result
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
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

CPT Code	Description
0211U	Oncology (pan-tumor), DNA and RNA by next-generation sequencing, utilizing formalin-fixed paraffin-embedded tissue, interpretative report for single nucleotide variants, copy number alterations, tumor mutational burden, and microsatellite instability, with therapy association
0239U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free DNA, analysis of 311 or more genes, interrogation for sequence variants, including substitutions, insertions, deletions, select rearrangements, and copy number variations
0242U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free circulating DNA analysis of 55-74 genes, interrogation for sequence variants, gene copy number amplifications, and gene rearrangements
0244U	Oncology (solid organ), DNA, comprehensive genomic profiling, 257 genes, interrogation for single-nucleotide variants, insertions/deletions, copy number alterations, gene rearrangements, tumor-mutational burden and microsatellite instability, utilizing formalin-fixed paraffin-embedded tumor tissue
0245U	Oncology (thyroid), mutation analysis of 10 genes and 37 RNA fusions and expression of 4 mRNA markers using next-generation sequencing, fine needle aspirate, report includes associated risk of malignancy expressed as a percentage
0250U	Oncology (solid organ neoplasm), targeted genomic sequence DNA analysis of 505 genes, interrogation for somatic alterations (SNVs [single nucleotide variant], small insertions and deletions, one amplification, and four translocations), microsatellite instability and tumor-mutation burden
0262U	Oncology (solid tumor), gene expression profiling by real-time RT-PCR of 7 gene pathways (ER, AR, PI3K, MAPK, HH, TGFB, Notch), formalin-fixed paraffin-embedded (FFPE), algorithm reported as gene pathway activity score
0285U	Oncology, response to radiation, cell-free DNA, quantitative branched chain DNA amplification, plasma, reported as a radiation toxicity score
0287U	Oncology (thyroid), DNA and mRNA, next-generation sequencing analysis of 112 genes, fine needle aspirate or formalin-fixed paraffin-embedded (FFPE) tissue, algorithmic prediction of cancer recurrence, reported as a categorical risk result (low, intermediate, high)
0288U	Oncology (lung), mRNA, quantitative PCR analysis of 11 genes (BAG1, BRCA1, CDC6, CDK2AP1, ERBB3, FUT3, IL11, LCK, RND3, SH3BGR, WNT3A) and 3 reference genes (ESD, TBP, YAP1), formalin-fixed paraffin-embedded (FFPE) tumor tissue, algorithmic interpretation reported as a recurrence risk score
0296U	Oncology (oral and/or oropharyngeal cancer), gene expression profiling by RNA sequencing at least 20 molecular features (e.g., human and/or microbial mRNA), saliva, algorithm reported as positive or negative for signature associated with malignancy
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-…
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

CPT Code	Description
0313U	Oncology (pancreas), DNA and mRNA next-generation sequencing analysis of 74 genes and analysis of CEA (CEACAM5) gene expression, pancreatic cyst fluid, algorithm reported as a categorical result (i.e., negative, low probability of neoplasia or positive, high probability of neoplasia)
0314U	Oncology (cutaneous melanoma), mRNA gene expression profiling by RT-PCR of 35 genes (32 content and 3 housekeeping), utilizing formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as a categorical result (i.e., benign, intermediate, malignant)
0315U	Oncology (cutaneous squamous cell carcinoma), mRNA gene expression profiling by RT-PCR of 40 genes (34 content and 6 housekeeping), utilizing formalin-fixed paraffin-embedded (FFPE) tissue, algorithm reported as a categorical risk result (i.e., Class 1, Class 2A, Class 2B)
0326U	Targeted genomic sequence analysis panel, solid organ neoplasm, cell-free circulating DNA analysis of 83 or more genes, interrogation for sequence variants, gene copy number amplifications, gene rearrangements, microsatellite instability and tumor mutational burden
0329U	Oncology (neoplasia), exome and transcriptome sequence analysis for sequence variants, gene copy number amplifications and deletions, gene rearrangements, microsatellite instability and tumor mutational burden utilizing DNA and RNA from tumor with DNA from normal blood or saliva for subtraction, report of clinically significant mutation(s) with therapy associations
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
81228	Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of genomic regions for copy number variants, comparative genomic hybridization [CGH] microarray analysis
81229	Cytogenomic (genome-wide) analysis for constitutional chromosomal abnormalities; interrogation of genomic regions for copy number and single nucleotide polymorphism (SNP) variants, comparative genomic hybridization (CGH) microarray analysis
81277	Cytogenomic neoplasia (genome-wide) microarray analysis, interrogation of genomic regions for copy number and loss-of-heterozygosity variants for chromosomal abnormalities
81425	Genome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis
81426	Genome (e.g., unexplained constitutional or heritable disorder or syndrome); sequence analysis, each comparator genome (e.g., parents, siblings) (List separately in addition to code for primary procedure)
81427	Genome (e.g., unexplained constitutional or heritable disorder or syndrome); re-evaluation of previously obtained genome sequence (e.g., updated knowledge or unrelated condition/syndrome)
81445	Targeted genomic sequence analysis panel, solid organ neoplasm, DNA analysis, and RNA analysis when performed, 5-50 genes (e.g., ALK, BRAF, CDKN2A, EGFR, ERBB2, KIT, KRAS, NRAS, MET, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed
81450	Targeted genomic sequence analysis panel, hematolymphoid neoplasm or disorder, DNA analysis, and RNA analysis when performed, 5-50 genes (e.g., BRAF, CEBPA, DNMT3A, EZH2, FLT3, IDH1, IDH2, JAK2, KRAS, KIT, MLL, NRAS, NPM1, NOTCH1), interrogation for sequence variants, and copy number variants or rearrangements, or isoform expression or mRNA expression levels, if performed
81455	Targeted genomic sequence analysis panel, solid organ or hematolymphoid neoplasm, DNA analysis, and RNA analysis when performed, 51 or greater genes (e.g., ALK, BRAF, CDKN2A, CEBPA, DNMT3A, EGFR, ERBB2, EZH2, FLT3, IDH1, IDH2, JAK2, KIT, KRAS, MLL, NPM1, NRAS, MET, NOTCH1, PDGFRA, PDGFRB, PGR, PIK3CA, PTEN, RET), interrogation for sequence variants and copy number variants or rearrangements, if performed
81479	Unlisted molecular pathology procedure
81504	Oncology (tissue of origin), microarray gene expression profiling of > 2000 genes, utilizing formalin-fixed paraffin-embedded tissue, algorithm reported as tissue similarity scores

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

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

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

Description of Services

Technologies used for molecular profiling of cancers vary, and can include, but are not limited to, tests that evaluate variations in the genes, such as Chromosome Microarray 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 (e.g., epi-Colon, Clonoseq, DermTech PLA). 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. In turn, this may help with the decision of whether to include adjuvant chemotherapy.

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” which may correspond to a likelihood of breast cancer recurrence within 10 years. The test was initially developed for women with early-stage invasive breast cancer with ER+ cancers that are lymph node-negative, 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).

Poorvu et al. (2020) evaluated women less than 40 years of age with early-stage ER+ and HER2- breast cancer to determine if the 21-gene recurrence score (RS) could inform chemotherapy recommendations. The prospective TAILORx study enrolled 509 patients and the RS assay was performed either clinically (189 patients) or on banked specimens (320 patients). The median follow-up time was 6 years. Of the 509 patients, 300 (59%) had N0 breast cancer 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 node-negative and limited node-positive disease.

Wang et al. (2019) examined the value of Oncotype Dx when determining the prognosis in female breast cancer patients with tumor stage 1-2 (tumor is 20-55mm), positive in 1-3 lymph nodes and no evidence of metastasis ($T_{1-2}N_1M_0$). 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 were linked to recurrence score (RS) results from assays performed by Genomic Health. All cases with RS had negative HER2, and the authors selected female ER-positive invasive ductal carcinoma cases in T1-2N1M0 stage with Oncotype RS results diagnosed between 2004 and 2012. Patients were categorized into low-risk (RS<11), intermediate-risk (RS 11–25), and high-risk (RS >25) groups. The median patient age was 59 years. Of these patients, 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 patients, 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 patients (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 node positive patients.

Altman et al. (2018) examined the utility of Oncotype Dx in women with estrogen receptor positive and HER2 positive breast cancer. Patients were identified from the Surveillance and Epidemiology End Results program database that met criteria and were stratified using the TAILORx recurrence score cutoffs. Of patients that 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 patients, 67% had chemotherapy, 30% of intermediate risk, and 19% of low risk patients. However, this study does not provide information on the clinical utility of Oncotype Dx in women with HER2 positive breast cancer since clinical outcomes were not captured.

Wolmark et al. (2016) assessed the utility for a 21 gene recurrence score (RS) in predicting distance recurrence (>5 years) in stages I and II breast cancer in high and low expressing ESR1 groups within a cohort of 3,060 patients 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 1-3 node positives, the risk of distant recurrence was 7.9%. In those with 4 or more nodes positive, the risk of distant recurrence was 16.7%.

Albain et al. (2010) studied the use of Oncotype Dx in node positive breast cancer. The authors used 367 samples banked from the phase 3 trial SWOG-8814 for postmenopausal women with node-positive, estrogen-receptor-positive breast cancer. This trial showed that chemotherapy with cyclophosphamide, doxorubicin, and fluorouracil (CAF) before tamoxifen (CAF-T) added survival benefit to treatment with tamoxifen alone. The samples available for study represented 40% of the 927 patients in the tamoxifen and CAF-T groups, with sufficient RNA for analysis (tamoxifen, n=148; CAF-T, n=219). There was no benefit identified in the CAF group who had a low recurrence score, but those with a high recurrence score had a strong correlation with an improvement in disease-free survival, after adjustment for number of positive nodes. The authors concluded that a high recurrence score may be prognostic for tamoxifen-treated patients with positive nodes and predicts significant benefit of CAF. A low recurrence score suggests that women might not benefit from anthracycline-based chemotherapy, despite positive nodes.

PAM-50

PAM-50, also known as Prosigna® (NanoString Technologies, Seattle, WA) is a breast cancer prognostic 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 node-negative (Stage I or II) or node-positive (Stage II), hormone receptor-positive breast cancer. 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 4 or more positive nodes (Gnant et al., 2013; Parker et al., 2009).

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 breast cancer distant recurrence risk. The assay analyzes tumor tissue (fresh, frozen or formalin-fixed paraffin-embedded) 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.

The randomized, phase 3 clinical MINDACT trial included 6693 women with early-stage breast cancer with the primary goal to assess whether, among patients 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, whereas those at high clinical and genomic risk did receive such therapy. In patients 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 breast cancer 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 breast cancer 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- 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.

Sestak et al. (2020) retrospectively investigated a cohort of patients 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 EndoPredict (EPclin), either alone or in combination with clinical parameters, for distant recurrence (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-positive, HER2-negative breast cancer. As part of that group, 470 (19.5%) had ILC, 1944 (80.5%) had invasive ductal carcinoma (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.

In a comparison of comparison of EndoPredict (EP) and EPclin with Oncotype DX recurrence score for prediction of risk of distant recurrence after endocrine therapy, Buus et al. (2016) concluded that EP and EPclin were highly prognostic for distance recurrence in endocrine-treated patients with ER+, HER2-negative disease. The researchers found that EPclin provided more prognostic information than recurrence score, which they determined was partly but not entirely because of EPclin integrating molecular data with nodal status and tumor size.

Breast Cancer Index (BCI)

Breast Cancer Index (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 formalin-fixed, paraffin-embedded (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 + breast cancer 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 extended endocrine therapy 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 extended endocrine therapy. 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+ breast cancer.

A study by Bartlett et al. (2019) examined the use of BCI to predict benefit from extended endocrine therapy (EET) in patients that were randomized in a previous trial called the Adjuvant Tamoxifen-To Offer More? (aTTom) trial. In the original aTTom trial, there were 6956 patients with, 583 HR+ N+ patients 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 patients 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 patients, 49% were classified as BCI (H/I)-High and had a significant benefit from 10 versus 5 years of EET as the risk of recurrence was 27% and 37% for patients with 10- and 5-year EET, respectively. BCI (H/I)-Low patients 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.

Sestak, et al. (2018) who provided a secondary analysis of data obtained from the Anastrozole or Tamoxifen Alone or Combined randomized clinical trial, 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 risk of recurrence (ROR), Breast Cancer Index (BCI), EndoPredict (EPclin), 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 to10 years and 5 to10 years after diagnosis. The analysis included 774 post-menopausal women with estrogen positive, HER2 negative disease. Five hundred and ninety-one had node-negative disease. All genomic signature tests provided significantly more information that the clinical treatment score, the recurrence score and the 4 marker immunohistochemical score alone. The most valuable tests were the PAM 50 and BCI. In the 183 patients with 1-3 positive nodes, 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 patients consider chemotherapy or extended endocrine testing (NCCN, Breast 2020).

Zhang et al. (2017) examined the predictive ability of BCI results, when integrated with tumor size and grade (BCIN), to accurately identify outcomes in a well annotated retrospective series of node positive patients. A total of 402 patients with 1-3 positive nodes who were treated with adjuvant endocrine therapy with or without chemotherapy using a prespecified model. The primary endpoint was time-to-distant recurrence (DR). BCIN classified 20% of patients as low risk with a 15-year DR rate of 1.3% and 321 patients 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 node positive patients with a limited risk of recurrence over 15 years that could avoid extended endocrine treatment. Further studies on combined genomic and clinical algorithmic predictions are needed on node positive patients.

Sestak et al. (2016) conducted a retrospective analysis to examine cross-stratification between Breast Cancer Index (BCI) and the Oncotype DX Recurrence Score (RS) to directly compare their prognostic accuracy at the individual patient level. Six hundred and sixty-five patients with hormone receptor-positive (HR^+) and lymph node-negative 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 patients with significant and clinically relevant rates of DR. These results indicate that additional subsets of women with HR^+, lymph node-negative breast cancer identified by BCI may be suitable candidates for adjuvant chemotherapy or extended endocrine therapy.

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 fixed paraffin embedded (FFPE) blocks from hormone positive, node negative patients were used in this study. The multi-institutional cohort (n=358) was from estrogen receptor positive, node negative breast cancer patients 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.

National Comprehensive Cancer Network (NCCN) Clinical Guidelines

The National Comprehensive Cancer Network (NCCN) clinical guidelines for breast cancer indicate that “gene expression assays can 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 for prognosis and prediction of chemotherapy benefit”, as it has been clinically validated. The panel also notes that other prognostic multigene assays can be considered to help estimate risk of recurrence, but these have not been validated to predict response to systemic chemotherapy. NCCN notes that the Breast Cancer Index (BCI) test is predictive of benefit of extended adjuvant endocrine therapy (NCCN, Breast 2021).

Other Breast Cancer Profiling Assays

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.

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 radiation therapy (RT) in individuals who had undergone breast conserving surgery (BCS) for a diagnosis of ductal carcinoma in situ (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 DCISionRT score 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.

Choosing the optimal treatment approach for individuals diagnosed with ductal carcinoma in situ (DCIS) has been a significant challenge and a topic of active research. A major goal is to understand the risk of recurrence 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 breast conserving surgery (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 breast cancer 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 patient selection for treatment, since the study was not randomized or rule-based. Further research is needed to provide more evidence to support routine DCISionRT testing.

Bremer et al. (2018) 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 decision score (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 (RT) was evaluated as a function of DS, by risk group. Study population included 526 individuals diagnosed with a primary DCIS and treated with breast conserving surgery (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 DCIS score designed to represent the risk of breast cancer recurrence within 10 years of original diagnosis (0 to 100) is then calculated for the individual.

In a review of the literature regarding prognosis and treatment of ductal carcinoma in situ (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 patients 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. Overall, there is limited data available on the clinical efficacy of Oncotype DX DCIS and more studies are needed.

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 ER+ early stage breast cancer patients 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)

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

- For patients older than 50 years and whose tumors have Oncotype DX recurrence scores less than 26, and for patients 50 years or younger whose tumors have Oncotype DX recurrence scores 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 patients 50 years or younger with Oncotype DX recurrence scores of 16 to 25, clinicians may offer chemoendocrine therapy (Type: evidence based, benefits outweigh harms; Evidence quality: intermediate; Strength of recommendation: moderate).
- Patients with Oncotype DX recurrence scores 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 patients 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 a patient has ER/PgR–positive, HER2-negative, node-negative breast cancer, 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 a patient has ER/PgR–positive, HER2-negative, node-positive breast cancer, the MammaPrint assay may be used. It should be used in patients with one to three positive nodes and 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 patients should be informed that a benefit of chemotherapy cannot be excluded, particularly in patients 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 breast cancer, ASCO (Harris et al., 2016a; Harris et al., 2016b) found sufficient evidence of clinical utility for the biomarker assays Onco*type* DX, EndoPredict, PAM50, Breast Cancer Index, and urokinase plasminogen activator and plasminogen activator inhibitor type 1 in specific subgroups of breast cancer. No biomarker except for estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 was found to guide choices of specific treatment regimens. Treatment decisions should also consider disease stage, comorbidities, and patient preferences.

For this guideline, the ASCO panel considered only prognosis and prediction in patients with newly diagnosed, nonmetastatic, primary breast cancer. 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 breast cancer 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 patients 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 breast cancer. 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 Breast Cancer Index, can be used.
- Expression of uPA-PAI1 or multigene panels, such as MammaPrint, Oncotype DX, EndoPredict, Prosigna or Breast Cancer Index, may be used in conjunction with all clinicopathological factors to guide systemic treatment decisions in patients where these decisions are challenging, such as luminal B-like/HER2-negative and node-negative/nodes 1–3-positive breast cancer [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.

Thyroid Cancer

There have been multiple studies, prospective and retrospective, for the commercially available molecular classifiers for indeterminate and suspected malignant thyroid nodules, such as the Afirma Gene Expression Classifier, and next generation sequencing test panels, such as ThyGenX/ThyraMir and ThyroSeq v3.

In a parallel randomized clinical trial, Livhits et al.(2021) sought to compare the diagnostic performance between DNA-RNA testing (ThyroSeq v3 multigene genomic classifier) and RNA testing (Afirma genomic sequencing classifier). The trial included patients in the UCLA Health system showing indeterminate cytology after thyroid biopsy from August 2017 to January 2020,as per the Bethesda System for Reporting Thyroid Cytopathology category III or IV. A total of 346 patients (median age 55 and 76.9% female) were randomized to 1 of the 2 tests. Benign call rate was 61% (95% CI, 53%-68% for the DNA-RNA test and 53% (95% CI, 47%-61%) for the RNA test alone. RNA and DNA-RNA tests had specificities of 80% (95% CI, 72%-86%) and 85% (95% CI, 77%-91%), respectively (P = .33). PPVs of the RNA and DNA-RNA test were 53% (95% CI, 40%-67%) and 63% (95% CI, 48%-77%) respectively (P = .33). The DNA-RNA test showed no statistically significant difference in PPV when compared to the prior version of the test (ThyroSeq v2 next-generation sequencing). The RNA test had a higher positive predictive value (PPV) in comparison with the previous version of the test (Afirma gene expression classifier). Eighty-seven (51%) patients who underwent RNA testing and 83 (49%) patients who underwent DNA-RNA testing were able to avoid diagnostic thyroidectomy. Ultimately, twenty percent of indeterminate result nodules were malignant. The researchers concluded that both tests (RNA and DNA-RNA) displayed high specificity and resulted in 49% of patients whose biopsies showed indeterminate results avoiding further diagnostic surgery. There was no statistically significant difference in performance of RNA testing vs. DNA-RNA testing observed in this study, though prior clinical trials had indicated that the previous version of the DNA-RNA test had greater specificity than the previous version of the RNA test.

Endo et al. (2019) analyzed the Afirma Gene Sequencing Classifier (GSC) assay that was developed to improve PPV versus the Gene Expression Classifier assay. The researchers analyzed all patients with cyto-I nodules and molecular testing with either

GEC or GSC and clinical information was obtained for 343 GEC-tested nodules and 164 GSC-tested nodules. The GSC assay was found to have a significantly higher benign call rate (76.2% vs. 48.1%, $p < 0.001$), PPV (60.0% vs. 33.3%, $p = 0.01$), and specificity (94.3% vs. 61.4% $p < 0.001$) than the GEC. The researchers concluded that this study showed an improved specificity and PPV while maintaining high sensitivity and NPV for GSC compared with GEC.

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, patients 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 cross-sectional cohort study, Duick et al. (2012) demonstrated that obtaining a GEC test (Afirma) in patients with cytologically indeterminate nodules was associated with a reduction in the rate of diagnostic thyroidectomies. The authors reported that approximately one surgery was avoided for every two GEC tests run on thyroid fine-needle aspirations (FNA) with indeterminate cytology. Data was contributed retrospectively by 51 endocrinologists at 21 practice sites. Compared to a 74% previous historical rate of surgery for cytologically indeterminate nodules, the operative rate fell to 7.6% during the period that GEC tests were obtained. The rate of surgery on cytologically indeterminate nodules that were benign by the GEC reading did not differ from the historically reported rate of operation on cytologically benign nodules. The four primary reasons reported by the physicians for operating on nodules with a benign GEC reading were, in descending order, large nodule size (46.4%), symptomatic nodules (25.0%), rapidly growing nodules (10.7%) or a second suspicious or malignant nodule in the same patient (10.7%). According to the authors, these reasons are concordant with those typically given for operation on cytologically benign nodules.

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%.

Labourier et al (2015) studied surgical specimens and preoperative FNAs (n = 638) for 17 validated gene alterations in the BRAF, RAS, RET and PAX8 genes combined with a 10-miRNA gene expression classifier that provided positive (malignant) or negative (benign) results. Mutations were detected in 69% of nodules with malignant outcome. Among mutation-negative specimens, miRNA testing correctly identified 64% of malignant cases and 98% of benign cases. The authors reported the diagnostic sensitivity and specificity of the combined algorithm was 89% and 85%, respectively. They calculated that with a thyroid cancer prevalence of 32%, the NPV would be 94%, and could help reduce unnecessary surgeries by 69%.

The National Comprehensive Cancer Network (NCCN) guideline for Thyroid Carcinoma (NCCN, Thyroid 2021) indicates that molecular diagnostics may be helpful for thyroid nodules with indeterminate cytology or to reclassify follicular lesions. They note that molecular markers should be interpreted with caution and used in conjunction with individualized clinical, radiographic and cytologic features. Molecular profiling is not recommended for Hurthle cell neoplasms, as studies have shown that historically, molecular diagnostics do not perform well for these neoplasms. 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. The NCCN panel recommends that molecular testing be used to help make decisions regarding systemic treatment and to determine whether the individual may be eligible for clinical trials. Some mutations may also have prognostic importance.

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/PPARγ rearrangements, and a mRNA expression profile of 167 genes, and concluded that more data was needed to fully understand how such tests can impact clinical management. They conclude that there is currently no single optimal molecular test that can definitively rule in or rule out malignancy in all cases of indeterminate cytology.
- Follicular Neoplasm/Suspicious for Follicular Neoplasm Cytology-after consideration of clinical and sonographic features, molecular testing may be used to supplement malignancy risk assessment data in lieu of proceeding directly with surgery.
- Suspicious for Malignant Cytology-After consideration of clinical and sonographic features, mutational testing for *BRAF* or the seven-gene mutation marker panel (*BRAF*, *RAS*, *RET/PTC*, *PAX8/PPARγ*) may be considered in nodules with SUSP cytology if such data would be expected to alter surgical decision-making. Molecular testing using the 167 GEC has a PPV that is similar to cytology alone (76%) and a NPV of 85% and it is therefore not indicated in patients with this cytological diagnosis.
- Malignant cytology-while studies have been presented in the literature that suggest that *BRAF* and other multi-gene panels may be useful in prognosis and treatment decisions, more studies are needed to establish the impact of molecular profiling involving multiple mutations or other genetic alterations on clinical management of patients 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 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.

Hematological Malignancies

Leukemias

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. (2015) 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.

Koh et al. (2014) utilized a CGH+SNP array platform to study the presence of CNVs and LOH in 15 children with acute myeloid leukemia (AML) and 3 with myelodysplastic syndrome (MDS). Cytogenetic analysis revealed CNV in 11 regions in 8 patients. SNP+CGH found 14 CNV in 9 patients, and cryptic LOHs in 3 of 5 patients with normal cytogenetics. Overall, 9 patients were found to have abnormalities not detected by routine cytogenetics. 3 patients with AML and terminal LOH of >10Mb had significantly inferior relapse-free survival time, suggesting that SNP+CGH testing can provide additional prognostic information.

Puiggros et al. (2012) studied 70 patients with chronic lymphocytic leukemia (CLL) by routine cytogenetics, FISH, and genomic arrays to determine if genomic arrays could replace current testing standards. Routine cytogenetics found 31% genomic anomalies in patients, and FISH found 69%. Genomic arrays, Cytogenetics Whole-Genome 2.7M Array and CytoScan HD Array, found anomalies in 79% and 80%, respectively. Arrays missed small deletions at 11q and 17p due to their limited sensitivity in these regions. The authors concluded that arrays should remain a complementary tool to routine cytogenetics and FISH to prevent a negative impact on patients who harbor genetic anomalies that would be missed by this technology.

Hagenkord et al. (2010) examined the optimal SNP array probe density for clinical use in CLL to identify actionable genetic variation missed by FISH and conventional chromosome analysis. The validation cohort consisted of 18 archived sample and 11 clinical samples that were simultaneously tested with standard FISH for CLL. Where possible, cytogenetic and flow cytometry was also performed. Affymetrix SNP arrays of low (10K2.0), medium (250K Nsp) and high (SNP6.0) density were utilized. Ultimately the medium density array was validated for clinical use and was found in 98.5% concordance with standard FISH. In particular, a region of acquired uniparental disomy (UPD) with two mutation copies of TP53 was identified that was not found by FISH or routine cytogenetics. The authors concluded that SNP array karyotyping provides high resolution CNV analysis, identification of UPD and detects lesions missed by FISH.

Boultwood et al. (2010) used a SNP array to analyze 41 chronic myeloid leukemia (CML) patients using 53 bone marrow or blood sample. 32 were in chronic phase and 21 were in blast crises. The samples were analyzed for uniparental disomy (UPD) and copy number variants, with quality control comparisons with 100 healthy controls of different ethnicities for SNP array hybridization intensities, and 45 healthy controls as a reference set. Across the samples 44 regions of UPD were identified, with chromosome 8 having the highest frequency. 10 regions of copy number variation were identified in 4 of 21 patients with blast crises, and none were observed for those in chronic phase. The authors noted that 32 regions of UPD were noted in 23 of 45 healthy controls on chromosomes 15 and 22. Therefore only regions of UPD were reported for CML patients that weren't found in the controls, and this emphasized to the authors that SNP analysis, particularly for UPD, requires inclusion of constitutional controls. UPD is not identifiable by other testing methods, but is important as the acquired homozygosity of disease genes may contribute to disease progression. In this cohort, UPD was found in 1 patient at 20q11 that includes the ASXL1 gene, a tumor suppressor gene associated with early events in CML. Sequencing exon 12 in all patients found that 6 of 41 had ASXL1 mutations, which is likely a newly identified molecular abnormality for CML.

Clinical Practice Guidelines

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).

Myelodysplastic Syndrome

Song et al. (2017a) 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.

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.

Thiel et al. (2011) notes that 40% of those with MDS have a normal karyotype and may have a different prognosis that those who have an abnormal karyotype. The availability of CGH now allows for the identification of cryptic genomic abnormalities and having this information may have a prognostic or treatment impact. They studied 107 MDS patients with a normal karyotype and found that 39% of patients had cryptic genomic imbalances, including regions that are known to be impacted in MPS such as del4q, del5q, and del7q. Most alterations were verified by other methods. Overall, these patients had inferior survival and outcomes similar to those with cytogenetically visible aberrations when compared to the rest of the patients in this cohort with no identifiable cytogenetic abnormalities.

Multiple Myeloma

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.

Tiu et al. (2011) examined the analytical validity and clinical utility of SNP arrays in individuals with myelodysplastic syndromes when performed in parallel with cytogenetics vs. cytogenetics alone. They analyzed 430 patients within the MDS spectrum which included 250 with MDS, 95 with MDS/myeloproliferative overlap neoplasm, and 85 with acute subsequent AML. Overall, the combined SNP array + karyotype had a higher diagnostic yield of chromosomal defects at 74%, compared to karyotype alone at 44%. Novel lesions were identified by array in 54% with normal cytogenetics and 62% of those with abnormal cytogenetics. The presence and number of SNP identified lesions proved to be an independent predictor of outcome and tended to have worse survival outcomes. The authors concluded that concurrent use of routine cytogenetics with a SNP array improves diagnostic yield and prognostic information compared to cytogenetics alone.

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 (NCCN, Multiple Myeloma 2022).

Detection of Minimal Residual Disease (MRD) in Hematologic Malignancies

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.

Ladetto et al. (2014) compared real time quantitative polymerase chain reaction (RQ-PCR) to NGS for identifying clonotype identification, clonotype identity and comparability of MRD results. A total of 378 samples from 55 patients with acute lymphoblastic leukemia (ALL), mantle cell lymphoma (MCL) or multiple myeloma (MM) were analyzed. RQ-PCR identified 45 clonotypes, and NGS found 49, and were identical or >97% homologous in all cases. Both consistently had a sensitivity level of $1x10^{-5}$ and MRD results were concordant in 79.6% of cases. NGS showed at least the same level of sensitivity as RQ-PCR without the need for patient specific reagents, and may be a useful tool for monitoring in ALL, MCL and MM.

Determining the response to treatment is an important aspect of managing multiple myeloma. NCCN Multiple Myeloma guidelines include assessing MRD as part of the management algorithm and states that MRD has been identified as an important prognostic factor. NCCN notes that a validated next generation sequencing assay or next generation flow could be used for determining MRD. The ideal time is after each treatment stage (e.g., after induction, high dose therapy/ASCT, consolidation, maintenance). Two consecutive assessments are not necessary, one test is sufficient after each treatment stage. Only individuals who appear to have complete response and have no evidence of progression or new bone lesions should have MRD assessment (NCCN, Multiple myeloma 2022).

NCCN guidelines for ALL recommend MRD at a sensitivity of 10^{4}or better and NGS is listed as one the recommended methods for MRD assessment (NCCN, Acute Lymphoblastic Leukemia 2021 and NCCN, Pediatric Acute Lymphoblastic Leukemia 2022). 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 (NCCN, ALL 2021). 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.

The NCCN guidelines for AML state that the role of MRD is evolving in both prognosis and treatment and that clinical trial participation is encouraged (NCCN, Acute Myeloid Leukemia 2021). 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.

Lung Cancer Tissue Testing

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 (FoundationOne) 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).

NCCN guidelines for NSCLC recommend molecular testing all patients with advanced or metastatic disease for the following mutations to guide the selection of therapy (NCCN, Non-small cell lung cancer 2021): EGFR, ALK, ROS1, BRAF, MET, and RET (in additional to testing for PDL-1 expression). NCCN recommends that when feasible, testing be performed via a broad, panel-based approach, most typically performed by next generation sequencing (NGS). In addition, the guidelines include a discussion of the role of plasma cell-free/circulating tumor DNA testing. NCCN states that cell-free/circulating tumor DNA testing should not be used in lieu of a tissue diagnosis as the analytical standards have not been established. However, NCCN also suggests that the use of cell-free/circulating tumor DNA testing can be considered in specific clinical circumstances, most notably: if a patient is medically unfit for invasive tissue sampling; or in the initial diagnostic setting, 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 all patients in which an oncogenic driver is not identified (NCCN, Non-small cell lung cancer 2021).

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 the area of 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
  - Non - squamous, 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.

Melanoma

Cutaneous Melanoma

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.

In a 2019 retrospective cohort study done in follow-up to earlier studies involving the Pigmented Lesion Assay (PLA), Ferris et al. document 12-month follow up data and seek to further confirm clinical utility of this commercially available, non-invasive gene expression test. Previous studies had found no missed melanomas in three- and six-month follow up periods. A 12-month chart review was performed to further follow up on 734 pigmented lesions that had been found to have negative PLA results from 5 US dermatology centers. Although 13 of these lesions had undergone biopsy and were submitted for histopathologic investigation during the follow up period, none of the lesions had a histopathologic diagnosis of melanoma. The PLA test's utility was also studied further in a registry of 1575 participants from 40 US dermatology offices. Overall, 99.9% of PLA negative lesions were monitored clinically, avoiding surgical procedure. Biopsy was performed in 96.5% of all PLA positive lesions. The authors concluded that the PLA's high negative predictive value and clinical utility was confirmed in this long-term follow up study and can be used to guide the management of pigmented lesions to avoid unnecessary biopsies. There were limitations, however, including the assumption that patients who did not return to follow-up at the site of PLA testing within 12 months (34% of patients fell into this group) were truly negative. Another concern is that some PLA negative lesions may not have undergone adequate assessment within the 12-month follow-up. Lastly, of the 13 authors, 9 have an affiliation with DermTech, the maker of the test.

Zager et al. (2018) 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.

The clinical utility of a two gene-gene expression assay by DermTech was studied by Ferris et al. (2017). A noninvasive adhesive skin patch test looking at the gene expression of *LINC/PRAME*, known as the pigmented lesion assay (PLA), was compared to the findings of 45 dermatologists who evaluated clinical and dermoscopic images of the same lesions and based on their observations, recommended biopsy or not. All samples were biopsied, and readers were blinded to the histopathology. Sixty samples were included that were obtain from March 2014 to November 2015 and represented which 8 were melanomas and 52 were nonmelanomas. The biopsy concordance using only the dermatologist review was 95%. When the PLA results were included, the biopsy concordance improved to 98.6%. This clinical utility was further explored in a real-world analysis in an observational cohort of 381 patients (Ferris, et al., 2018). The PLA test was positive in 51 patients, and all had a biopsy that resulted in 37% diagnosed with melanoma. In the 330 negative PLA group, nearly all were managed by monitoring. Three had biopsies, and none were found to be melanoma. The authors calculate that 93% of samples diagnosed histologically as melanoma were positive for both *LINC/PRAME*. *PRAME* only and *LINC* only positives were melanomas histologically melanomas in 50% and 7% of cases respectively.

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.

Wiesner et al. (2016) provided a review on the diagnostic, prognostic, and therapeutic value of understanding genomic alterations in spitzoid tumors. Spitzoid tumors are composed of large spindle shaped or epithelioid melanocytes and are biologically distinct from melanocytic naevi and melanoma. Naevi and melanoma may have BRAF, NRAS mutations or inactivation of NF-1, Spitz tumors often have genomic rearrangements or HRAS mutation, or inactivation of BAP1. The number of genomic alterations correlates with the degree of abnormal histology and CGH analysis or FISH can accurately classify benign and malignant Spitz tumors. However, most of melanocytic tumors are histologically distinguishable as benign or malignant, so CGH provides no diagnostic value in these situations. Limited data exists on using CGH to differentiate benign from malignant in ambiguous melanocytes, but the authors report that prior publications and their own experience shows that ambiguous tumors have more genetic aberrations than benign lesions, but fewer than malignant, so the value is limited to up grading or down grading the risk of malignancy, but doesn't necessarily give clear answers.

Kutzner et al. (2012) evaluated the use of CGH and FISH in evaluating 27 histologically ambiguous "distinct morphological variant of superficial spreading melanoma, termed 'melanomas composed exclusively or predominantly of large nests' (MLN)". Of the 27 original samples, the authors concluded that 11 met the definition of an MLN. The others were considered to be conventional spreading melanomas. They were found equally in men and women, and the average age was 61 years. The majority of MLN mirrored the typical features of melanoma, and some clinicians in the group noted that in patients with multiple melanocytic lesions, the MLNs were very different from other pigmented lesions and raised the clinical suspicion of melanoma. Eight of the 11 MLN were evaluated by CGH and 10 were also evaluated by FISH. All cases analyzed by CGH had multiple chromosome aberrations, and no one aberration was associated with the morphology of large nests and was similar to the group of conventional spreading melanomas. FISH was only positive in 4 cases, which were also abnormal on CGH. Cases that were abnormal by CGH, but normal on FISH, were abnormal in chromosomal regions not covered by FISH. The authors concluded that while the histological appearance created difficulties in a definitive diagnosis, "most of the MLN were correctly diagnosed as malignant melanomas by clinicians on the basis of clinical criteria." In cases that continue to confound after conventional histological examination, CGH can be useful to confirm a diagnosis.

Raskin et al. (2011) used CGH and FISH to evaluate atypical Spitz tumors to differentiate between melanoma and Spitz nevi. Sixteen patients with histologically ambivalent melanocytes were evaluated in the study, and of these, 8 has positive sentinel lymph node biopsy, 1 of which also had distant metastasis. Also evaluated were 8 patients with Spitz nevi, and 3 patients with melanoma (2 spitzoid, 1 superficial spreading). Chromosomal aberrations were found in 7 patients with ambivalent melanocytes, and there was no difference between the positive and negative lymph node biopsy groups. One had a fatal outcome. Chromosome abnormalities were also found in 2 spitzoid melanomas, and 1 conventional melanoma. The majority of aberrations found in the ambivalent group were not the ones commonly found in melanomas, suggesting that this may be a unique clinical entity. FISH failed to detect one spitzoid melanoma, 1 fatal metastatic case, and the other chromosomally aberrant ambivalent cases. It was positive in 1 spitzoid melanoma and 1 conventional. Overall, the authors concluded that CGH may offer better diagnostic aid with better sensitivity and specificity than FISH in atypical Spitz tumors.

NCCN (Melanoma 2021) indicates that for diagnostic testing, prognostic testing, and somatic testing, there is agreement that any testing should be in adjunct to other histopathological testing. 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

- 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.
- Available noninvasive pre-biopsy imaging and molecular technologies have not been prospectively compared for diagnostic accuracy. Pre-diagnostic noninvasive genomic 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 who are without evidence of disease (NED), unless required to guide adjuvant or other systemic therapy or consideration of clinical trials.
- BRAF mutation testing is recommended for patients with stage III at high risk for recurrence for whom future BRAF-directed therapy may be an option.

Uveal Melanoma

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 uveal melanoma (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 uveal melanoma 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) evaluated the clinical validity and utility of DecisionDx-UM in a prospective, multicenter, study (supported by Castle Biosciences, Inc.). 70 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.

Minca et al. (2014) noted that monosomy 3 and MYC amplification at 8q24 are strong prognosticators of outcomes in uveal melanoma (UVM) and is commonly detected by FISH. They hypothesized that CMA would be an alternative to FISH and have advantages in identifying loss of heterozygosity, partial chromosome loss and other aberrations that FISH can't detect. They analyzed CMA using SNP+CGH (Roche-NimbleGen OncoChip) on enucleations from formalin-fixed paraffin-embedded tissue (FFPET) for 34 patients and/or frozen tissue (FZT) for 41 patients. CMA was successful in 30 of 30 of 34 FFPET and all 41 FZT. In 27 paired FFPET/FZT samples 96% were concordant for at least 4 of 6 major chromosome abnormalities, and 93% were concordant for one (- chromosome 3). CMA was concordant with FISH in 90% of FFPET and 93% of FZT. Partial -3q was detected in two FISH negative cases and whole chromosome LOH for 3, 4 and 6 in one case. Results of UVM SNP+CGH genotyping were significantly correlated with clinical outcome and reliably predicted metastasis, time to progression, and survival. The authors concluded that SNP+CGH is a practical method for UVM prognostication, and provides additional data with relevance to biology, diagnosis and prognosis.

In a prospective multi-center validation study, Onken et al. (2012) evaluated the prognostic performance of a 15 gene expression profiling (GEP) assay that assigned primary posterior uveal melanomas to prognostic subgroups: class 1 (low metastatic risk) and class 2 (high metastatic risk). A total of 459 patients were enrolled. Analysis was performed to compare the prognostic accuracy of GEP with Tumor-Node-Metastasis (TNM) classification and chromosome 3 status. Patients were managed for their primary tumor and monitored for metastasis. The GEP assay successfully classified 446 of 459 cases (97.2%). The authors concluded that the GEP assay had a high technical success rate and was the most accurate prognostic marker among all of the factors analyzed. The GEP provided a highly significant improvement in prognostic accuracy over clinical TNM classification and chromosome 3 status. Further studies are needed to determine the clinical utility of these tests and the role they have in clinical decision-making.

NCCN guidelines from 2021 cover the staging and management of uveal melanoma. They state 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 2021).

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.

Cancers of Unknown Primary Site

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.

In a systematic review of loss-of-heterozygosity based topographic genotyping with PathfinderTG®, Trikalinos et al. (2010) found no studies that demonstrated longer survival, longer time to tumor recurrence, or fewer adverse outcomes as a result attributable to unnecessary harmful interventions because of this testing. The authors reported several limitations with eligible studies including limited sample size and lack of patient selection criteria.
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) 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) clinical practice guidelines for occult primary (cancer of unknown primary site) 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. The use of next-generation sequencing (NGS) may be considered after initial determination of histology has been made to identify potentially actionable mutations that could guide treatment decisions. NCCN suggests that pathologists and oncologists collaborate on the judicious use of these modalities on a case-by-case basis, with the best individualized patient outcome in mind (NCCN, Occult primary (cancer of unknown primary [CUP]) 2021).

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.

Colorectal Cancer (CRC)

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.

Venook et al. (2013) conducted a validation study of the 12-gene recurrence score in cancer and leukemia group B (CALGB) 9581 of 1,713 randomly assigned patients with stage II colon cancer to treatment with edrecolomab or observation and found no survival difference. The analysis reported included all patients with available tissue and recurrence (n=162) and a random (approximately 1:3) selection of nonrecurring patients. RS was assessed in 690 formalin-fixed paraffin-embedded tumor samples with quantitative reverse transcriptase polymerase chain reaction by using prespecified genes and a previously validated algorithm. Association of RS and recurrence was analyzed by weighted Cox proportional hazards regression. The researchers concluded that 12-gene RS predicts recurrence in stage II colon cancer in CALGB 9581, which is consistent with the importance of stromal response and cell cycle gene expression in colon tumor recurrence. RS appears to be most discerning for patients with T3 MMR-I tumors, although markers such as grade and lymphovascular invasion did not add value in this subset of patients.

In a validation study 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, Yothers et al. (2013). Recurrence Score was assessed in 892 fixed, paraffin-embedded tumor specimens (randomly selected 50% of patients with tissue). Data were analyzed by Cox regression adjusting for stage and treatment. Based on the results, the authors concluded that 12-gene Recurrence Score predicts recurrence risk in stage II and stage III colon cancer and provides additional information beyond conventional clinical and pathologic factors. Incorporating Recurrence Score into the clinical context may better inform adjuvant therapy decisions in stage III as well as 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 (2014) 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. Larger, prospective studies are needed to further confirm the performance of this test.

NCCN clinical practice guidelines for colon cancer from 2021 states, in a footnote for pedunculated or sessile polyp (adenoma) with invasive cancer, that "It has not been established if molecular markers are useful in treatment determination (predictive markers) and prognosis." The guidelines state that there is insufficient data to recommend the use of multigene assay panels to estimate risk recurrence in colon cancer or determine adjuvant therapy in colon or rectal cancer patients. The NCCN panel encourages clinical trial enrollment to generate further data on these tests. (NCCN, Colon cancer 2021, Rectal cancer 2021).

Prostate Cancer

Decipher, Oncotype DX Prostate, Prolaris, and Promark

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.

In a prospective observational study, Marascio et al. (2020) sought to determine impact of genomic classifier (GC) testing on treatment management in men with prostate cancer who had undergone prostatectomy. Two cohorts of men diagnosed with prostate cancer and treated with radical prostatectomy (RP) were included in the study. All participants received Decipher tumor testing. One cohort of 3910 men focused specifically on clinical utility (CU) and measured change in decision making related to treatment by evaluating pre-and postgenomic treatment recommendations from urologists across various practice settings. The second cohort focused on clinical benefit (CB); this group included 102 men and examined differences in clinical outcome. The CB group came from a single academic institution which followed pre-defined best practices based on GC results. Participants were required to have undetectable PSA after RP and one or more adverse pathologic (AP) feature (e.g., extracapsular extension, seminal vesicle involvement or positive margins). The researchers reported that, in the CU cohort, the most frequent recommendation made by providers prior to Decipher testing was observation (69%). GC testing resulted in a change in recommendation for 39% of patients in this cohort. In the CB group, patients and providers were recommended to follow the tumor board best practice guidance, but they were not mandated to do so. Based on these GC-related best practices, adjuvant radiotherapy (ART) was recommended for 61% of men in the CB cohort and overall, best practice was followed for 74%. The authors concluded that using GC significantly altered treatment decision-making. Implementation of best practices based on results of GC testing led to greater than expected improvements in biochemical endpoints with no higher risk of adverse outcomes for those individuals whose results indicated lower or intermediate risk. Although the results are promising, the study had limitations. The CU cohort did not receive recommendations for “best practice” based on the GC results; however, the authors felt this was also a strength as it provides real-world estimates of the impact of this type of testing in practice. Additionally, the study reported on time to early biochemical recurrence only. Further independent validation was recommended by the researchers, as additional follow up may detect differences in longer term endpoints.

Berlin et al. (2019) evaluated the utility of the Decipher 22-feature genomic classifier (GC) to identify patients with intermediate-risk prostate cancer (IR-PCa) who may be treated with radiation therapy alone. They identified 121 patients that fell into this risk category and only were treated with radiation therapy and not hormone therapy. The assay was performed on biopsy samples. Of the 121 patients, 33 (27%) and 87 (72%) of men were classified as having favorable and unfavorable IR-PCa, respectively (1 case unclassifiable). After performing the assay, GC scores were high in 3 favorable IR-PCa and low in 60 unfavorable IR-PCa. The researchers determined that higher GC scores versus the NCCN risk sub-groups was associated with biochemical relapse (p=0.007) and metastasis (p=0.004). The researchers concluded that Decipher demonstrated predictive validity for disease recurrence and recommended a prospective clinical trial to demonstrate clinical utility.

Kim et al (2019) assessed the use of Decipher for the risk stratification for men on active surveillance to determine increased risk of disease progression and metastasis due to delayed therapy. A cohort of 266 men with very low/low and favorable-intermediate risk prostate cancer was selected. Decipher and Cancer of the Prostate Risk Assessment (CAPRA) were compared as predictors of adverse pathology. Decipher from the 266 diagnostic biopsies (65% NCCN-very-low/low and 35% favorable-intermediate) was an independent predictor of adverse pathology (p=0.025) when adjusting for CAPRA. No data on clinical outcomes were reported.

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.

Klein et al. (2016) retrospectively analyzed prostatectomy tissue of 337 Gleason 3+3 patients. To compare clinico-pathologic variables across pathologic Gleason score categories, Fisher's exact test or analysis of variance F test were used. Distributions of Decipher scores among different clinico-pathologic groups were compared using Wilcoxon rank sum test. The association of Decipher score and adverse pathology was examined using logistic regression models. Among men who had Gleason 3+3=6 disease only, 269 (80%) had low Decipher scores with 43 (13%) and 25 (7%) harboring intermediate and high scores respectively. Thus, a small proportion of histologic Gleason 6 tumors harbor molecular characteristics of aggressive cancer. The authors note that molecular profiling of such tumors at diagnosis may better select patients for active surveillance at the time of diagnosis and trigger appropriate intervention during follow-up.

Glass et al. (2016) published long-term outcomes to a previously reported validation study on Decipher. Study subjects (n=224) had aggressive prostate cancer with at least 1 of several criteria such as preoperative prostate specific antigen 20 ng/ml or greater, pathological Gleason score 8 or greater, stage pT3 disease or positive surgical margins at prostatectomy. Of the 224 patients treated 12 experienced clinical recurrence, 68 had biochemical recurrence and 34 experienced salvage treatment failure. At 10 years after prostatectomy the recurrence rate was 2.6% among patients with low Decipher scores but 13.6% among those with high Decipher scores (p=0.02). When CAPRA-S and Decipher scores were considered together, the discrimination accuracy of the ROC curve was increased by 0.11 compared to the CAPRA-S score alone (combined c-index 0.84 at 10 years after radical prostatectomy) for clinical recurrence. The authors conclude that Decipher improves the ability to predict clinical recurrence in prostate cancer and adds precision to conventional pathological prognostic measures. Long-term studies are needed to validate these results.

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 prostate cancer (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 despite of 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 National Comprehensive Cancer Network (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.

Marrone et al. (2015) did a literature review of the Decipher test, a 22 gene expression assay designed to predict the metastatic rate of prostate cancer within 5 years of a radical prostatectomy. They utilized PubMed to search for peer reviewed literature that discussed the analytic validity, clinical validity and clinical utility of Decipher. Eight studies were identified, but no guidelines Analytical validity was identified by the authors in a single conference abstract, and the correlation between genomic classifier scores between matched biopsies was 74%. Clinical validity was described in all included studies, and the authors found that the data represented that the genomic classifier was able to adequately discriminate between those men that developed metastatic prostate cancer within 5 years and those that did not. Clinical utility was another matter, however. The authors found that additional evidence was needed to show that outcomes were improved in men whose post-surgical treatment was guided by Decipher results when compared to standard of care.

NCCN clinical practice guidelines for prostate cancer (NCCN, Prostate cancer 2022) 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 is recommended 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 resistance/recurrence after radical prostatectomy (category 2B evidence.)

In the discussion section of the NCCN guidelines, it 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.”

Other Prostate Cancer Assays

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.

A study from McKiernan et al (2016) evaluated the performance of the ExoDx Prostate IntelliScore 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. ExoDx Prostate IntelliScore 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.

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. was evaluated. 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.

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 prostate cancer (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 Urological Association (AUA) with the American Society for Radiation Oncology (ASTRO) and the 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 clinical 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.

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 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 biomarkers (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).
- 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).

In 2018, Bekelman et al. (2018) 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).

Pancreatic Cancer

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 1382 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.

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

Singhi et al. (2018) studied the clinical validity of using pre-operative pancreatic cyst fluid (PCF) for next generation sequencing (NGS) of KRAS, GNAS, TP53, PIK3CA and PTEN genes in order 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.

NCCN Pancreatic Adenocarcinoma guidelines (2021) include a footnote recommending tumor/somatic gene profiling in cases of metastatic or locally advanced disease to identify potential uncommon mutations. Recommendations further include specific testing for fusions (ALK, NRG1, NTRK, ROS1), mutations (BRAF, BRCA1/2, HER2, KRAS, PALB2) and mismatch repair deficiency.

Other Cancers and Clinical Indications

Molecular profiling has many theoretical clinical applications in the field of oncology. Published clinical studies have addressed the use of molecular profiling including, but not limited to, the following:

- Acute myeloid leukemia (Port et al., 2014; Link et al., 2012)
- Adrenocortical cancer (Zheng et al., 2016; Ross et al., 2014a)
- Chronic myeloid leukemia (Keramatinia et al., 2017)
- Colorectal cancer
- Circulating tumor cells (Yang et al., 2018; Merker et al., 2018) Gastric and gastrointestinal cancer (West et al., 2017; Ali et al., 2015, Vignot et al., 2015; Miura et al., 2014)
- Gynecological cancer (Rodriguez-Rodriguez et al., 2016; Ross et al., 2013)
- Head and neck cancer (Wang et al., 2017; Chung et al., 2015)
- Lung cancer
- Melanoma (Cutaneous and Uveal)
- Myelodyplastic and myeloproliferative syndromes
- Non-melanoma skin cancers
- Pancreatic cancer (Zhou et al., 2017; Chmielecki et al., 2014; Chantrill et al., 2015)
- Thyroid cancer
- Urothelial carcinoma/urinary bladder adenocarcinoma (Roy et al., 2017; Ross et al., 2014b; Millis et al., 2015)

There is insufficient published evidence to support the use of molecular profiling for these cancers, technologies or sample types. The main evidence deficiencies are insufficient data on analytical validity, clinical validity, and clinical utility.

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.

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.

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 2021 Consensus Statement from the International Association for the Study of Lung Cancer (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 circulating tumor DNA (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.

A 2020 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.

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.

Another renal cell carcinoma study 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.

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.

Another study by Lam et al (2019) 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.

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.

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.

One liquid biopsy test, Guardant360, evaluates cell-free tumor DNA for 73 different genes. 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 ctDNAs 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.

Cohen et al. (2017) and Su et al. (2018) researched different methods for detection of T790M in plasma cell-free DNA for lung cancer. The researchers used a combination of peptide nucleic acid (PNA) and Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) to monitor cell-free DNA T790M in EGFR-mutant patients. The cohort included 103 tumor and cell free DNA T790M samples. Detection sensitivity of cfDNA T790M was 67.4% and overall concordance was 78.6%. Among 65 T790M-positive tumors, 15 were negative in cfDNA (23.1%). Seven of 38 T790M-positive cfDNA samples were negative in the tumors (18.4%).

Kim, et al. (2017) 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.

Another study also evaluated rapid plasma genotyping for the detection of EGFR and KRAS in advanced lung cancer (Sacher et al. 2016). Blood samples were taken from patients with advanced non - squamous non-small-cell lung cancer (NSCLC). The patients either (1) had a new diagnosis and were planned for initial therapy or (2) had developed acquired resistance to an EGFR kinase inhibitor and were planned for re-biopsy. Test was performed for EGFR exon 19 del, L858R, T790M, and/or KRAS G12X. All patients underwent biopsy for tissue genotyping, which was used as the reference standard for comparison. Of 180 patients with advanced NSCLC, 120 cases were newly diagnosed; 60 had acquired resistance. Tumor genotype included 80 EGFR exon 19/L858R mutants, 35 EGFR T790M, and 25 KRAS G12X mutants. The plasma test had a positive predictive value of 100% (95% CI, 91%-100%) for EGFR 19 del, 100% (95% CI, 85%-100%) for L858R, and 100% (95% CI, 79%-100%) for KRAS, but lower for T790M at 79% (95% CI, 62%-91%). The sensitivity was 82% (95% CI, 69%-91%) for EGFR 19 del, 74% (95% CI, 55%-88%) for L858R, and 77% (95% CI, 60%-90%) for T790M, but lower for KRAS at 64% (95% CI, 43%-82%). Sensitivity for EGFR or KRAS was higher in patients with multiple metastatic sites and those with hepatic or bone metastases, specifically. The researchers concluded that this rapid plasma testing detected EGFR and KRAS mutations rapidly with high specificity needed to select therapy and avoid repeat biopsies. In addition, this testing may also detect EGFR T790M missed by tissue genotyping due to tumor heterogeneity in resistant disease.

Riediger et al (2016) studied tumors over time through the use of plasma DNA. The researchers aimed to identify early indications for therapy response or tumor progression. Lung adenocarcinoma patients who were treated with TKIs had serial plasma samples taken. Through digital PCR, EGFR and KRAS mutations were quantified in the circulating DNA. The DNA levels were compared to the treatment courses and variations were found in 15 patients. The study concluded that serial assessment of EGFR mutations in the plasma of these lung cancer patients was able to determine treatment response and tumor progression earlier than other methods.

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.

A 2021 Hayes Precision Medicine Research Brief found that there is insufficient evidence to perform a comprehensive assessment of the Galleri multi-cancer early detection test at this time.

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.

Leighl et al.(2019) published the results of a clinical utility study which sought to compare comprehensive cell-free DNA (cfDNA) with physician standard of care (SOC) tissue genotyping to identify guideline-recommended biomarkers in patients with NSCLC. The study included prospectively enrolled participants with metastatic NSCLC who had not previously been treated, and who had undergone SOC tissue genotyping per physician discretion. Pretreatment blood samples were used for comprehensive cfDNA analysis with Guardant 360. Of 282 total enrollees, the physician discretion SOC genotyping with tissue samples identified a biomarker in 60 patients (21.3%.). Guideline recommended biomarker was identified in 77 patients (27.3%) using the cfDNA test (P<0.0001 for non-inferiority). In patients with positive tissue samples, an 80% cfDNA clinical sensitivity for any guideline-recommended biomarker was identified. In the case of FDA-approved targets (EGFR, ALK, ROS1, BRAF), concordance was >98.2% with 100% positive predictive value for cfDNA versus tissue sampling. Use of cfDNA in addition to tissue sampling increased detection from 60 to 89 patients, an increase of 48%. In addition, cfDNA average turnaround time was significantly faster than tissue (9 vs 15 days, P<0.0001). This was the largest cfDNA study in previously untreated metastatic NSCLC. The researchers concluded that the cfDNA test identifies guideline-recommended biomarkers at least as well as SOC tissue genotyping and does so more rapidly and completely.

In a 2019 publication, Aggarwal et al 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.

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.

Another study of ctDNA testing for 70 genes and NSCLC was performed by Thompson, et al. (2016). A total of 112 plasma samples were obtained from 102 prospectively enrolled patients with advanced NSCLC. Matched tissues samples, when available, were also evaluated. The investigators found 275 alterations in 45 genes, and at least one alteration in the ctDNA for 86 of 102 patients (84%), with EGFR variants being most common. This testing detected 50 driver and 12 resistance mutations, and mutations in 22 additional genes for which experimental therapies, including clinical trials, are available. Tissue sequencing was only successful for 50 patients (49%). Overall concordance for all variants covered and detected by both platforms was 60%. Actionable EGFR mutations were detected in 24 tissue and 19 ctDNA samples, yielding concordance of 79%.

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.

The use of circulating tumor DNA (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 patients 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 circulating tumor DNA (ctDNA) to test for risk of metastatic recurrence and predictive ability of pathologic complete response (pCR) in early breast cancer patients. A retrospective ancillary ctDNA study was performed on samples that had been prospectively collected from high-risk early breast cancer patients that were part of the multicenter neoadjuvant I-SPY2 TRIAL. Eligibility requirements included tumor size ≥2.5-cm and stage II/III breast cancer. Patients 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 patients 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 circulating tumor DNA (ctDNA) is associated with colorectal cancer (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 patients were cleared of disease. In the post-therapy period, ctDNA-positive patients were more than 40 times more likely to have a recurrence of their disease than the ctDNA-negative patients. 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 patient 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)

Merker et al. (2018) published a joint review from the American Society of Clinical Oncology (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 reviewed. The literature review included 77 references. The literature review 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.

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 November 9, 2021)

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Policy History/Revision Information

Date	Summary of Changes

07/01/2022	**Applicable Codes**
	• Updated list of applicable CPT codes to reflect quarterly edits:
	  ○ Added 0326U, 0329U, and 0331U
	  ○ Revised description for 0016M
	Supporting Information
	• Archived previous policy version CS152NJ.O

Instructions for Use

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

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